

Changes in skin reactivity, specific IgE and IgG levels after one year of immunotherapy in olive pollinosis

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Changes in specific skin reactivity, specific IgE and specific IgG after immunotherapy (IT) were investigated in olive pollinosis. Thirty patients, receiving IT with commercial extracts, were studied in comparison with a control group of seven patients, receiving only drug therapy. Skin reactivity, IgE and IgG were assessed before starting IT and 1 year later. Definite changes in the three considered parameters occurred in patients given IT with *Olea europaea* extracts; no variation was observed in the control group. The specific skin reactivity, evaluated by means of quantitative skin prick tests, significantly decreased (Skin Index geometrical mean from 2.73 to 0.88, $P < 0.001$); the specific IgE, measured by RAST, were surprisingly decreased (from 7.76 to 4.74 PRU/ml, $P < 0.001$); the specific IgG, measured by ELISA, in basic conditions were detectable only in nine patients of 30, while, after IT, they were found in almost all patients with a remarkable increase (from 5.48 to 266.89 AU/ml, $P < 0.001$). No correlation was found among the changes in the considered parameters, suggesting that, at least in olive pollinosis, specific skin reactivity, specific IgE and specific IgG are three variables depending on IT but reciprocally independent.

Key words: allergens; IgE; IgG; immunotherapy; pollen, skin tests.

Accepted for publication 1 November 1990

CLINICAL ASPECTS

Olive pollen is one of the most important allergenic agents in countries with a Mediterranean climate. Immunotherapy (IT) has been widely employed in the treatment of olive pollinosis but the clinical and laboratory effects have been scarcely investigated, so far. Changes in specific skin reactivity, specific IgE and specific IgG after 1 year of IT with commercial extracts of *Olea europaea* were investigated in order to speculate on their biological significance. Differing from what was observed in a control group composed of drug-treated subjects, patients receiving IT showed a significant decrease in specific skin reactivity and in specific IgE. Specific IgG, scarcely detectable before IT, were remarkably increased after the treatment. These three variables did not vary correspondingly. Thus, at least in olive pollinosis, one may conclude that changes in specific skin reactivity, specific IgE and specific IgG can be considered as dependent effects of the IT activity but reciprocally independent.

Olive pollen is one of the most important allergenic agents in countries with a Mediterranean climate. Since olive (*Olea europaea* L.) should be

regarded as an almost strictly cultivated species, the prevalence of olive pollinosis may vary considerably from region to region within the same

climatic environment, depending on the quantity of cultivation of this tree. Bari, southern Italy, is one of the sites where this pollen has been recognized in considerable amount (11). Olive pollens usually appear in the atmosphere of Bari during the first fortnight of May, changing within a few days from very poor counts to several hundred or thousand grains/cubic meter; after reaching a peak a couple of weeks later, the pollen season declines, lasting in all 30–40 days. In this area, the prevalence of olive allergy can be estimated at around 30–50 % of all patients with pollinosis, although it should be underlined that about 80 % of these subjects show other associated allergic sensitizations. Immunotherapy (IT) has been widely employed in the treatment of olive allergy; nevertheless its effects have been poorly investigated from both a clinical and a laboratory point of view.

IT research may regard efficacy and safety and may be performed by clinical methods and laboratory tests, *in vivo* and *in vitro* (8). In this paper three different laboratory parameters are examined in a selected group of 30 olive-sensitized patients, undergoing IT with commercial extracts. Specific skin reactivity, as an *in vivo* method, allergen-specific IgE and allergen-specific IgG, as *in vitro* methods, were studied before and after one cycle of treatment. A second group of seven patients, treated with drug therapy only but receiving the same exposure to the pollens, was studied as a control group. The relationships among the changes in the three considered parameters were statistically evaluated.

MATERIAL AND METHODS

Periods and study design

The recruitment of the patients developed over the 1985 pollen season (May–June) and lasted for some months more, until September 1985. The subjects satisfying the recruitment conditions were randomly allocated to two groups: a trial group (Group A), to receive IT from October–November to April and, in addition, drug therapy (antihistamines, nebulized bronchodila-

tors and DSCG), during the symptomatic season, and a control group (Group B), to receive only drug therapy during the ensuing season. Quantitative skin prick tests and a peripheral venous blood drawing for specific IgE and IgG determinations were performed twice in each patient: the first time in October–November 1985, 3 months after the end of allergenic exposure and just before Group A patients started IT, and the second time, 1 year later, in October–November 1986, when all patients had been exposed to the successive pollen season (which ended 3 months before) and when Group A patients had received a complete cycle of IT.

Admission criteria

The enrollment criteria were:

- 1) A clear clinical history, with special emphasis on the concurrence of symptomatic and aerobiological periods, without concern for the nature of the symptoms: patients could enter the trial regardless of whether they had rhinoconjunctivitis or rhinoconjunctivitis plus asthma.
- 2) A positive skin prick test for olive pollen. Polysensitized patients were also considered under the conditions that: a) the associated sensitizations were clinically insignificant or b) the associated sensitizations caused symptoms in a completely different period of the year, and c) their treatment did not conflict with a potential IT for olive pollen. Freeze-dried, BU standardized extracts from Pharmacia AB, Uppsala, Sweden were used in skin prick testing, at a concentration of 100,000 BU/ml (1,000 BU is the allergen concentration eliciting a wheal of the same size as that of histamine 1 mg/ml); these extracts were reconstituted in H₂O/glycerol (50/50 v/v), with phenol 5 mg/ml; the allergen panel included *Phleum pratensis*, *Parietaria judaica*, *Artemisia vulgaris*, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog and *Cladosporium herbarum*. The tests were performed quantitatively (see below); the mean wheal diameter was calculated 15 min after pricking; a mean diameter > 3 mm was assumed as a positive result.
- 3) Eligibility for receiving IT, based on the rules generally accepted in this matter (8) as well as

on practical considerations, such as patient compliance.

4) No previous IT for olive pollen.

5) Adult age.

6) Residence in the area of Bari, so that all patients would have a similar pollen exposure.

Sample drawing

An investigated population of 50 people was formed from the eligible cases seen at the Department of Allergic Diseases, University Hospital of Bari, during the months from May 1985 to September 1985. The patients were allotted serially a number from the table of random numbers and allocated to the two groups with a ratio IT-treated/controls equal to 4/1; in practice patients with numbers ending in 2 and 5 were allocated to the control group.

Patients

After considering patients who refused to enter the trial (10 cases) and cases who dropped out (3 cases, because of noncompliance, between 2 and 5 weeks after having started the treatment), the two groups were as reported in Table 1.

Immunotherapy

IT was performed with commercial extracts of *Olea europaea* from four companies (Table 2); the way of administration was essentially pre-seasonal: the first dose was given in October-November, then a cycle of weekly administrations followed; doses were roughly doubled at each

time. After reaching the highest dose (usually according to the manufacturer's recommendations), a short maintenance course was undertaken. The maintenance dose was the highest reached and was given first after 2 weeks, then after 3 weeks and finally monthly, until the treatment was stopped at the end of April, just before the beginning of the pollen season.

Quantitative skin prick testing

Specific skin reactivity was evaluated quantitatively (15). Briefly, a drop of extract was applied in duplicate on the volar surface of the right forearm; skin was then pricked by gently pressing an Østerballe-type lancet through the drop, at an angle of 90°; the same operator performed the tests before and after IT. The results were read 15 min after pricking; the perimeter of the wheals (if present) was marked with a soft ball-point pen and transferred to suitable forms by means of tape. A freeze-dried, BU standardized extract of *Olea europaea* from Pharmacia AB, Uppsala, Sweden, (Pharmalgen *Olea europaea*) was employed at four 10-fold increasing concentrations, from 100 to 100,000 BU/ml (see above). The extracts were reconstituted in glycerol/H₂O (50/50, v/v), with phenol 5 mg/ml; this solution was also used as a negative control extract; a solution of histamine chloride 1 mg/ml was used as a positive control; glycerol diluent and histamine solution were from Pharmacia AB, Uppsala, Sweden. The wheal areas were successively measured and the average of the two homologous wheals was calculated. In the present paper the results referring to changes in specific skin reactivity are shown in the form of a Skin Index, calculated as the ratio between the average of the two 100,000 BU wheals and the average of the two histamine wheals. The absolute values of the 100,000 BU wheals in mm² are also reported.

IgE evaluation

Olea europaea-specific IgE were evaluated by a radio-allergo-sorbent test (RAST from Pharma-

Table 1.

Thirty-seven olive pollen sensitized patients. Clinical data

	IT-treated	Controls
Number	30	7
Sex	14 M, 16, F	3 M, 4 F
Age: mean	28.7	34
SD	12.5	11.6
Monosensitized	21	3
Polysensitized	9	4

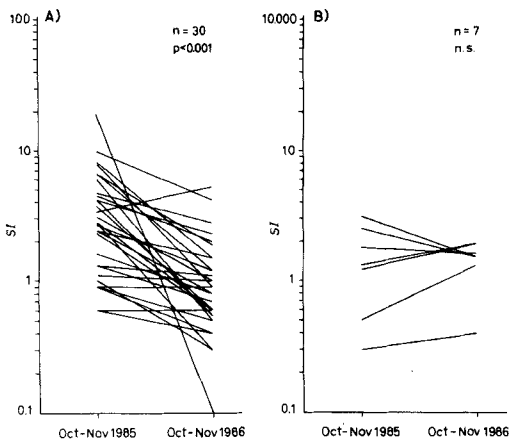


Fig. 1. Changes in specific skin reactivity, expressed as a Skin Index (SI), in IT olive-sensitized patients (A) and in controls (B). Statistical evaluation by Wilcoxon signed rank test for paired data.

cia AB, Uppsala, Sweden), according to the manufacturer's instructions. Sera displaying high levels of specific IgE were diluted in order to fall into a class 2 range. Results were expressed as Pharmacia RAST Units/ml (PRU/ml).

IgG evaluation

Preparation of the pollen extract. Dried *Olea europaea* pollen was kindly provided by Pharmacia (Uppsala, Sweden). The pollen extraction was performed essentially according to Giuliani et al. (9). Briefly, ether-soluble fatty compounds were removed by washing the pollen eight times with 1/5 (w/v) peroxide-free dry diethylether. Defatted pollen was extracted in 0.125 M ammonium bicarbonate, pH 8.0, containing 1 mM sodium azide for 20 h at 40°C. After extensive dialysis against 2 mM ammonium bicarbonate and finally against water, the extract was lyophilised. The protein content was evaluated by using the method of Bradford (4).

IgG assay. *Olea europaea*-specific IgG were evaluated by an ELISA test. Sera were tested in duplicate both with and without antigen. Microelisa plates (Dynatech, Plochingen,

Germany) were coated with the antigen 3 h at 37°C and then left over night at 4°C. For each well, either 0.2 ml of 20 µg/ml *Olea europaea* pollen extract solution in 0.05 M bicarbonate buffer, pH 9.6 (antigen buffer), or antigen buffer alone was added. After washing in phosphate-buffered saline/0.05% Tween 20 (PBS/Tween), the plates were incubated with 1/10 dilution in PBS/Tween of the human sera, 0.2 ml well, 1 h at 37°C. After washing, the plates were incubated for 1 h at 37°C with 0.2 ml/well of peroxidase-labeled anti-human IgG antibody (KPL, Gaithersburg, MD, USA) 1/5,000 in PBS/Tween. The plates were washed again and developed by adding 0.2 ml/well of ortho-phenyldiamine solution (0.5 mg/ml in 0.1 M citrate/0.2 M phosphate buffer, pH 7.2), containing 0.01% hydrogen peroxide. The reaction was stopped after 30 min, by adding 50 µl/well of 0.5 N sulphuric acid, and the plates were read at 495 nm in a Titertek Multiscan ELISA reader (Flow Laboratories, Milano, Italy). In each plate, a reference human serum obtained from an olive-sensitized patient and assigned 5,000 IgG arbitrary units (AU)/ml, was used at dilutions ranging from 1/5 to 1/20 in PBS/Tween. The amount of IgG AU/ml contained in each serum was calculated by regression analysis on the curve obtained with the reference serum. The sensitivity of this assay was of 20 IgG AU/ml; the reproducibility was approx. 15%, when evaluated in samples tested five times. Sera with high levels of specific IgG were diluted in order to fall into the linear part of the standard curve.

Ethics

The patients were informed about the outline and the purposes of the study and verbal consent was requested. The declaration of Helsinki was followed.

Statistical evaluation

Data are reported either individually or as geometrical means; standard deviations (SD) and

Table 2.
 Details about the four commercial extracts for IT, patients and dosages

Extract commercial name	Manufacturer	Manufacturer's Italian representative	Diluent	Units	Patients	No. weekly administrations	Top dose and maintenance dose	No. maintenance doses. Group average	Cumulative dosage. Group average
Pharmalgen	Pharmacia AB Uppsala, Sweden	Pharmacia SpA Cologno Monzese (Milano)	Saline/ Al(OH) ₃ Phenol 5 mg/ml	BU ¹	18	14	100,000 BU	3.55 SD 1.5	509,719 SD 182,259
None	Alergia e Immunologia Abellò Madrid, Spain	Neo Abellò Spa Ospiate di Bollate (Milano)	Saline/ Al(OH) ₃ Phenol 4 mg/ml	STU ²	2	14	800 STU	2.75 SD 1.06	4,173 SD 960
None	Allergopharma Joachim Ganzer KG Hamburg, Germany	Bracco Industria Chimica SpA Milano	Saline/ Al(OH) ₃ Phenol 4 mg/ml	PNU	4	12	10,000 PNU	3.25 SD 1.26	45,665 SD 7,489
Alhydrox	Dome, Hollister Stier Miles-Bayer Bridgend, UK	Bayropharm Italiana SpA Milano	Saline/ Al(OH) ₃ Phenol 4 mg/ml	PNU	6	12	6,000 PNU	3.92 SD 1.2	34,085 SD 8,591

¹BU: Biological Units. 1,000 BU is the allergen concentration eliciting a wheal of the same size as that of histamine 1mg/ml.
²STU: Standard Treatment Units. 1,000 STU is assumed as the maximal tolerable dose (as assessed by the manufacturer).
 For *Olea europaea*, 1,000 STU are equal to 25 BU, according to Brighton et al. (5): 100 BU is the allergen concentration eliciting a wheal of 75 mm², on average, in 30 selected patients.

variation coefficients (VC) were also calculated. Non-parametric statistical analysis was used to evaluate the significance of the differences before and after IT (Wilcoxon signed rank test for paired data) as well as the correlation between: 1) changes in specific skin reactivity (Skin Index) and changes in specific IgE; 2) changes in specific skin reactivity and changes in specific IgG; 3) changes in specific IgE and in specific IgG (Spearman's correlation test). The changes are expressed as ratios between post IT values and pre-IT values. The Spearman's correlation test was also employed to examine the correlation between absolute values of Skin Index and specific IgE, before IT as well as after IT.

RESULTS

Specific skin reactivity

The changes in specific skin reactivity (expressed as a Skin Index) are reported in Fig. 1. Among olive IT patients (Group A), 27 of 30 showed a decrease in Skin Indexes, two remained constant and only one had an increase; the two geometrical means significantly differed, decreasing from 2.73 before IT (SD 3.83,

VC 140) to 0.88 after IT (SD 1.16, VC 132). In contrast, in the seven subjects who did not undergo IT (Group B), the allergen-induced skin reactivity was slightly increased at the end of the study, although the observed difference was statistically insignificant (geometrical means from 1.21 to 1.31; SD from 1.01 to 0.51; VC from 83 to 66).

The geometrical averages of the 100,000 BU wheal areas were: in Group A, 29.74 mm² before IT and 17.62 mm² after IT; in Group B, 21.27 mm² and 24.47 mm², respectively.

Specific IgE

The changes in specific IgE are shown in Fig. 2. A decreasing trend was observed in the IT group. The geometrical means were: 7.76 PRU/ml before IT and 4.74 PRU/ml after, with a Wilcoxon's test for paired data proving positive; SD were 25.85 and 21.33; VC were 333 and 450, respectively. Four subjects showed an increase in IgE levels after IT, but only one had a marked augmentation, from 16.5 to 102.5 PRU/ml. Two subjects, with undetectable specific IgE levels before the treatment, remained negative after IT.

A minor and statistically non-significant increase was found in the control group (geometrical means from 2.66 to 2.81 PRU/ml; SD from 9.90 to 11.71; VC from 372 to 417).

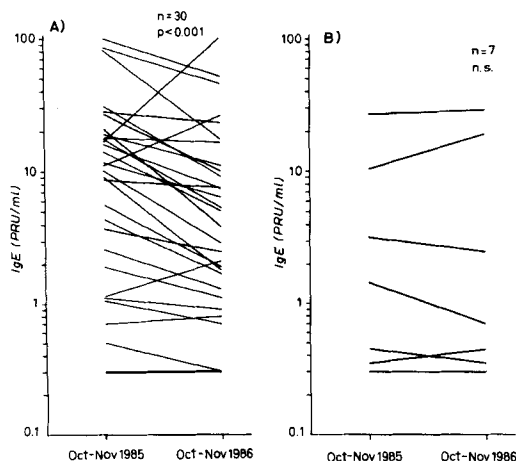


Fig. 2. Changes in specific IgE, expressed as PRU/ml, in IT olive-sensitized patients (A) and in controls (B). Statistical evaluation by Wilcoxon signed rank test for paired data.

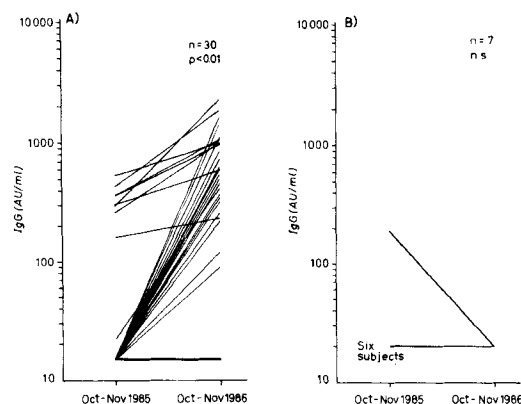


Fig. 3. Changes in specific IgG, expressed as AU/ml, in IT olive-sensitized patients (A) and in controls (B). Statistical evaluation by Wilcoxon signed rank test for paired data.

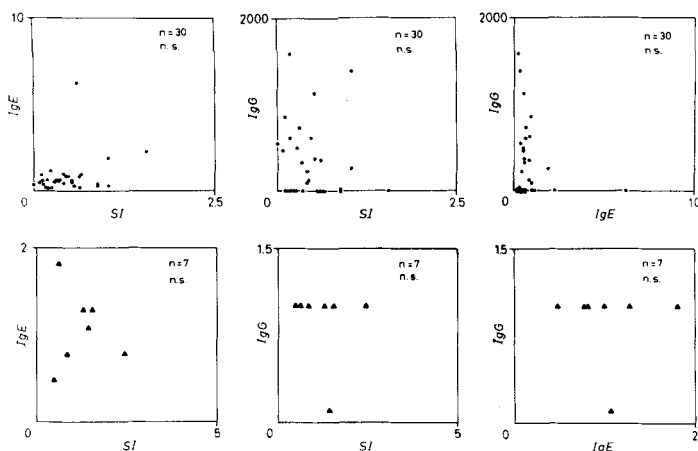


Fig. 4. Correlations between changes in Skin Index (SI) and specific IgE, SI and specific IgG, specific IgE and specific IgG, in IT olive-sensitized patients (top) and in controls (bottom). The changes are expressed as ratios between post-IT values and pre-IT values. Statistical evaluation by non-parametric Spearman's correlation test.

Specific IgG

Fig. 3 reports the findings of the allergen-specific IgG investigation. In the IT group, only nine of 30 patients showed a measurable amount of specific IgG before treatment, while after treatment all the subjects had detectable levels of IgG, except three who still remained negative. The geometrical mean values changed remarkably from 5.48 AU/ml to 266.89 AU/ml; SD were 161.16 and 571.79. In the control group six of seven had undetectable levels of specific IgG both at the beginning and at the end of the observation period, while the remaining patient showed a marked decrease (from 189 down to less than 20 AU/ml).

As regards the relationships among the three considered variables, in pre-treatment conditions the non-parametric Spearman's rank test failed to show any significant correlation between Skin Indexes and specific IgE, both in Group A and Group B patients; the correlation was still non-significant after the treatment. The fact that in basic conditions specific IgG were undetectable in most of the patients, made testing correlations between Skin Indexes and specific IgG and between specific IgE and specific IgG unreliable.

When the changes observed after IT were investigated, no correlation could be demonstrated by the same statistical test between: 1) Skin Indexes and specific IgE; 2) Skin Indexes and specific IgG; 3) specific IgE and specific IgG, in the IT patients (Fig. 4). As expected, no correlation among the changes in the above-mentioned parameters could be found in the control patients (Fig. 4).

DISCUSSION

Changes in specific skin reactivity, specific IgE and specific IgG levels in serum represent three of the most widely investigated biological effects of IT.

A decrease in immediate skin reactivity has been proved after IT with a number of allergenic extracts, including mites (1), animal danders (10), moulds (12), *Hymenoptera* venoms (3) and pollens (2, 17). The present paper also clearly demonstrates this phenomenon in olive pollinosis, as indicated by the significant difference between the Skin Index geometrical averages calculated before and after one cycle of IT.

The consequences of the allergenic IT on the circulating specific IgE are more controversial, despite the many studies on this subject. Out of the wide range of often conflicting results, the

most usual IgE response pattern after several years of treatment is an increase shortly after starting the therapy, followed by a slow decrease (6, 8, 16). In olive IT we found a definite decline 1 year after starting the treatment. Since olive IT has been sparsely investigated so far and, to our knowledge, no *in vitro* studies have been reported, the possibility of making a direct comparison is difficult. Moreover, the short-term and seasonal effects have not been examined in this paper. This restricts the analysis to only two well-defined and consistent but arbitrary points: before IT and 1 year later (i.e. after 1 cycle of IT, a new seasonal exposure and 5 months of discontinuation). Nevertheless, we consider our data convincing: the decline referred to 24 of the 30 treated subjects, and the post-treatment geometrical average significantly differed from the pre-treatment one, although a slightly larger dispersion of the individual values, as indicated by the coefficient of variation (from 333 % to 450 %), should not be neglected. Finally, the controls showed no decrease.

It has been shown that a marked rise in serum level of allergen-specific IgG is the most constant immunological change during IT (1, 2, 3, 6, 7, 8, 10, 13, 14). We found that specific IgG in olive pollinosis were scarcely detectable in untreated patients; in basic conditions the employed assay proved negative for 21 of 30 patients belonging to the IT group and in six of seven patients in the control group (73 % in all). IT resulted in a striking augmentation of these immunoglobulins in 27 subjects, while among the controls the only positive subject decreased his specific IgG and the six negative subjects remained unchanged. These results are consistent with the majority of other reports on this subject. There is an almost general agreement that allergen-specific IgG are undetectable or detectable only at low levels in allergic untreated patients; when allergenic IT has been established, specific IgG start to increase slowly a few months after the onset, reaching considerable values in 1 year. After that they remain elevated during the time of the treatment. In our study, the after-IT data notably indicated that the changes observed could not be con-

sidered as a mere transitory consequence of the IT, since these changes had been detected after a relatively prolonged suspension of the treatment (this also applies to the two other parameters studied).

Finally, the statistical evaluation of the data showed that although definite changes in the three parameters occurred in patients given IT with *Olea europaea* extracts, these changes could not be correlated. This suggests that, at least in this pollinosis, specific skin reactivity, specific IgE and specific IgG are three variables depending on IT but reciprocally independent. The fact that those patients developing higher levels of IgG after IT did not necessarily have a greater decrease in IgE should be underlined. The lack of reverse correlation between changes in IgE and IgG indicate the absence of any significant reciprocal interference in the IgG and IgE evaluation techniques, since high levels of specific IgE could be detected in sera displaying high levels of IgG.

ACKNOWLEDGMENTS

This work was supported in part by CNR, Rome, P. F. FATMA, S. P. 2.4.

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