

Desensitization Effect of Preseasonal Seven-Injection Allergoid Immunotherapy with Olive Pollen on Basophil Activation: The Efficacy of Olive Pollen-Specific Preseasonal Allergoid Immunotherapy on Basophils

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Key Words

Allergen-specific immunotherapy · Allergen-specific nasal challenge · Allergoid · Basophil activation test · *Olea europaea* pollen

Abstract

Background: It has previously been demonstrated that subcutaneous immunotherapy with allergoids positively affects clinical and immunological parameters even after 7 pre-seasonal injections. However, its effect on basophil activation remains unclear. We investigated the effect of pre-seasonal allergoid immunotherapy on basophils and concomitantly assessed its clinical and immunological efficacy in olive pollen-monosensitized patients. **Methods:** This study enrolled 437 consecutive patients with respiratory allergy and positive skin prick tests (SPTs); 212 (48.5%) patients were sensitized to olive pollen, and 33 (7.5%) patients were sensitized to olive pollen only. Of these patients, 23 received preseasonal immunotherapy with an olive pollen allergoid. The olive pollen-specific basophil activation, the titrated nasal provocation test, the nasal symptom score, and olive pollen-specific IgE, IgG1 and IgG4 levels were evaluated before immunotherapy and 8 months after the end of immunother-

apy in the follow-up visit. **Results:** In comparison to baseline evaluation, 7 preseasonal injections of an allergoid resulted in a significant decrease in the percentage of basophils expressing CD63 (29 vs. 7%, respectively, $p < 0.0001$) and a significant increase in the titrated nasal provocative dose (1/10 vs. 1/1, respectively, $p < 0.01$). SPT induration diameters caused by an olive pollen extract decreased (12 mm at baseline vs. 5.5 mm at follow-up, $p < 0.005$), as did nasal symptom score (7 at baseline vs. 3 at follow-up, $p < 0.01$). Olive pollen-specific IgE (17.5 vs. 50 kU/l, $p < 0.012$), IgG1 (0.16 vs. 2.9 $\mu\text{g/ml}$, $p < 0.0001$) and IgG4 (0.07 vs. 1.92 $\mu\text{g/ml}$, $p < 0.0001$) levels significantly increased. **Conclusions:** Immunotherapy with 7 preseasonal injections of an olive pollen allergoid decreases olive pollen-specific basophil activation over 8 months, an effect observed in vitro and in vivo.

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Introduction

Allergen-specific immunotherapy (SIT) is a powerful treatment for patients suffering from IgE-mediated allergic diseases because it not only reduces allergic symptoms and intake of anti-allergic medication, but also may alter

the natural history of the disease [1]. However, high allergen concentrations are needed to provoke an adequate immune response and clinical efficacy with SIT, which may cause an increase in the risk of IgE-related allergic adverse events [2–4]. Using unmodified allergen extracts for subcutaneous immunotherapy (SCIT), a large number of injections and slow dose increases are necessary to achieve high allergen concentrations. Therefore, allergoids were developed by modification with formaldehyde and/or glutaraldehyde resulting in extremely reduced IgE binding capacity but still intact immunogenicity, leading to fewer side effects [5–7]. Because mast cells and basophils express high-affinity IgE receptors, reduced IgE binding has significant clinical implications. Release of IgE-related mediators by mast cells and basophils is less of a concern with allergoid immunotherapy allowing the use of higher doses of allergens. Allergens at higher doses appear to be preferentially processed by macrophages and dendritic cells rather than B lymphocytes. While macrophages and dendritic cells are associated with Th1 type cytokines, B lymphocytes interact with Th2 cells inducing secretion of allergenic IL-4 and IL-5. Additionally, reduced IgE binding of allergoids is associated with lesser FcεRI- and FcεRII-mediated antigen uptake, processing and presentation [8, 9]. As a result, allergoids allow administration of high-dose allergens during a short-term buildup phase.

There are two application schedules designated as pre-seasonal and perennial immunotherapy in the literature. The pre-seasonal schedule is generally performed in patients with pollinosis while the perennial schedule is preferentially used in mite-induced allergic rhinoconjunctivitis and/or asthma. Both pre-seasonal and perennial immunotherapy with allergoids have been shown to be effective in terms of clinical parameters, including rhinoconjunctivitis and/or asthma symptom and medication scores [10–18], conjunctival [10, 12], nasal and skin reactivity [13, 17, 18] as well as the immunological parameters. Moderate reductions in allergen-specific IgE, remarkable increases in allergen-specific IgG1 and IgG4 [10, 11, 13] and a significant decline in IL-4 levels in allergen-stimulated peripheral blood mononuclear cell culture supernatant have been shown in several studies [13]. However, the effect of allergoid immunotherapy on basophil responsiveness in sensitized patients remains unclear. In this study, we firstly investigated the efficacy of pre-seasonal SCIT with 7 injections of an olive pollen allergoid on basophils via flow-cytometric basophil activation tests conducted in parallel with analyses of the clinical and immunological effects in olive pollen-monosensitized patients.

It has been estimated that there are >100 million olive trees in Turkey, corresponding to 1 in every 8 olive trees on the planet [19]. Olive pollen-sensitized patients commonly apply to the Ege University Allergy Polyclinic, the only public allergy clinic serving the mainly middle-class adult population of Izmir. Therefore, treatment of this widespread allergy by a pre-seasonal rather than by perennial SCIT would result in a significant public health benefit in Turkey.

Patients and Methods

Patients

The current study evaluated 437 consecutive patients with moderate/severe allergic rhinitis and/or asthma symptoms according to the Allergic Rhinitis and Its Impact on Asthma guidelines [20] presenting to the Division of Allergy and Clinical Immunology of the Medical Faculty of Ege University between July 2005 and July 2006. All patients showed at least one positive skin prick test (SPT) reaction out of a panel of allergens, which is standardized in biologic units, including grasses (*Cynodon dactylon*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca pratensis* and *Anthoxanthum odoratum*), weed pollens (*Chenopodium album*, *Plantago lanceolata*, *Artemisia vulgaris*, *Taraxacum vulgare* and *Parietaria officinalis*), tree pollens (*Betula verrucosa*, *Quercus robur*, *Alnus glutinosa*, *Corylus avellana*, *Populus alba*, *Platanus orientalis* and *Olea europaea*), mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), molds (*Alternaria*, *Aspergillus* and *Cladosporium* species) and animal epithelia (*Felis domesticus* and *Canis familiaris*; Allergopharma Joachim Ganzer KG, Reinbek, Germany). Histamine (10 mg/ml) and saline were used as positive and negative controls, respectively. A wheal size ≥ 3 mm versus the negative control was regarded as positive, and the highest induration diameter was recorded. Only patients who showed an isolated positive SPT reaction to olive pollen were enrolled in the trial. For inclusion, they had to report allergic symptoms during the *O. europaea* pollination season, from mid-April through mid-June, for at least the preceding 2 years [21]. Patients were asked to complete daily diary cards to record their nasal symptom score from mid-April till mid-June 2006 while taking cetirizine 10 mg once daily. They did not record a medication score, because they were not allowed to take cetirizine >10 mg once daily or any other medication. The severity of each nasal symptom (pruritus, sneezing, nasal discharge and congestion) was recorded according to the following scale with a daily maximum score of 12, with 0 points, no symptoms; 1 point, mild symptoms; 2 points, moderate symptoms, and 3 points, severe symptoms [22]. The average daily nasal symptom score was obtained by summing the daily scores and dividing by the number of days at the end of the season (fig. 1).

In November 2006, titrated nasal provocation tests (NPTs), olive pollen-specific IgE, IgG1 and IgG4 tests and olive pollen-specific basophil activation tests were performed in the study patients.

At the follow-up in November 2007, we repeated SPT to olive pollen, titrated NPT and the basophil activation test to olive pol-

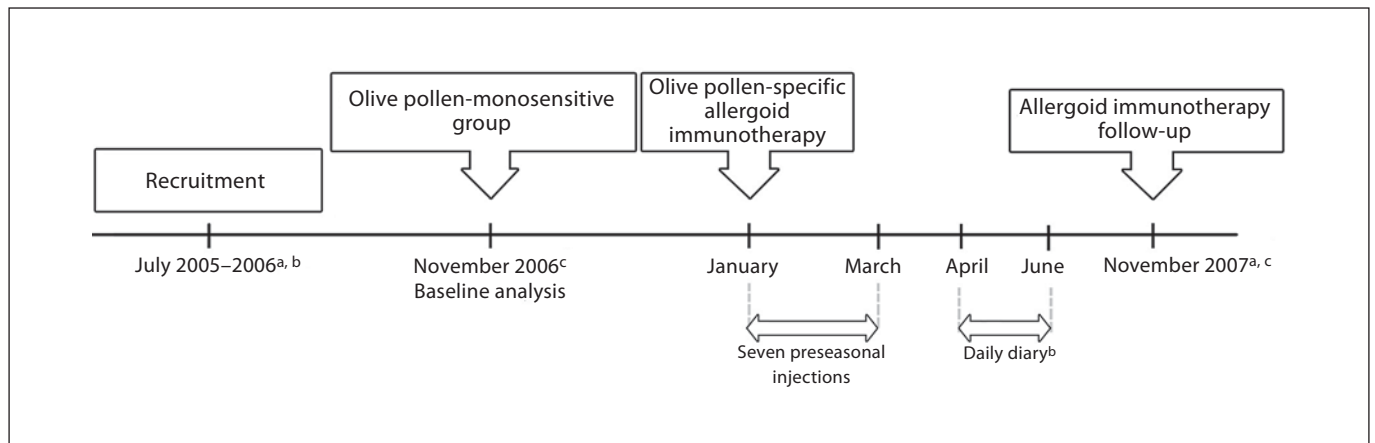


Fig. 1. Study design and timeline. The current study evaluated 437 consecutive patients with moderate/severe allergic rhinitis and/or asthma symptoms according to the Allergic Rhinitis and Its Impact on Asthma guidelines [20] who had a positive SPT to aeroallergens; these patients presented at the Division of Allergy and Clinical Immunology of the Medical Faculty of Ege University

between July 2005 and July 2006. Out of 437 patients, only 33 were monosensitized to the olive pollen and enrolled in the study. Of the 33 patients, 23 patients chose immunotherapy, but 10 patients preferred anti-symptomatic treatment. ^aSPT; ^bnasal symptom score; ^cbasophil activation test, olive pollen-specific immunoglobulins, titrated NPT.

len, and assessed the levels of olive pollen-specific IgE, IgG1 and IgG4. Patients were again asked to complete daily diary cards from mid-April till mid-June 2007 while taking once-daily cetirizine.

The study protocol (fig. 1) was approved by the Ege University Ethics Committee and written consent was obtained from all individuals participating in the study.

Immunotherapy

The investigational product was a preparation of *O. europaea* treated with formaldehyde under controlled conditions to produce an allergoid, which was then co-precipitated with aluminum hydroxide. The resulting allergoid adsorbate was supplied as a suspension in two concentrations, strength A 1,000 therapeutic units/ml and strength B (10,000 therapeutic units/ml). Subcutaneous SIT started with 0.1 ml of strength A followed by an approximately doubling of the dose at weekly intervals up to 0.6 ml of strength B containing 11 µg Ole e 1. Preseasonal SCIT (Allergovit®; Allergopharma Joachim Ganzer KG) was offered to all olive pollen-monosensitized patients. Of these, 23 patients chose immunotherapy, but 10 patients preferred anti-symptomatic treatment. SIT was started in January 2007 to reach the highest dose before the beginning of the pollen season. Adverse reactions were graded according to the European Academy of Allergy and Clinical Immunology classification [23].

Flow-Cytometric Basophil Activation Test

Venous blood samples were anticoagulated with ethylenediaminetetraacetic acid and centrifuged at 4°C for 10 min at 550 g. The leukocyte-enriched buffy coat below the serum was collected and centrifuged again, before the supernatant was decanted and the remaining leukocyte-enriched fraction was aliquoted into three tubes. Olive pollen-specific basophil activation was investigated by incubating 25 µl of leukocytes with 25 µl of olive pollen

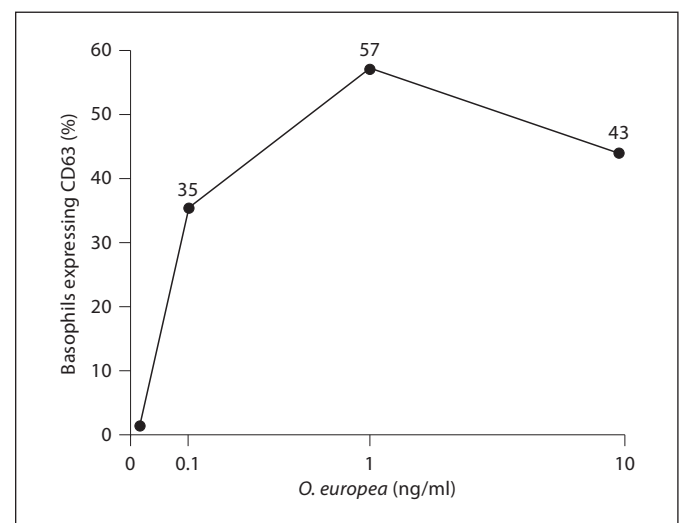


Fig. 2. Mean percentage of CD63-positive basophils after incubation with *O. europaea* pollen (0.1–10 µg/ml) in *O. europaea* pollen-monosensitized patients (n = 6).

in a final concentration of 1 ng/ml (Buhlmann Laboratory, Allschwil, Switzerland). The optimal concentration of 1 ng/ml was determined in a dose-response study on 6 olive pollen-monosensitized patients (fig. 2). Further, 1 tube with 25 µl phosphate-buffered saline served as negative and 1 tube with 25 µl anti-IgE as positive control. All 3 tubes were incubated for 40 min at 37°C before they were stored on ice, and 10 µl ice-cold phycoerythrin (PE)-conjugated anti-CD63 and fluorescein isothiocyanate (FITC)-conjugated anti-IgE mAb (Buhlmann Laboratory) were

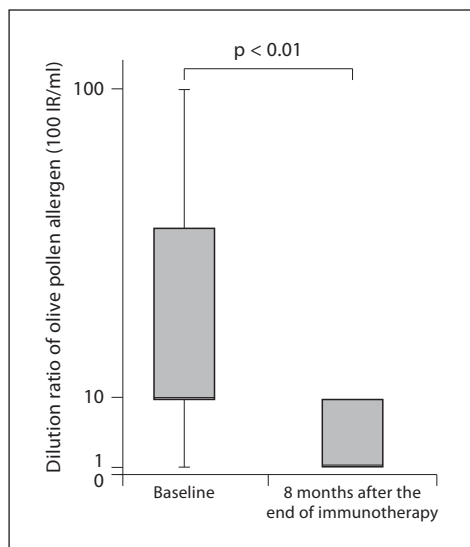


Fig. 3. The ratio of positive dilutions in NPT significantly decreased 8 months after the end of allergoid immunotherapy. Box plots indicate the middle 50% of the data (median value depicted with a line), with the upper and lower box edges marking the 75th and 25th percentiles, respectively ($n = 20$). Statistical significance ($p < 0.01$) was determined with the Wilcoxon signed-rank test.

added. Finally, flow-cytometric analysis was performed within 2 h on a FACSCalibur™ flow cytometer (Becton Dickinson Immunocytometry System, San Jose, Calif., USA).

For calculation of basophil activation, the IgE-positive cell population (= basophils) was gated, and subsequently the expression of CD63 (= activated basophils) was analyzed on this gated cell population. An average of 500 IgE-positive cells was studied per sample and results were given as percentage of basophils expressing CD63 according to the following formula: (number of IgE+ and CD63+ basophils)/number of IgE+ basophils. Patients exhibiting at least 15% CD63 expression with olive allergen after subtraction of the negative control value were regarded as allergic to olive pollen.

To evaluate the effect of SIT on basophil activation, the flow-cytometric basophil activation test was carried out before SIT and 8 months after the end of immunotherapy in the follow-up visit (fig. 3).

Titrated Intranasal Provocation Testing

NPT with *O. europaea* allergen extract (Stallergenes, Paris, France) was performed in the absence of nasal symptoms after the season using nasal applicators spraying 0.1 ml each time. The NPT was started with normal saline. Individuals who had no symptoms and who did not decrease in the nasal flow rate by >20% following saline application were exposed to allergen provocation. A nonphenolic aqueous solution of *O. europaea* allergen extract at a concentration of 100 IR/ml containing 10 µg/ml of Ole e 1 allergen was used. Serial dilutions of the allergen extract were prepared immediately before NPT using a nonphenolic physiologic saline diluent (Stallergenes). Allergen provocation was start-

ed with 0.1 IR/ml olive pollen allergen and increasing 10-, 100-, and 1,000-fold (1, 10 and 100 IR/ml *O. europaea* allergen, respectively). At each step, nasal and eye symptoms were recorded during a 15-min observation period, and the changes in the nasal flow rate were measured by active anterior rhinomanometry (Jäger Rhinoscreen, Hochberg, Germany). Symptoms were scored as follows: sneezing: 0–2 times, 0 point; 3–4 times, 1 point, and ≥ 5 times, 3 points; itching: 1 point for itching of the nose, ear or palate, respectively (maximum 3 points); rhinorrhea: none, 0 point; mild, 1 point; moderate, 2 points, and severe, 3 points; nasal block: none, 0 point; mild, 1 point; moderate, 2 points, and severe 3 points; eye symptoms (watering, itching and redness): 1 point, total 1 point. When the symptom score reached or exceeded 5 or reached 4 with a decrease in nasal flow of 40% according to the basal value, the test was regarded as positive. NPT was discontinued when there was a positive reaction or the maximum allergen concentration was reached [24].

O. europaea Pollen-Specific IgE, IgG1 and IgG4

To measure *O. europaea* pollen-specific IgE venous blood samples were analyzed in all olive pollen-monosensitized patients. The results were graded as follows (CAP system, Pharmacia, Uppsala, Sweden): class 0, <0.35 U/ml; class I, 0.35–0.7 U/ml; class II, 0.7–3.5 U/ml; class III, 3.5–17.5 U/ml; class IV, 17.5–50 U/ml; class V, 50–100 U/ml, and class VI, >100 U/ml.

Olive pollen-specific IgG1 and IgG4 levels were measured by enzyme-linked immunosorbent assay at Allergopharma Laboratories.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows 15.0 (SPSS Inc., Chicago, Ill., USA). Descriptive statistics were used to summarize clinical and demographic characteristics of the patients. Results are expressed as means \pm SD. Wilcoxon's signed-rank test was used to compare baseline and follow-up data (after SIT). A value of $p < 0.05$ was considered statistically significant.

Results

To determine the aeroallergen sensitization pattern and the frequency of *O. europaea* pollen hypersensitivity, SPT results were analyzed in 437 consecutive patients. The results showed that 139/437 patients (31.8%) were sensitized to one aeroallergen (monosensitized), 91 (20.8%) patients were sensitized to two aeroallergens (oligosensitized) and 207 (47.4%) patients were sensitized to three or more aeroallergens (polysensitized). The most frequent sensitization among patients was to grass (63.2%), followed by *O. europaea* pollen (48.5%) and mites (37.3%). Of the 437 patients, 212 (48.5%) were found to be sensitized to olive pollen in conjunction with other aeroallergens, while 33 patients (7.5%) were sensitized to olive pollen only. Thirty-one (94%) of these olive pollen-monosensitized patients showed olive pollen-specific IgE of

class I–VI. The demographic characteristics of these 33 olive pollen-monosensitized patients are presented in table 1.

Immunotherapy

SCIT with the olive pollen allergoid was offered to the 33 olive pollen-monsensitized patients but only 23 (69.7%) patients chose immunotherapy, while 10 patients preferred anti-symptomatic treatment only. However, only 20 of them were able to complete the immunotherapy regimen.

Safety

Two patients experienced moderate systemic reactions of grade II (1 patient with generalized urticaria, coughing and dyspnea 2 h after the 6th immunotherapy injection and the other had generalized urticaria 1 h after the 5th injection) [23]. The patient with generalized urticaria withdrew consent for immunotherapy, and SCIT was discontinued in another patient due to an unexpected pregnancy. None of immunotherapy injections resulted in large local reactions or subcutaneous nodules at the injection sites.

Flow-Cytometric Basophil Activation Test

Basophil activation test was performed to correlate basophil activation and olive pollen sensitization and to determine whether SIT correlates with decreased basophil responsiveness. Before SIT, 30 (90.9%) of the 33 patients showed 15% or more CD63 expression with olive allergen, and 2 patients (6.1%) exhibited <15% CD63 expression. One patient who responded neither to olive pollen allergen nor to the positive control was designated a non-responder. Subsequently, this patient's data were excluded from further analysis. Compared to baseline values, CD63 expression was significantly lower following stimulation with 1 ng/ml olive pollen allergen (fig. 4). Before immunotherapy, median CD63-positive expression was amounted to 29% (range 18–67%), while 8 months after the end of immunotherapy the median value was 7% (range 4–12%, $p < 0.0001$).

Clinical Parameters

As shown in table 2, clinical parameters were compared before immunotherapy and 8 months after the end of immunotherapy in the follow-up visit. Nasal symptom scores decreased after immunotherapy compared to baseline. At the follow-up visit, SIT-treated patients showed decreased skin test reactivity and significantly increased allergen concentrations to cause a positive NPT reaction (fig. 3).

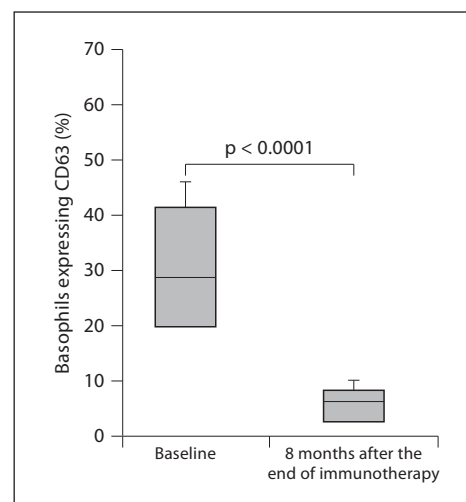


Fig. 4. Eight months after the end of allergoid immunotherapy, basophils from olive pollen-monosensitive patients exhibited reduced CD63 expression following stimulation by olive pollen allergen in the basophil activation test. Box plots indicate the middle 50% of the data (median value depicted with a line), with the upper and lower box edges marking the 75th and 25th percentiles, respectively ($n = 19$). An outlier data point 1.5–3 box lengths from the upper edge of the box is depicted with a round symbol. Statistical significance ($p < 0.0001$) was determined with the Wilcoxon signed-rank test.

Table 1. Demographics of olive pollen-monosensitive patients

Variable	Patients
Total	33
Female	24
Male	9
Age (mean \pm SD), years	36.1 \pm 9.7
Range	16–53
Duration of rhinitis (mean \pm SD), years	6.27 \pm 6.30
Range	2–27

O. europaea Pollen-Specific IgE, IgG1 and IgG4

The olive pollen-specific IgE, IgG1 and IgG4 levels markedly increased, and the olive-specific IgE/IgG4 ratio decreased 8 months after SIT (table 2).

Discussion

Olive pollinosis was detected in 1 of every 2 patients (48.5%) treated at our Allergy Polyclinic for allergic rhinitis and/or asthma symptoms. In the Mediterranean

Table 2. The effects of olive pollen allergoid immunotherapy on clinical and immunological parameters

	Baseline	8 months after the end of immunotherapy	p value
Nasal symptom score ^a	7 (3–9)	3 (3–9)	0.001
SPT reactivity, mm ^a	12 (6–25)	5.5 (0–12)	0.005
Allergen dilution ratio in NPT ^a	1/10 (1/100–1/1)	1/1 (0–1/10)	0.01
Olive-specific IgE, kU/l ^a	17.5 (0.35–100)	50 (0.7–100)	0.012
Olive-specific IgG1, µg/ml	0.16 (0.07–4.5)	2.9 (0.21–19.4)	0.0001
Olive-specific IgG4, µg/ml ^a	0.07 (0.07–0.43)	1.92 (0.07–12.4)	0.0001
Olive-specific IgE:olive-specific IgG4 ^a	44.8 (0–450)	1.6 (0.1–25)	0.001
CD63 expression, % ^a	29 (18–67)	7 (4–12)	0.0001

Comparisons between measurements taken at baseline and at the 1-year follow-up after immunotherapy were carried out using the Wilcoxon signed-rank test. $p < 0.05$ was considered statistically significant.

^a Data are given as medians (ranges).

and Aegean regions, olive pollinosis is an important health problem due to the extensive cultivation of olive trees [19, 25]. In our study, patients were most frequently sensitized to grass (63.2%), followed by *O. europaea* pollen (48.5%) and mites (37.3%). Our patients exhibited sensitization patterns similar to other Mediterranean countries [26–29].

SIT is known to reduce basophil responsiveness [30], but specific basophil reactivity to allergen is not commonly analyzed during follow-up. Wasp venom immunotherapy was not reported to affect basophils at the end of a 5-day buildup course, but 6 months later CD63 expression was significantly decreased [31]. Similarly, decreased basophil reactivity to peanut protein was observed in peanut-allergic children 6 months after oral immunotherapy [32]. Here, we demonstrated for the first time that preseasonal immunotherapy with an olive pollen allergoid reduced olive pollen-specific basophil activation 8 months after the end of immunotherapy.

In parallel with reduced olive pollen-specific basophil activation, significant increases in allergen-specific IgG1 and IgG4 levels were observed after allergoid immunotherapy. Similarly, Lalek et al. [33] demonstrated that perennial SCIT with a birch pollen allergoid led to reduced CD63 expression, which correlated with patient symptoms assessed by a visual analog scale. They also firstly revealed the reduced inhibitory effect of the patient's serum after elimination of the IgG antibodies. This study stresses the prominent role of IgG antibodies in decreased basophil activation to allergen challenge in the basophil activation test. The blocking IgG antibodies most likely bind to allergen and thus reduce free allergen concentra-

tions in the vicinity of mast cells and basophils [33]. Cady et al. [34] demonstrated that IgG antibodies produced during subcutaneous immunotherapy with a cat extract also inhibited basophil responses to allergen via binding to both FcγRIIA and inhibitor FcγRIIB immunoglobulin Fc receptors expressed by basophils.

All olive pollen-mono-sensitized patients in the study at hand showed positive responses to olive pollen in titrated NPT, with significantly reduced reactivity at the follow-up visit. Similarly, skin test reactivity significantly decreased. Olive pollen-specific IgE levels increased in comparison to baseline (table 2). However, results of specific IgE levels were inconsistent after SIT with different allergoids. For example, Guerra et al. [35] reported no change in specific IgE levels 1 year after pollen allergoid immunotherapy, whereas Pastorello et al. [36] observed a significant increase in mean specific IgE levels after 3 and 4 months of pollen SCIT. Keskin et al. [13] did not detect any differences in grass pollen-specific IgE between levels at baseline and after 1 year of immunotherapy. On the other hand, we observed significant differences from baseline in SPT and nasal reactivity, nasal symptom score and the allergen-specific IgE:IgG4 ratio (table 2). Therefore, the achievement of diminished specific IgE levels may not be an obligatory criterion in the evaluation of efficacy of immunotherapy (fig. 5).

Allergoids are known to be allergen preparations with diminished allergenicity but retained immunogenicity. Consistent with this observation, Corrigan et al. [10] did not report any serious drug-related adverse event or anaphylaxis in patients suffering from allergic rhinitis and/or asthma who received grass pollen allergoid immuno-

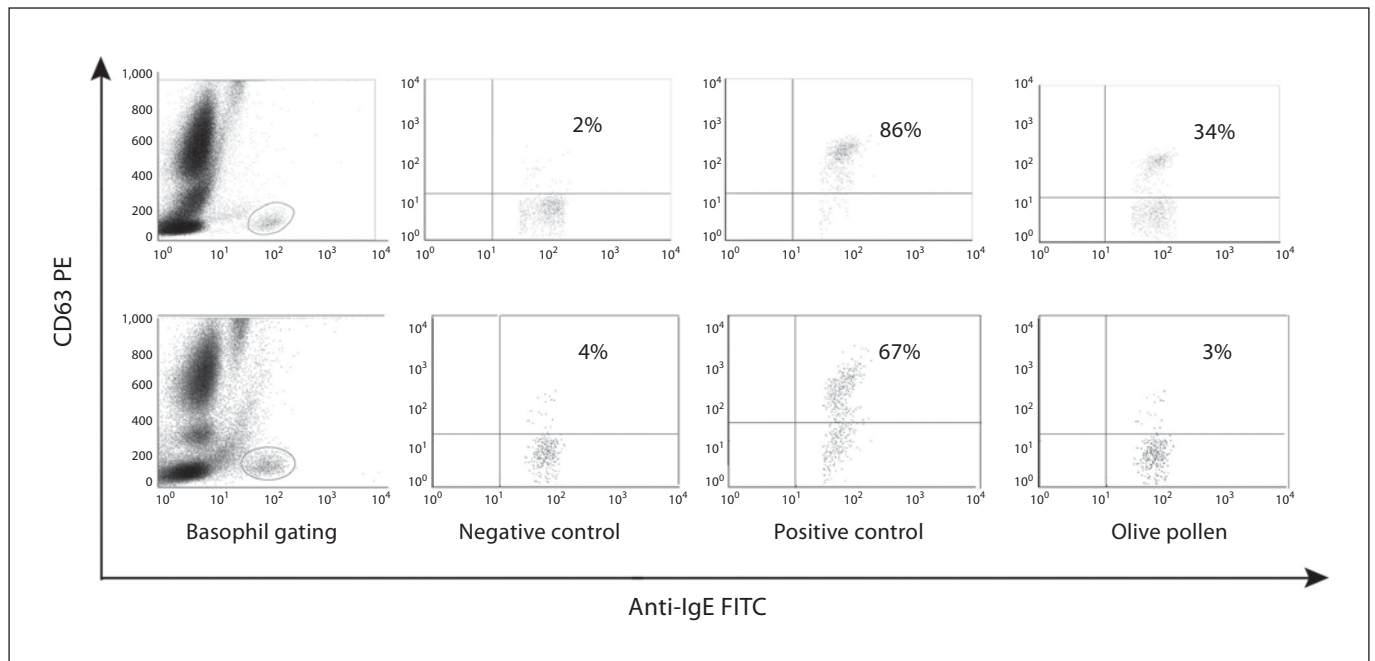


Fig. 5. Diminished *O. europaea* pollen-specific activation of basophils from an olive-pollen monosensitized subject who received the preseasonal 7-injection regimen. The percentage of CD63-positive basophils following stimulation with 1 ng/ml olive pollen allergen was detected at baseline (a) and 8 months after the end of immunotherapy (b). The basophil population was gated (circled) by the expression of FITC-conjugated anti-IgE (x-axes). The ex-

pression of PE-conjugated CD63 (y-axes) by the gated population is presented as the percentage of basophils expressing CD63 according to the following formula: number of FITC anti-IgE+ and PE anti-CD63+ basophils/number of FITC anti-IgE+ basophils. Patients exhibiting CD63 expression $\geq 15\%$ than the negative control with olive allergen were regarded as allergic to olive pollen.

therapy for 3 years. Safety of the high-dose pollen allergoid Allergovit was confirmed by a recent review article. The risk of developing a serious systemic reaction was noticeably reduced with Allergovit compared to an unmodified semi-depot preparation of the same manufacturer (every 7,500th vs. every 5,000th patient) in the years 1996–2000 [37]. In the study at hand, 2 of 23 patients receiving SCIT experienced a moderate systemic reaction after the 5th or 6th injection. We therefore concluded that systemic symptoms can occur at or near the highest dose of olive pollen-specific allergoid immunotherapy.

In conclusion, we have demonstrated that olive pollinosis affects an important proportion of atopic adults in Izmir, Turkey. The preseasonal application of 7 injections of the olive pollen allergoid is a short-term treatment modality that has positive effects on clinical and immunological parameters, including basophil activation. It would be of value to design a study in a larger patient cohort with a double-blind, placebo-controlled trial design, thus enabling direct comparison of the effects obtained by immunotherapy.

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Disclosure Statement

No potential conflicts of interest were disclosed.

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