Rhinitis, sinusitis, and upper airway disease

Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber

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Background: The efficacy and safety of a 5-grass-pollen sublingual immunotherapy (SLIT) tablet (Stallergènes SA, Antony, France) have been evaluated in clinical studies during the pollen season. The allergen challenge chamber (ACC) has been developed as a pharmacodynamic assessment tool to control the environmental allergens and to avoid all problems associated with unpredictable pollen seasons.

Objective: We sought to evaluate the onset of action and efficacy of 300-IR (index of reactivity) SLIT tablets by using an ACC.

Methods: Patients with grass pollen–induced rhinoconjunctivitis were randomized into the active or placebo groups. A standardized allergen challenge with grass pollen and symptom evaluation every 15 minutes was performed at baseline, 1 week, and 1, 2, and 4 months of treatment. The primary end point was the average rhinoconjunctivitis total symptom score (ARTSS). Allergen-specific basophil activation, T-cell proliferation, and plasmatic IgE and IgG responses were assessed before and after treatment.

Results: In the intention-to-treat population (n = 89) a significant treatment effect was achieved after the first month (P = .0042) and second month (P = .0203) and was maintained through to the fourth month (P = .0007). In the active group the ARTSS (means ± SDs) decreased at each challenge: week 1, 7.40 ± 2.682; month 1, 5.89 ± 2.431; month 2, 5.09 ± 2.088; and month 4, 4.85 ± 1.999. An improvement (vs placebo) of 29.3% for the mean ARTSS (median, 33.3%) was observed at end point. Furthermore, the induction of grass pollen allergen–specific IgGs was associated with clinical response. The most frequent adverse reactions were local: oral pruritus, ear pruritus, and throat irritation.

Conclusions: In this ACC study the 300-IR 5-grass-pollen SLIT tablets had a significant effect on rhinoconjunctivitis symptoms (vs placebo) from the first month of treatment onward.

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Key words: Grass pollen, tablets, sublingual immunotherapy, allergen challenge chamber, Vienna Challenge Chamber

A 5-grass-pollen 300-IR (index of reactivity) sublingual immunotherapy (SLIT) tablet (Stallergènes SA, Antony, France) has demonstrated its efficacy and safety in a series of multicenter clinical trials in adult and pediatric populations with seasonal allergic rhinoconjunctivitis (SAR) triggered by grass pollen.1-3 These standard outdoor clinical trials evaluated the symptoms of SAR during the pollen season and, as such, were inevitably influenced by unpredictable variations in pollen levels, antigenicity, and exposure. In fact, variations in the patients’ degree of pollen exposure occur through both local variations in pollen counts and differences in individuals’ daily routines and pollen-avoidance strategies (eg, staying indoors during the pollen peak). The lack of standardized exposure and natural year-to-year variations in the dates of pollen season onset and peaks makes it difficult to perform outdoor studies designed to measure the onset of action of SLIT and thus determine the optimal preseasonal treatment duration required for efficacy.

In Europe the main pollination period covers about half the year, from spring to autumn. For grasses, the flowering period starts at the beginning of May for countries in which pollination is early and finishes at the end of July for the latest countries. In Mediterranean areas flowering usually starts and ends 1 month earlier compared with other European areas. Pollination occurs about 2 to 3 weeks earlier at sea level than in mountainous regions. On the whole, in Europe grass flowering notably starts in June. The pollen season tends to vary from year to year because of fluctuations in climatic factors, but the maximum atmospheric concentration of grass pollen usually occurs 1 to 2 months after the start of the main flowering season.4,5 The current study assessed the efficacy and onset of action of 5-grass-pollen tablets under controlled conditions provided by an allergen challenge chamber (ACC; also known as an environmental exposure unit) to overcome these variations. An ACC is a specially designed room used to expose study participants to a fixed, predetermined allergen concentration for a set period of time.6 ACCs also allow identical repeated exposures and thus assessment of changes over time in an individual’s response.
In recent years, ACCs have been used in a variety of studies evaluating different aspects of SAR therapeutics, such as the onset of action of antiallergic treatments \(^\text{7,9}\) and the efficacy and safety of drug candidates.\(^\text{6,10,11}\) The current draft guideline on the clinical development of specific immunotherapy products for the treatment of allergic diseases published by the European Medicines Agency’s Committee for Medicinal Products in Human Use cites the ACC as a pharmacodynamic assessment tool that can potentially be used to provide supportive evidence of clinical efficacy.\(^\text{12,13}\) ACC studies investigating the effect of various antihistamines (desloratadine, cetirizine, azelastine, and fexofenadine) have shown that symptom scores with placebo and active treatments are similar to those obtained in outdoor clinical trials. Additional information from ACC trials might significantly contribute to a better determination of a medication’s clinical profile, especially in terms of onset and duration of action.\(^\text{12,14}\)

A Cochrane collaboration meta-analysis of SLIT in patients with rhinitis demonstrated evidence of efficacy with reduction in symptoms and use of symptomatic medication.\(^\text{15}\) It has been previously shown that a preseasonal and coseasonal treatment with sublingual grass pollen tablets is effective and safe in the treatment of grass pollen SAR.\(^\text{1,2,16,17}\) The aim of this study was to demonstrate the placebo-controlled efficacy of a 5-grass-pollen 300-IR SLIT tablet and provide the first ever determination of the onset of action of SLIT tablets under the controlled, stable conditions found within an ACC.

**METHODS**

**Patients**

Eligible patients were men and women aged between 18 and 50 years with a documented history of moderate-to-severe seasonal grass pollen–related allergic rhinoconjunctivitis for at least the 2 previous pollen seasons. At screening, patients were required to demonstrate grass pollen sensitization through a positive specific skin prick test response (wheal diameter > 3 mm) to a 5-grass pollen extract (Stallergènes SA) and a specific serum IgE level of at least 0.70 kU/L for timothy grass (assayed with the UniCAP system; Phadia, Uppsala, Sweden). In addition, patients had to show a symptomatic reaction to an allergen challenge test at baseline (ie, before the administration of any study treatment), which was defined as a rhinoconjunctivitis total symptom score (RTSS) of at least 7 (of 18) within the 2-hour challenge (see the “Efficacy variables” section for more details of the RTSS). The main exclusion criteria were as follows: allergic rhinoconjunctivitis caused by a cosensitization likely to significantly influence symptoms throughout the study and asthma requiring treatment other than rarely a short-acting inhaled B2-agonist. All patients provided written informed consent before study entry. The study was carried out in accordance with the Declaration of Helsinki (1964, as amended in 2004) and good clinical practice (CPM/ICH/135/95) and was approved by the appropriate independent ethics committee and regulatory authorities.

**Study design**

This was a randomized, double-blind, parallel-group, placebo-controlled, single-center trial performed between the 2007 and 2008 grass pollen seasons. After an initial screening visit and a baseline allergen challenge, eligible patients were randomized 1:1 to receive either a 300-IR SLIT tablet or placebo. Patients underwent an allergen challenge in the chamber with grass pollen before treatment (the baseline challenge). A 2-hour baseline challenge was chosen, which was sufficient for qualification, to avoid unnecessary priming and to keep the patients’ burdens as low as possible (no rescue medication was allowed). Additional challenges were performed after 1 week and 1, 2, and 4 months of treatment (each lasting 4 hours, Fig 1).

**Immunotherapy**

The investigational product was a 300-IR 5-grass-pollen SLIT tablet, (orchard, meadow, perennial rye, sweet vernal, and timothy grasses; Stallergènes SA) taken once daily. The IR is a measure of the biologic potency of an allergen extract assessed based on skin reactivity. The dosage of the 300-IR tablet corresponded to approximately 20 μg of group 5 major allergens.

Patients were told to take the sublingual pollen extract or placebo tablets once a day before eating or drinking and, preferably, at the same time of day throughout the 4-month treatment period. The patients were further instructed to leave the tablet under the tongue and not to swallow until the tablet had completely dissolved. Treatment was taken daily at the dose of 300 IR from day 1 and for 4 months. The doses were administered under medical supervision on every scheduled visit in the study. Patients were observed for 30 minutes to check for any local or systemic reactions. Antihistamines, decongestants, antileukotrienes, cromones, corticosteroids, and topical nasal or ocular treatments were prohibited during the treatment period. There was no necessity for rescue medication because the trial was performed out of season.

**Allergen challenge and study measurements**

The allergen challenge was carried out in the validated Vienna Challenge Chamber (VCC) at the department of the Allergy Center of Vienna West (Vienna, Austria). The methods for the VCC are described in this article’s Methods section in the Online Repository at www.jacionline.org.

During the challenge the patients scored the 6 individual rhinitis and conjunctivitis symptoms every 15 minutes on computer keypads. Nasal airflow was measured every 30 minutes by means of active anterior rhinometry. Nasal secretion was determined every 30 minutes by collecting and weighing used tissues; patients were given preweighed packs of paper tissues, which they used to blow their noses as necessary. FEV\(_1\) was measured every hour by using standard spirometric procedures (with reference values given by the European Community for Coal and Steel).

Initial measurements (except nasal secretion weight) were performed before patients entered the chamber. Blood was taken before treatment initiation and after 2 and 4 months of treatment. This biologic sample was subjected to a range of prespecified immunologic analyses (see the “Assessment of immunologic changes” section in the Methods section of this article’s Online Repository).

**Efficacy variables**

The RTSS includes the 6 most common symptoms of allergic rhinoconjunctivitis: sneezing, rhinorrhea, nasal pruritus, nasal congestion, ocular pruritus, and tearing. Each symptom was evaluated by the patient with a score ranging from 0 to 3, as follows: 0, absent symptoms (no sign/symptom evident); 1, mild symptom (sign/symptom is clearly present/minimal awareness and easily tolerated); 2, moderate symptom (definite awareness of sign/symptom that is bothersome but tolerable); and 3, severe symptom (sign/symptom that is hard to tolerate and causes interference with daily activities). The RTSS is the sum of the 6 individual symptom scores and thus varies from 0 to 18. The RTSS was recorded every 15 minutes during the 4-hour allergen exposure challenge (2 hours at baseline). The average rhinoconjunctivitis total symptom score (ARTSS) for each patient was calculated for each challenge as
the average of the RTSSs across the challenge’s 16 time points (8 time points for baseline challenge). The primary efficacy variable was the ARTSS during the allergen challenge after 4 months of treatment or at end point. The secondary efficacy variables were nasal airflow, nasal secretion weight, and cutaneous reactivity. Immunologic parameters were exploratory variables.

Safety
Subjects were questioned about the occurrence, onset, severity (mild, moderate, and severe) and outcome of all adverse events (AEs) during the study. AEs were monitored throughout the study and coded according to the MedDRA dictionary (version 10.1, http://meddramsso.com). AEs were classified according to severity and their relationship to the study medication.

The safety population included all patients who were randomized and had received at least 1 dose of investigational product.

Statistical analysis and study populations
Analysis of the primary efficacy variable was performed for both the intention-to-treat (ITT) and per-protocol (PP) populations, with the ITT analysis considered primary. An analysis of covariance was performed on the primary efficacy variable (ARTSS at 4 months or at end point), with treatment as the main factor and baseline ARTSS as the covariate (see the “Assessment of the statistical analysis and study populations” section in the Methods section of this article’s Online Repository).

RESULTS
Population
Of a total of 97 screened patients, 89 were randomized into either the 300-IR (n = 45) SLIT tablet group or the placebo group (n = 44). Eighty-two patients completed the treatment phase. In all, 7 patients discontinued the study before completion: 3 in the SLIT arm (2 consent withdrawals and 1 unrelated AE [ie, oral inflammation in the context of dental surgery]) and 4 in the placebo arm (2 consent withdrawals and 2 AEs). The flow of patients through the study is summarized in Fig 2.

Demographic data in the 2 study arms are presented in Table I, and baseline ARTSSs are presented in Table III. There were no between-group differences in terms of age, BMI, and mean ARTSS at the baseline allergen challenge. There was a higher proportion of women in the placebo group than in the SLIT group (63.6% vs 53.3%, respectively). The mean treatment duration was close to 4 months in both treatment groups (Table I).

Primary efficacy variable (ARTSS at 4 months)
In the course of the baseline challenge, individuals started free of symptoms and reached the worst symptoms after 90 to 120 minutes. Both groups reacted to the same amount (Fig 3, A). For the ITT population, the 300-IR group had a significantly lower ARTSS (means ± SDs) during allergen challenge after 4 months of treatment (or at end point) than the placebo group (4.85 ± 1.967 vs 6.87 ± 3.114). The difference in adjusted means for the 300-IR group versus the placebo group was −1.97, with a 95% CI of −2.99 to −0.94 (P = .0003); this represents a relative mean improvement of 29.3% (median, 33.3%) compared with placebo. This SLIT versus placebo difference in
the adjusted mean ARTSS was confirmed ($P = .0008$) in the PP population ($n = 83$) and in a sensitivity analysis ($P = .0006$) performed for the completed patients ($n = 82$, Table II).

Because all the following allergen challenges during the treatment period lasted 4 hours when patients had maximum symptoms during the last 2 hours of challenges, the mean values during the 4-hour challenges were higher than the mean values observed during baseline challenge.

A complementary analysis of ARTSSs was performed taking into account the 2 first hours of each challenge; thus ARTSSs (0-2 hours) after 1 week and 1, 2, and 4 months of treatment could be compared with baseline ARTSSs. Thus the percentage of improvement at the end point (compared with baseline) could be calculated only for the first 2 hours of challenge. It was 41.8% in the 300-IR group and 18.5% in the placebo group.

Onset of action
For the ITT population ($n = 89$), a significant treatment effect was achieved after the first month ($P = .0042$) and maintained at 2 ($P = .0203$) and 4 ($P = .0007$) months. In the active group the ARTSS decreased at each challenge (ie, $7.40 \pm 2.68$ at week 1, $5.89 \pm 2.43$ at month 1, $5.09 \pm 2.08$ at month 2, and $4.85 \pm 1.99$ at month 4), whereas the lowest mean ARTSS was observed at month 2 in the placebo group ($6.21 \pm 2.94$; Fig 3, B).

Nasal airflow and nasal secretion weight
There was no significant difference between the 300-IR and placebo groups in the mean change in nasal airflow or nasal secretion weight after 4 months of treatment.

Cutaneous reactivity
Skin prick tests for the 5 grass pollens were performed at screening and after 1, 2, and 4 months of treatment. The reduction in wheal size at the end point (mean ± SD) was small: $-1.11 \pm 2.72$ mm in the 300-IR group and $-0.40 \pm 2.34$ mm in the placebo group. The intergroup difference did not reach statistical significance. However, the sample size was not calculated in terms of this parameter.
Immunologic analyses

Immunologic changes were assessed in peripheral blood before (visit 3) and after (visit 7) 4 months of SLIT by using the 5-grass-pollen extract. There was no difference in basophil activation (determined on the basis of CD203c expression) in response to grass pollen allergens between patients receiving the active treatment and those receiving placebo. Likewise, no changes in basophil activation were noticed for the 2 groups of patients before or after treatment. No significant changes in T-cell proliferation were observed in response to grass pollen allergens before (visit 3) and after (visit 7) 4 months of treatment. Lastly, patients exposed to active treatment (but not placebo) displayed a substantial increase in allergen-specific plasma IgE (Fig 4, A) and IgG (Fig 4, B) responses after immunotherapy. Interestingly, specific IgG (but not IgE) values were found to be higher in patients exhibiting the best clinical response to treatment. Defining clinical responders as the 25% of patients with the highest relative improvement in ARTSSs from baseline, the median titers of IgG were 1.700 and 2.060 mg/L at visit 3 and 3.680 and 2.915 mg/L for SLIT from baseline, the median titers of IgG were 1.700 and 2.060 mg/L at visit 3 and 3.680 and 2.915 mg/L for SLIT.

Safety

A total of 73 treatment-emergent adverse events (TEAEs) were reported by 27 (60.0%) patients in the active group, and 39 TEAEs were reported by 14 (31.8%) patients in the placebo group. No AEs occurred during the allergen challenges. All treatment-related AEs were mild in the 300-IR group, and there were no serious AEs. These events generally appeared in the first few days of treatment and lasted less than 2 weeks. The most commonly observed TEAEs are summarized in Table III and consisted of oral pruritus, throat irritation, and headache, each of which was reported by 16 of the 89 subjects in the safety population. According to the European Academy of Allergology and Clinical Immunology recommendations, no systemic related possible, probable, or certain TEAEs were observed in the 300-IR group, and 1 episode of mild rhinoconjunctivitis was experienced by a patient in the placebo group.

Three patients withdrew from the study because of TEAEs: 1 in the SLIT group (oral inflammation after dental surgery not related to treatment) and 2 in the placebo group (pneumonia and headache). Pulmonary function, as assessed based on FEV1 at each allergen challenge, was not modified in either group.

Overall, the safety results were consistent with those reported in previous studies of the same grass pollen SLIT tablet.

DISCUSSION

This double-blind, placebo-controlled study provides new and important information about the onset of action of the 300-IR 5-grass-pollen SLIT tablet. To the best of our knowledge, no other data on this key SLIT parameter have been published. Previous studies have shown that SLIT is effective but were not designed to clarify the optimal period for which it should be administered before the beginning of the pollen season (ie, the preseasonal treatment period). This study is the first to document the onset of action of SLIT under controlled conditions and shows that 300-IR SLIT tablets can provide a statistically significant improvement in SAR symptoms after as little as 1 month of treatment. Interestingly, a nonsignificant trend toward superiority over placebo (P = .0647) was apparent after just 1 week of treatment.

Our current study showed that treatment with a 5-grass-pollen SLIT tablet was associated with less intense symptoms (relative to placebo) after a controlled, out-of-season grass pollen challenge in individuals with a history of SAR and a previous positive challenge result with grass pollen. Because no rescue medications were allowed during the study, the symptom score is a pure assessment of efficacy. This study supports previous findings that a 300-IR 5-grass-pollen SLIT tablet improved symptoms of pollen-related rhinoconjunctivitis significantly in patients treated 4 months before the pollen season. The relative mean improvement was 29.3% (median, 33.3%) compared with placebo. For the first time, an evaluation of grass pollen SLIT tablets in the absence of any rescue medication in either the placebo or active treatment groups has been carried out. This was possible because the study was performed (1) outside the pollen season and (2) in an ACC under closely monitored conditions. We observed a placebo effect over time, which we ascribe to interactions between the VCC trial subjects and a potential influence of their experience on reporting of symptoms.

Some mild local AEs were observed after direct, once-daily administration of 300-IR SLIT tablets. These local irritations occurred in 35.6% of the actively treated patients and lasted only for a few days. These results completed the assessment of the safety of the 5-grass-pollen SLIT tablets, which has already been demonstrated in previous clinical trials in adults and children with SAR.

Before our study, only 2 other studies had investigated immunotherapy outcome with controlled environmental exposures (to ragweed and birch pollen). Ragweed pollen exposure in a controlled setting demonstrated that specific immunotherapy significantly reduced symptoms of ragweed-induced allergic rhinitis. Evaluation was made after at least 2 years of subcutaneous immunotherapy and featured a positive control group of immunotherapy-naive subjects with ragweed allergy. In a double-blind,
placebo-controlled study a short (4 months) course of birch pollen SLIT was found to be efficacious and safe.14

The main advantage of the ACC is a defined, constant, and reproducible allergen concentration18 under a definable, stable climatic condition. ACC studies have typically been designed to evaluate the onset and duration of action of antiallergic treatments.

These results are essential for determining the optimal administration regimen for this immunotherapy. Indeed, the symptom score improves from the first week of treatment with grass pollen SLIT tablets, with a significant effect at 1 month, a plateau after 2 months, and maintenance of the effect at 4 months. However, these challenges do not exactly represent real-life conditions, with their multiple and fluctuating sources of allergen and changing climatic parameters. The single allergen source, the lack of seasonal priming, subject demographics closed by the ACC area, trial context, and short duration are also critical points of ACC studies.6 They might not reflect the natural pathologic process and environmental factors contributing to an individual’s development of allergic rhinoconjunctivitis.

The absence of statistical differences between the 2 groups on objective measurements (nasal airflow and secretions) differs with the finding of symptom improvement. In a previous publication assessing the efficacy of birch immunotherapy during allergen exposure,14 nasal airflow was significantly different between the active and placebo groups, although the difference did not reach significance for secretions. Several clinical studies have previously shown that nasal peak inspiratory flow and clinical scoring are weakly correlated and are complementary tools to evaluate allergic rhinitis.20,21 Nasal secretion measurements are weakly correlated with objective and subjective measurements of rhinorrhea and congestion.22 Nasal symptom scoring remains the most relevant way to assess the efficacy of a treatment for allergic rhinitis and might yield highly significant differences versus placebo without differences in complementary objectives (eg, peak nasal inspiratory flow and secretion weight).

Although detailed analyses of the concomitant immunologic changes are currently being performed by using a battery of purified grass pollen allergens, we have only reported herein on immune responses to the 5-grass-pollen extract. No significant changes in either basophil activation or T-cell proliferation were noticed within the 4-month treatment period. As reported previously,1 allergen-specific IgEs and IgGs were clearly induced in patients receiving the active treatment but not in those receiving placebo. Although not further discussed here, our results also suggest that some specific IgGs might be relevant for clinical efficacy.

In conclusion, for the first time ever, the efficacy and onset of action of a 5-grass-pollen SLIT tablet has been assessed (vs placebo) in an ACC. The effect of this 300-IR 5-grass-pollen SLIT tablet versus placebo on rhinoconjunctivitis symptoms is statistically significant from the first month of treatment onward.

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Clinical implications: In this ACC trial the effect of a 5-grass-pollen 300-IR SLIT tablet on rhinoconjunctivitis symptoms was statistically significant (vs placebo) from the first month of treatment onward.

REFERENCES
METHODS

ACC

The allergen challenge was carried out in the validated VCC, a department of the Allergy Center Vienna West, Vienna, Austria. The VCC is a specially designed sealed room in which a precisely defined and monitored airborne concentration of allergen (1450–1500 pollen grains/m³, the level typically found in the Austrian countryside at a height of 1.5 m during pollen peaks) can be administered to subjects continuously and maintained over a period of hours. The volume of the VCC was approximately 37.2 m³.

The pollen was a standardized mixture consisting of equal proportions of pollen from 4 grass species: orchard (Dactylis glomerata), meadow (Poa pratensis), perennial rye (Lolium perenne), and timothy (Phleum pratense) grasses. No placebo challenge was performed because we focused on the differences between the 2 groups of patients (treated or not). Distribution of allergen within the VCC was kept constant by means of an automatic, turbulent airflow supply unit; the pollen concentration was measured every 5 minutes with a modified Burkard pollen trap (Burkard Manufacturing Co, Ltd, Hertfordshire, England). All patients thus inhaled similar amounts of allergen during each allergen session. Frequent cleaning between challenge sessions decreased the possibility of allergen adhesion with irregular exposure concentrations. The air temperature was maintained at approximately 24°C ± 0.7°C, and the relative humidity was approximately 40% ± 1%.

Patients were observed through 4 windows during exposure, and supervising personnel were accessible to patients through an intercom system. In the VCC up to 20 subjects can be challenged at a time and during the study. The number of subjects varied between 15 and a maximum of 20 subjects belonging to the 2 groups in different daily sessions.

Statistical analysis and study populations

Analysis of the primary efficacy variable was performed for both the ITT and PP populations, with the ITT analysis considered as primary. The secondary efficacy variables were analyzed for the ITT population only. The ITT population consisted of all patients who had been randomized and taken at least 1 dose of the investigational product or placebo. The PP population included all subjects who completed the study according to the protocol and did not display any major protocol deviation, as well as patients who withdrew for a related AE.

A sample size of 34 subjects per treatment group would have a power of 81% to detect a difference in ARTSS (mean of the sums of the 6 individual symptom scores at each time point during the allergen exposure) of 2.4 between the 300-IR and placebo treatments (mean score with placebo, 8; mean score with 300 IR, 5.6 [ie, an improvement of 30%]), assuming an overall α value of .05 and a common SD of 3.4.

Assuming a 20% screening failure rate and a 15% dropout rate, 100 subjects had to be screened to have 40 randomized subjects in each group at the start of the study.

Unless otherwise stated, all statistical tests were 2-sided and performed at the 5% level of significance. In cases of premature withdrawal during the treatment period, the last observation carried forward method was applied. The treatment effect was expressed as the difference in least-square means between the 2 treatment groups, together with the corresponding 95% CI.

The onset of action was defined (by using a repeated-measures analysis of covariance mixed model) as the first allergen challenge at which (1) the mean ARTSS in the SLIT group became significantly different from that in the placebo group and (2) a significant difference was maintained in the subsequent challenges. Results of immunologic parameters were summarized descriptively and graphically with notched box plots.

Assessment of immunologic changes

Basophil activation tests were performed with an Allergenicity Kit (Beckman Coulter, Roissy, France). Briefly, whole blood was incubated for 15 minutes at 37°C with fluorescein isothiocyanate–labeled anti-CRTH2, phycoerythrin-labeled anti-CD203c, and phycoerythrin–cyanin 7–labeled anti-CD3 mAbs in the presence of serial dilutions of 5-grass-pollen extract (1–0.0001 μg/mL total protein; Stallergènes SA). PBS and anti-IgE were used as negative and positive controls, respectively. Nonactivated and activated basophils were identified with a FC500 flow cytometer chemokine receptor-homologous molecule (Beckman Coulter, Fullerton, Calif) as CRTH2⁺CD203cdim and CRTH2⁺CD203cbright cells, respectively.

Allergen-specific plasma IgE and IgG antibodies were analyzed by using the ImmunoCAP 1000 system (Phadia) with the 5-grass-pollen extract as the allergen. Plasma samples were tested undiluted for specific IgE and at 1:100 dilution for specific IgG.