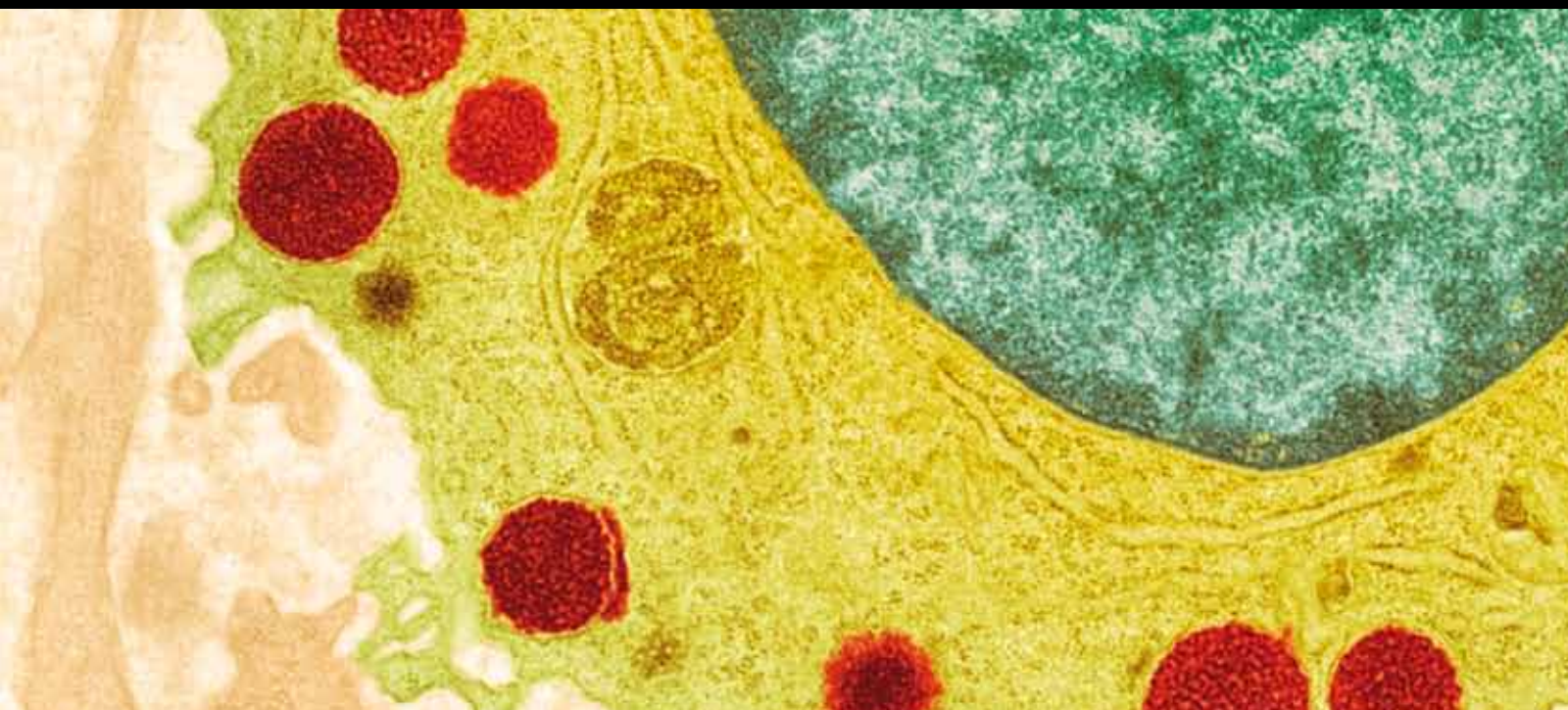


MUCOSAL IMMUNITY AND SUBLINGUAL IMMUNOTHERAPY IN RESPIRATORY DISORDERS

GUEST EDITORS: NERIN N. BAHCECILER, SEVAL GUNESER KENDIRLI, NAZAN COBANOGLU,
AND AARIF O. EIFAN





Mucosal Immunity and Sublingual Immunotherapy in Respiratory Disorders

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Guest Editors: Nerin N. Bahceciler, Seval Guneser Kendirli, Nazan Cobanoglu, and Aarif O. Eifan



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Editorial

Mucosal Immunity and Sublingual Immunotherapy in Respiratory Disorders

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The prevalence of allergic diseases, specially respiratory allergic diseases such as allergic rhinitis and asthma, has been increasing worldwide for the last 2 decades [1, 2]. Although avoidance of the responsible allergen, anti-inflammatory, and symptomatic treatment modalities has shown great efficacy in the treatment of allergic respiratory disorders, cessation of pharmacotherapy usually results in recurrence of signs and symptoms, with a demand to restart the treatment.

Currently, allergen-specific immunotherapy (SIT) is the only available curative choice with the capacity of altering the natural course of allergy [3, 4]. Although SIT by the subcutaneous route has been extensively used and has shown marked efficacy since its discovery, it was associated with uncommon, but severe or even fatal, systemic reactions [5]. Consequently, alternative, noninjective allergen delivery routes have been proposed, and allergen delivery through mucosal surfaces was suggested as a possible mechanism for the induction of mucosal tolerance to allergens [5, 6]. Local mucosal routes such as oral, nasal, bronchial, and sublingual were investigated since then, and controlled trials failed to demonstrate satisfactory clinical efficacy and/or safety of oral, nasal, and bronchial allergen application; therefore those routes have been abandoned [7–11]. Meanwhile, the efficacy and safety of SIT via the sublingual route was well documented by a number of controlled trials both in children and adults with asthma and/or rhinitis [12, 13]. Since then, sublingual immunotherapy (SLIT) in the liquid drop formulation has been tested in a large number of double-blinded, placebo-controlled studies, and those studies were included in Cochrane meta-analyses [14–16] demonstrating efficacy both in children and adults with allergic rhinitis or asthma sensitized to house dust mite or various pollens. Thereafter, orodispersible grass-pollen tablets were

developed and recent well-designed, well-powered, double-blinded, placebo-controlled studies demonstrated efficacy and safety of tablet formulation [17–20].

Some of those studies improved our understanding of the underlying immunological mechanisms in addition to the proven safety and efficacy. Recent studies demonstrated that SLIT exerts its immune-suppressive effect through the induction of Treg cytokines such as IL-10 and TGF- β [21, 22]. This effect starts on the uptake of allergen by oral mucosal Langerhans cells through high-affinity IgE receptors [6]. More recent studies demonstrated increase in expression of Foxp3+ cells in the sublingual mucosa, which was accompanied by the systemic immunologic response during SLIT [23].

Hereby in this issue, data on clinical implications, efficacy, compliance, monitorization of delivery, and immunological mechanisms of allergen SIT delivered by the mucosal-mainly sublingual route will be presented.

Nerin N. Bahceciler

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Letter to the Editor

Comment on “Therapeutic Effects and Biomarkers in Sublingual Immunotherapy: A Review”

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Numerous sublingual immunotherapy studies have shown efficacy using a wide variety of dosing regimens. Despite a few grade III and one anaphylactic reaction due to a patient over-dose, there have been no fatal reactions resulting from sublingual immunotherapy treatment. Although safer than SCIT, SLIT is still immunotherapy. Special consideration should be given to what will ensure the highest level of safety for the patient given his or her history, exam and allergy test results. Dosing levels for sublingual immunotherapy should be based on what is therapeutically effective for each individual patient and adjusted accordingly throughout the treatment course.

In the recent review article “Therapeutic effects and biomarkers in sublingual immunotherapy: a review,” it is stated “several case reports have also described anaphylactic shock or severe fatal reactions induced by sublingual [1–4] administration of allergens.” We reviewed the references included in the article and although there were systemic reactions, there are no case reports of fatal outcomes. The first reference speculated the reaction was due to dose-concentration allergen exposure during a high pollen season while using a high-dose protocol for sublingual immunotherapy [1]. The second reference included two cases of severe reactions from the standardized dosed tablet, Grazax. The first, a pediatric patient, was prescribed the tablet in addition to an existing SCIT regimen. Despite reactions using the same strength for moderately and strongly allergic patients, the authors remarkably suggest tablets are the safest approach for SLIT, especially in children. Sublingual immunotherapy with drops allows for increases or decreases of dosing dependent on the severity of allergy. In the second, an adult patient, it was unclear whether she was being cotreated with SCIT and SLIT. After attempted grass and birch SCIT, the patient was unable to tolerate both so SCIT for grass was discontinued. After the following year grass season, the patient began taking Grazax and did not take the first dose under the supervision of a physician. She had an immediate reaction suggesting the starting dose was too high for her severe allergies [2].

The third reference discussed two adolescent cases in which neither patient previously tolerated SCIT but was dosed using an ultrarush protocol resulting in grade III reactions [3]. The fourth reference did include a case of anaphylaxis; however, the individual discontinued her maintenance dose for three weeks and then continued taking SLIT at six times the prescribed dose, 60 drops at once [4].

The article also states later in the same paragraph “despite the few case reports of severe fatal events, life threatening severe fatal reactions have not been found in clinical trials.” To our knowledge, there have been no fatal events with sublingual immunotherapy. We respectfully request for you to correct the inaccurate statements that SLIT has caused severe fatal reactions. In medical parlance, fatal means death, and while the authors cite anaphylactic and grade III reactions to sublingual immunotherapy, there is no literature available to support the claim of “fatal.”

Although safer than SCIT, SLIT is still immunotherapy. Particular caution needs to be used for patients with prior systemic reactions to SCIT. Doses should be monitored and may need to be modified during treatment. First doses should be administered in the physician's office.

In our experience, multiantigen sublingual immunotherapy treatment that is dosed using the patient's allergen test results is both effective and maintains an excellent safety profile. Threshold dosing can be administered using a

number of environmental allergens [5, 6]. We have observed a combination of clinical symptom improvements, reduced skin test reactivity, and decreases in specific IgE levels in our forty-year history in treating 125,000 patients from the United States.

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Clinical Study

Can Serum-Specific IgE/Total IgE Ratio Predict Clinical Response to Allergen-Specific Immunotherapy in Children Monosensitized to House Dust Mite?

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Background. Allergen-specific immunotherapy (SIT) is one of the important regimens for the treatment of allergic diseases. Predictive tests for the clinical response to SIT are limited. In this study we aimed to evaluate whether specific IgE/total IgE levels can predict clinical improvement in monosensitized patients to house dust mite treated with immunotherapy. **Patients and Methods.** We analyzed 32 patients who had undergone 2 years of SIT. Serum t-IgE and s-IgE levels, and serum s-IgE/t-IgE ratios were calculated and tested for correlation with clinical response to SIT. Asthma symptom score (ASS), rhinitis symptom score (RSS), pulmonary functions and visual analogue scales (VAS) were evaluated at the beginning and after 2 years. **Results.** There were 17 boys and 15 girls with the mean age of 10.78 ± 3.03 years. The mean serum house dust mite s-IgE level was 128.62 ± 142.61 kU/L, t-IgE 608.90 ± 529.98 IU/mL, and s-IgE/t-IgE ratio 33.83 ± 53.18 . Before immunotherapy, ASS was 6.23 ± 1.63 , RSS; 8.20 ± 1.88 , VAS; 7.38 ± 2.01 , FEV1 (%); 89.14 ± 8.48 , PEF (%); 88.93 ± 13.57 , and after 2 years, these values were determined as 1.90 ± 1.10 , 3.05 ± 1.39 , 1.35 ± 1.24 , 97.6 ± 11.26 , and 97.0 ± 11.55 , respectively. s-IgE/t-IgE ratio was correlated with change in RSS ($r = -0.392$, $P = 0.08$) and VAS ($r = -0.367$, $P = 0.05$). **Conclusion.** Although SIT is very effective treatment, all patients do not benefit from treatment. We assumed that s-IgE/t-IgE ratio would be useful to predict the clinical response to SIT.

1. Introduction

Allergen-specific immunotherapy (SIT) has been used to treat IgE-mediated rhinoconjunctivitis, asthma, and venom hypersensitivity for almost a century. The clinical efficacy of SIT has been shown in several randomized, prospective, double blind placebo-controlled studies [1–5]. A number of studies have shown that SIT decreases clinical symptoms and improves lung function and quality of life in allergic diseases. SIT is only curative and specific method of treatment and may modify the natural course of the allergic disease [3–5].

Many complex immunologic changes occur during the course of SIT. Immunological changes induced by SIT are encompassing modulation of allergen-specific antibody responses, reduction in recruitment and activation of proinflammatory cells, and changes in the pattern of allergen-specific T-cell responses [6–9]. It has been postulated that

regulatory T (Treg) cells can control an established allergic response via distinct mechanisms. IL-10 and TGF- β decrease IgE production and enhance IgG4 and IgA production, respectively [1, 10–12]. Allergen-specific immunotherapy frequently induced a transient initial increase in serum-allergen specific IgE, and a subsequent decrease is observed in the following months or years of SIT [13, 14]. Moreover, successful SIT often elicits allergen-specific IgG4 and IgA responses [6, 15–17]. However, a significant decrease in the allergen-specific IgE/IgG4 ratio occurs after several months. Such changes in the IgE/IgG4 ratio have been found to correlate with a decrease in the late-phase skin reaction to the allergen and with the overall clinical efficacy of SIT in some studies [16–19]. It is unclear how serum total IgE (t-IgE) measurements, alone or in relation to s-IgE measurements, or blood eosinophil (b-eos) counts should be interpreted, as

well as what role they should play in selecting patients for SIT in clinical practice [20].

In this study, we aimed to evaluate whether specific IgE/total IgE levels can predict clinical improvement in monosensitized patients to house dust mite treated with immunotherapy.

2. Patients and Methods

We analyzed 32 children monosensitized to house dust mite with the diagnosis of asthma and/or allergic rhinitis that were on followup in Cukurova University, Faculty of Medicine, Division of Pediatric Allergy-Immunology, Adana, Turkey between January 2007 and September 2010. All patients underwent SIT by means of subcutaneous route as part of the therapy.

All patients presented with a clinical diagnosis of asthma and/or allergic rhinitis. Diagnosis of asthma and allergic rhinitis was defined according to GINA and ARIA criteria [21, 22]. Skin prick tests, serum t-IgE and s-IgE levels, and serum s-IgE/t-IgE ratios, asthma symptom scores (ASS), rhinitis symptom scores (RSS), pulmonary functions, and visual analogue scales (VAS) were evaluated at the beginning and after 2 years in all patients.

Immunotherapy was administered by means of subcutaneous route, and each patient received the maximum tolerated dose, as per the manufacturer's recommendations. The maintenance dose was 0.8 mL which corresponds to 3.84 mg of the major allergens of *D. pteronyssinus* and *D. farinae*.

Patients were divided into two groups according to the s-IgE/t-IgE ratio. We set up 16.2 as a cut-off point for s-IgE/t-IgE ratio, and Group 1 included patients who had s-IgE/t-IgE ratio <16.2, while Group 2 s-IgE/t-IgE ratio >16.2 [20].

2.1. SPT. A routine skin prick test was performed for all patients using kits containing common inhalant allergens (*D. pteronyssinus*, *D. farinae*, grass mix, tree mix, mold mix, and *Alternaria* species) (ALK-Abello, Madrid, Spain), and an induration with a diameter of ≥ 3 mm was accepted as a positive reaction.

2.2. Total IgE. Total IgE levels were assayed by microparticle enzyme immunoassay (Abott, USA).

2.3. spIgE. A blood sample was processed at the time of diagnosis and after 2 years of SIT. s-IgE levels were determined by using the fluoroimmunoassay technique (Unicap, Phadia, Uppsala, Sweden) according to the manufacturer's instructions. Serum s-IgE levels were determined with a detection limit of 0.35 kAU/L. In all patients, the s-IgE level was measured for the same allergens used in the skin prick tests.

spIgE/t-IgE ratio was calculated as

$$(\text{spIgE/t} - \text{IgE}) \times 100. \quad (1)$$

2.4. Asthma Symptom Score (ASS). Asthma symptom scores were determined based on the daytime symptoms and nocturnal awakening. In addition, the amount of as-needed β_2 -agonist (salbutamol) was recorded daily as the number of puffs. Daytime asthma symptoms and nocturnal awakenings were scored subjectively, as follows: 0, no symptoms during the day/night; 1, symptoms did not affect daily activities or nighttime sleep; 2, symptoms affected at least 1 daily activity or disturbed nighttime sleep; 3, symptoms affected 2 or more daily activities or disturbed sleep all night or most of the night. Use of β_2 -agonists was scored as follows: 0, none; 1, once a day; 2, between 2 and 3 times a day; 3, more than 3 times a day. The minimum score for each day was 0 (no symptoms during the day, no symptoms at night, and no use of β_2 -agonists), and the maximum score was 9 (severe symptoms during the day and at night, and more than 3 administrations of β_2 -agonists) [23].

2.5. Rhinitis Symptom Score. Each nasal symptoms including itching, congestion, sneezing, and rhinorrhea were scored subjectively as follows: 0, no symptoms; 1, symptom positive however did not affect daily activities or nighttime sleep; 2, symptoms were bothering but did not affect daily activities or nighttime sleep; 3, symptoms affected daily activities or disturbed sleep. Scores for each symptoms recorded and sum of the scores were calculated (0–12) [24].

2.6. VAS (Visual Analogue Scale). Visual analogue scale was evaluated on 10 cm card [25].

2.7. Pulmonary Functions. PFTs were performed according to the American Thoracic Society (ATS) Criteria by using ZAN 100 Spiromed (Oberthulba, Germany), and the results were expressed as percentages of the predicted values for age and height [26].

2.8. Determination of Changes in Clinical Parameters. Changes in ASS, RSS, VAS, and pulmonary functions throughout 2 years were calculated by the following formula:

Change in ASS

$$= \frac{\text{ASS (before treatment)} - \text{ASS (after treatment)}}{\text{ASS (before treatment)}} \times 100. \quad (2)$$

Decreases in RSS, FEV1, PEF, and VAS were calculated with same method.

2.9. Statistical Analysis. All statistical analysis was carried out using a computer software (SPSS version 11.0; SPSS; Chicago, Illinois, USA). Nonparametric Wilcoxon test was utilized to compare the groups. Spearman's correlation test was used to determine the statistical relationships between s-IgE/t-IgE ratio and changes in ASS, RSS, and VAS. The $P < 0.05$ value was considered as statistical significant.

3. Results

3.1. The Characteristics of the Patients. There were 17 boys and 15 girls with the mean age of 10.78 ± 3.03 years (range; 7–15 years). Sixteen patients had asthma, 4 patients had allergic rhinitis, and 12 patients had both asthma and allergic rhinitis. At beginning of the immunotherapy, the mean total IgE value was found to be 608.9 ± 529.987 IU/mL, sIgE and sIgE/t-IgE ratio were 128.62 ± 142.61 kUA/L (median: 69.05) and 33.83 ± 53.18 (median: 17.68), respectively. The characteristics of the patients were given in Table 1.

3.2. Changes in Clinical Symptoms, VAS, and Pulmonary Functions. We found statistically significant improvement in asthma symptom score, rhinitis symptom score, pulmonary functions, and VAS at the end of the 2 years of SIT (Table 2).

3.3. Correlation between the s-IgE/t-IgE Ratio and Changes in ASS, RSS, Pulmonary Functions, and VAS. We analyzed the correlations between the s-IgE/t-IgE ratio and changes in ASS, RSS, pulmonary functions, and VAS in order to determine whether this ratio would be useful to predict the efficacy of SIT. We found that s-IgE/t-IgE ratio was inversely correlated with the change in RSS but this was not statistically significant ($r = -0.392$, $P = 0.08$). On the other hand, significant inverse correlation was determined between the s-IgE/t-IgE ratio and change VAS ($r = -0.367$, $P = 0.05$). There was no significant correlation between the s-IgE/t-IgE ratio and changes in ASS and pulmonary functions (Table 3).

In addition, patients were divided into, two groups according to the s-IgE/t-IgE ratio. Previously Di Lorenzo et al. reported that serum s-IgE/t-IgE ratio of greater than 16.2 correlated with an effective clinical response to SIT [20]. Based on this data, we set up 16.2 as a cut-off point for s-IgE/t-IgE ratio [20]. Group 1 included patients who had s-IgE/t-IgE ratio <16.2 , while Group 2 included patients with s-IgE/t-IgE ratio >16.2 (Table 4). According to this cut-off point, we found significant inverse correlation between the s-IgE/t-IgE ratio and change in both RSS ($r = -0.432$, $P = 0.012$) and VAS ($r = -0.483$, $P = 0.01$). However, there were no significant correlations in Group 1 regarding these parameters. In addition, no statistically significant relation was found between the s-IgE/t-IgE ratio and changes in ASS and pulmonary functions in both groups.

4. Discussion

Allergen-specific immunotherapy has the potential to modify the natural course of allergic diseases [3–5, 27, 28]. Many different in vivo and in vitro tests have been used to determine the efficiency; however, predictive tests for the clinical response to SIT are limited.

Successful immunotherapy is accompanied by the suppression of numbers of T-helper 2 (Th2) effector cells, eosinophils, basophils, mast cells and neutrophils infiltration in target organs, induction of IL-10 and/or TGF- β + Treg cells, and increases in “protective” noninflammatory blocking antibodies, particularly IgG4 and IgA2 subclasses with

TABLE 1: The characteristics of the patients.

| Characteristics | |
|---------------------------------------|-------------------------------------|
| Age | 10.78 ± 3.03 (7–15 years) |
| Gender (F/M) | 17/15 |
| Diagnosis | N (%) |
| Asthma | 16/32 (50%) |
| Rhinitis | 4/32 (12.5%) |
| Asthma and rhinitis | 12/32 (37.5%) |
| Specific IgE (kuA/L) | 128.62 ± 142.61 (median: 69.05) |
| Total IgE | 608.9 ± 529.987 |
| (Specific IgE/total IgE) \times 100 | 33.83 ± 53.18 (median: 17.68) |

TABLE 2: The changes in ASS, RSS, pulmonary functions, and VAS following 2 years of SIT.

| Parameters | Before SIT | After 2 years of SIT | P value |
|------------|-------------------|----------------------|---------|
| ASS | 6.23 ± 1.63 | 1.90 ± 1.10 | 0.000 |
| RSS | 8.20 ± 1.88 | 3.05 ± 1.39 | 0.000 |
| FEV1 | 89.14 ± 8.48 | 97.60 ± 11.26 | 0.000 |
| PEF | 88.93 ± 13.57 | 97.0 ± 11.55 | 0.001 |
| VAS | 7.38 ± 2.01 | 1.35 ± 1.24 | 0.000 |

TABLE 3: Correlations between s-IgE/t-IgE ratio and change in ASS, RSS, pulmonary functions, and VAS.

| Correlations | R value | P value |
|--------------|---------|---------|
| ASS | -0.115 | 0.54 |
| RSS | -0.392 | 0.08 |
| FEV1 | 0.215 | 0.281 |
| PEF | 0.136 | 0.498 |
| VAS | -0.367 | 0.05 |

TABLE 4: Correlations between s-IgE/t-IgE ratio and change in ASS, RSS, pulmonary functions and VAS based on cut off value 16.2 for s-IgE/t-IgE ratio.

| | Correlations | R value | P value |
|--|--------------|---------|--------------|
| GROUP 1 (s-IgE/t-IgE ≤ 16.2) N = 16 | ASS | 0.239 | 0.410 |
| | RSS | 0.116 | 0.780 |
| | FEV1 | 0.121 | 0.694 |
| | VAS | 0.124 | 0.296 |
| GROUP 2 (s-IgE/t-IgE >16.2) N = 16 | ASS | -0.173 | 0.078 |
| | RSS | -0.432 | 0.012 |
| | FEV1 | 0.249 | 0.091 |
| | VAS | -0.483 | 0.01 |

inhibitory activity. This suppression occurs within weeks or months as a consequence of the appearance of a population of regulatory T cells that exert their effects by mechanisms involving cell-cell contact, but also by the release of cytokines such as IL-10 (increases IgG4) and TGF- β (increases specific

IgA). Mast cell and basophil desensitization are very early effects. Intermediate effects are related to changes in allergen-specific T cells, and late effects are related to B cells and IgE as well as mast cells, basophils, and eosinophils [6]. Several successful immunotherapy studies have shown an induction of peripheral tolerance and an anergic state in activated specific T cells [10–12, 27, 28]. On the other hand, a significant decrease in skin prick test reactivity can be also observed relatively late in the course [3–5]. Therefore, many clinicians want to find out objective and easily performed parameters to predict the efficacy of SIT. Recently, Di Lorenzo et al. demonstrated that the calculation of the serum s-IgE/t-IgE ratio could be useful for predicting the clinical response to SIT offering an advantage over measuring t-IgE and s-IgE levels in monosensitized patients for the following allergens: grass, *Parietaria judaica* [20]. To support this idea, in this study, we evaluate whether s-IgE/t-IgE ratio can predict clinical improvement in monosensitized patients to house dust mite treated with immunotherapy and found that s-IgE/t-IgE ratio would be useful to predict the clinical response to SIT especially in patients with allergic rhinitis. In our study, we demonstrated statistically significant correlation between the s-IgE/t-IgE ratio and change in VAS and RSS. Visual analogue scores and rhinitis symptom scores improved in children with s-IgE/t-IgE ratio higher than 16.2 at the end of the 2 years of SIT.

The continuous hyperreactivity in skin prick test might have negative impact on motivation of both physicians and patients. Therefore, different objective and practical parameters are needed to evaluate the clinical response to SIT. Nasal and/or bronchial provocation tests, diluted skin prick tests, SpiG4 levels, or other detailed tests are usually used in clinical studies or studies aimed to investigate the effectiveness of SIT. However, it is generally difficult to perform all these tests in routine practice because of ethical and economic issues. In this study, we evaluate whether this simple s-IgE/t-IgE ratio can predict clinical improvement in such patients routinely and we assumed that this ratio would be helpful to predict the efficacy of SIT in clinical practice.

Allergen-specific immunotherapy is of long-duration therapy, and at least 3–5-year treatment was recommended for the best clinical benefits [29, 30]. Because not all patients benefit from treatment, it is important to be able to have specific criteria to determine those patients who might best benefit from this therapy and decide when to discontinue SIT. We found that the serum s-IgE/t-IgE ratio would be a good predictor of clinical response to allergen-specific immunotherapy and this ratio might be useful for deciding when to discontinue the SIT as well. However further, studies in larger series are needed.

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Review Article

Therapeutic Effects and Biomarkers in Sublingual Immunotherapy: A Review

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Immunotherapy is considered to be the only curative treatment for allergic diseases such as pollinosis, perennial rhinitis, asthma, and food allergy. The sublingual route is widely applied for immunotherapy for allergy, instead of the conventional administration by subcutaneous route. A recent meta-analysis of sublingual immunotherapy (SLIT) has shown that this approach is safe, has positive clinical effects, and provides prolonged therapeutic effects after discontinuation of treatment. However, the mechanism of SLIT and associated biomarkers are not fully understood. Biomarkers that change after or during SLIT have been reported and may be useful for response monitoring or as prognostic indicators for SLIT. In this review, we focus on the safety, therapeutic effects, including prolonged effects after treatment, and new methods of SLIT. We also discuss response monitoring and prognostic biomarkers for SLIT. Finally, we discuss immunological mechanisms of SLIT with a focus on oral dendritic cells and facilitated antigen presentation.

1. Introduction

Allergic rhinitis is the most prevalent type I allergy, and pollen grains, mite, and mold are common causative allergens for seasonal or perennial rhinitis. Antihistamines, leukotriene inhibitors, and nasal steroids are commonly used to treat respiratory allergy, but these drugs sometimes have side effects that induce impaired performance [1, 2]. Almost 100 years have passed since the first report of immunotherapy for pollinosis in 1911 [3]. Subsequently, the protocol for allergen-specific immunotherapy has improved to increase efficacy and safety through coinjection or conjugation of allergens with an immunomodulatory adjuvant, premedication with an antihistamine or anti-human IgE antibody, or use of a rush protocol to shorten the duration of the up dosing phase [4–8]. The injection route for allergens has also been examined in trials of modified allergens to shorten the schedule and to increase the safety for immunotherapy [9, 10]. In the last few decades, sublingual administration has been

recognized as a route of administration of allergens that is safer than subcutaneous injection, and there is increasing evidence that the therapeutic effects of sublingual immunotherapy (SLIT) are comparable with those of traditional subcutaneous immunotherapy (SCIT) [11].

In this review, we focus on the therapeutic effects of SLIT and the problems to be solved in future clinical studies. We also discuss recent findings for prospective and response-monitoring biomarkers for SLIT, and we examine the cellular mechanisms of SLIT.

2. Safety and Therapeutic Effects of SLIT

Increasing numbers of clinical trials and meta-analyses have shown positive clinical effects and safety of SLIT. However, several case reports have also described anaphylactic shock or severe fatal reactions induced by sublingual administration of allergens [12–17]. In the reports, four patients experienced severe side effects with SCIT and discontinued the treatment

prior to SLIT [15, 16]. Patients who have experienced severe side effects in SCIT may be at risk for a severe fatal reaction in SLIT. To prevent an allergen overdose, a tablet or solid form for sublingual administration may be better than the use of an atomizer or dispenser for administration of liquid allergens, especially for young children. Despite the few case reports of severe fatal events, life-threatening severe fatal reactions have not been found in clinical trials [18]. Therefore, SLIT is considered to be a safe treatment in which reactions such as anaphylaxis can be avoided by using correct clinical protocols.

It takes a few weeks to six months to reach a maintenance dose using SCIT with a previous up dosing phase to reduce the risk of side effects [19]. In some studies, a build-up phase is used for SLIT before administering the maintenance dose of allergens. A comparison of the clinical effects and safety among four different SLIT regimes for grass pollen allergy using a mixture of extracts of five grass pollens (*Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, and *Poa pratensis*) concluded that a short build-up phase reduces the incidence of adverse events in administration of high-dose SLIT [20]. In this phase I study, the numbers of adverse events were compared among four active groups with a build-up phase repeating each concentration from 100 to 500 IR for 2 days ($N = 6$), a single daily build-up phase of 100 to 500 IR ($N = 6$), and no build-up phase for doses of 300 IR ($N = 6$) or 500 IR ($N = 5$). All groups showed mild and moderate adverse events, but only the group administered 500 IR without a build-up phase showed severe local adverse events (swelling of throat). A placebo group ($N = 7$) showed only mild adverse events. Another study compared the safety and efficacy among 3 SLIT groups with a build-up phase of 500 to 1,000 AU for 4 days, 300 to 1,200 AU for 4 days, and no build-up phase for a dose of 1,000 AU, using orosoluble tablets of a monomeric carbamylated allergoid [21]. Safety and efficacy were comparable among these groups, based on evaluation using a Visual Analog Scale (VAS), the Symptom Medication Score (SMS), and a nasal provocation test. An ultrarush schedule for SLIT has also been shown to be safe during the up dosing phase, but severe systemic and local adverse events may occur in the maintenance phase [22, 23]. In contrast, urticaria has been reported to occur in an ultrarush protocol [24]. The safety of this protocol may depend on the type and biological function of the causal allergens. It has also been suggested that the build-up phase for SLIT can be omitted or shortened compared to that for SCIT [25].

A recent meta-analysis found positive clinical effects of SLIT based on the results from 49 papers describing randomized, double-blind, and placebo-controlled (DBPC) trials [18]. The standardized mean differences (SMDs) for the symptom and medication scores were -0.49 ($P < 0.00001$) and -0.32 ($P < 0.00001$), respectively, in favor of active treatment (active; $N = 2,333$, placebo; $N = 2,256$). A meta-analysis of SLIT for grass pollinosis gave SMDs for the symptom (active; $N = 1,518$, placebo; $N = 1,453$) and medication (active; $N = 1,428$, placebo; $N = 1,358$) scores of -0.32 ($P < 0.0001$) and -0.33 ($P < 0.0002$), respectively, in favor of active treatment compared with placebo [26].

Both meta-analyses showed positive therapeutic effects of SLIT, especially for seasonal rhinitis, and these effects are comparable with those of SCIT [18, 27–29]. It has also been suggested that immunotherapy with SLIT and SCIT in combination may be beneficial [30]. In this study, 60 children with mild or moderate asthma or rhinitis who were monosensitized to house dust mite received injection of a mixture of *Dermatophagoides* allergens in a glycerinated solution. SCIT was used in a build-up phase for 16 weeks and was followed by SLIT three times a week as the maintenance phase. The clinical effects of SLIT were less than those of SCIT after 4 and 18 months and comparable after 12 months of treatment, based on the required dose of inhaled corticosteroids and the number of asthma attacks per year. SCIT and combination therapy of SLIT and SCIT significantly decreased the dose of inhaled corticosteroids and the number of asthma attacks at 4, 12, and 18 months and significantly improved the VAS for rhinitis. An advantage of SLIT is that sublingual self-administration can be performed at home during the maintenance phase, avoiding the need for patients to go to clinic for subcutaneous injection of allergens.

There is also increasing evidence for clinical effects after an extended period of SLIT and for prolonged clinical effects after treatment [31]. SLIT in 24 children with respiratory symptoms due to monosensitization to house dust mite showed a lack of positive clinical effects in the first year, but significant amelioration of rhinitis and asthma in the second and third years compared to the first year of treatment [32]. A study of 137 patients allergic to house dust mite also showed clinical effects in 2-year and 3-year SLIT and prolonged therapeutic effects at 4 and 3 years, respectively, after these treatments [33]. Scores for nasal airway resistance, secretion, symptoms, and skin prick test were significantly reduced at the end of the first year, and the nasal secretion score was significantly reduced at the end of the second year of treatment. Two-year SLIT significantly attenuated nasal airway resistance, secretion, sneezing, symptoms, and skin prick scores at 1 and 4 years after treatment compared with the respective scores at the start of treatment although all scores except for nasal airway resistance at 4 years after treatment were slightly, but significantly, higher than those at the end of treatment. Three-year SLIT significantly attenuated these scores at the end of treatment, and total score of nasal airway resistance, secretion, and sneezing, score for the nasal airway resistance, and symptom score at 3 years after treatment were similar to or lower than those at the end of treatment.

Carry-over effects of SLIT are supported by other studies. DBPC trials of 3-year SLIT for grass pollen allergy showed significantly decreased scores for symptom and the rhinoconjunctivitis quality-of-life questionnaire (RQLQ), and SMS and the medication score tended to decrease with active treatment compared with those for placebo at 1 year after SLIT [34, 35]. Our recent results also suggest a 1-year prolongation of clinical effects after 2-year SLIT for Japanese cedar pollinosis [36]. Analysis of 88 participants (SLIT; $N = 51$, placebo; $N = 37$) showed positive therapeutic effects in the second year of SLIT compared with placebo (reduction of SMS by 21%, $P = 0.02$) and at 1 year after treatment (23%, $P = 0.03$) (Figure 1). A recent phase III trial performed as

a large-scale randomized, DBPC study using a 75,000 SQ-T/2,800 BAU tablet in 257 subjects allergic to grass pollen also has shown that 3-year SLIT significantly decreased the mean rhinoconjunctivitis symptom and medication scores at 1 year after treatment compared with placebo. The results showed reductions of symptom scores of 31%, 36%, 29%, and 26% and reductions of medication scores of 38%, 45%, 40%, and 29% after 1, 2, and 3 years of treatment and after a follow-up year, respectively [37]. Long-lasting effects after 3-, 4-, and 5-year SLIT were evaluated in a 15-year prospective open controlled study in 59 patients with respiratory allergy for mite [38]. A decreased SMS of <50% of the baseline score (at the start of treatment) was found over the following 6 years after 3-year SLIT, and over 8 years after 4- and 5-year SLIT. The SMS after loss of the prolonged therapeutic effects increased to levels comparable with those in the control group. Significant clinical effects were obtained in a second course of SLIT given after the initial effects had vanished.

3. Unmet Problems in SLIT

Compliance with self-administration at home may be an important factor in the therapeutic effect of SLIT. Compliance with SLIT is likely to be similar to that for other self-administered drug treatments for allergy [11], and education on the SLIT protocol is needed for good compliance [39, 40]. Checking the compliance of each patient based on the amount of remaining vials or tablets may also be important for evaluating the efficacy of SLIT in clinical trials [34]. A device that reminds patients about intake of allergens may be useful to achieve good compliance in long term administration and to improve the efficacy of SLIT [41]. Delivery as a tablet or solid form may be better than an aqueous solution using an atomizer or dispenser to achieve good compliance and to hold allergens stably under the tongue because human error or bad conditions of a nozzle may lead to administration of an inaccurate amount of liquid drops. Such mistakes may also increase the risk of adverse reactions [17].

Bystander therapeutic effects of SLIT using allergens from a single source with polysensitized patients are uncertain. Inferior therapeutic effects for a polysensitized population have been reported compared with a monosensitized population [42]. Recent findings have shown that the use of both single and mixed allergen extracts improved mean QOL scores, increased threshold of a titrated nasal challenge, and decreased skin prick tests reactivity in polysensitized patients [43–45]. The efficacy of SLIT for polysensitized patients has also been found to be comparable with that for monosensitized patients [28]. Furthermore, SLIT for monosensitized (rhinitis only) and polysensitized (rhinitis and asthma) patients prevented or reduced additional sensitization compared with drug treatment [46, 47]. These preventive effects of SLIT were clearer for monosensitized patients. In contrast, SLIT for birch pollinosis was not effective against an already established apple allergy [48]. Mal d 1, a major allergen in apple, has 64% identity in amino acid sequence with Bet v 1, a major allergen in birch, and these allergens are cross-reactive in IgE-binding and T-cell activation. Bystander effects of SLIT using allergens from a single source for pa-

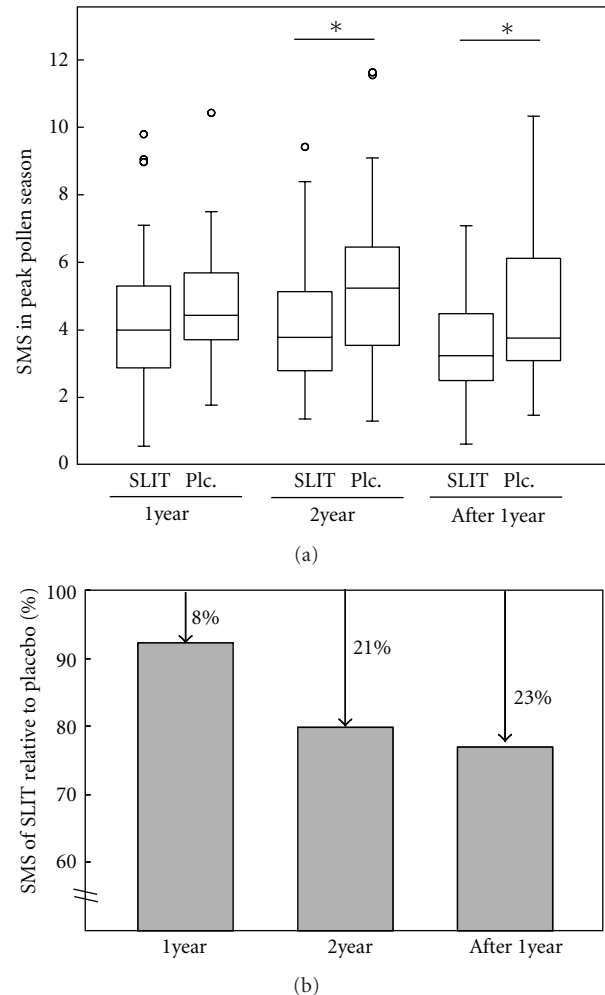


FIGURE 1: Clinical scores in 2-year SLIT and at 1 year after treatment [36]. (a) Average daily SMSs during a 2-year course of SLIT and at 1 year after treatment are plotted for the SLIT and placebo (Plc.) groups. * $P < 0.05$ (unpaired Student t -test). (b) Percentage average SMSs for the SLIT group based on a value of 100% for the placebo group.

tients with other established allergies may depend on the allergens used for immunotherapy and the degree of sensitization to the allergy. Further clinical trials and meta-analyses are needed to evaluate the bystander and prophylactic effects of SLIT.

In 2010, the World Allergy Organization defined a systemic reaction grading system for scoring of adverse reactions by SCIT to enable comparison of the severity of adverse events among clinical trials [49]. A similar approach to evaluation of clinical effects and adverse events in SLIT is needed to compare the clinical effects and therapeutic efficacy among studies that differ in allergen, dose, and method and protocol of administration. This will permit improved meta-analyses. Currently, it is difficult to optimize the SLIT protocol using results from multiple clinical trials that used different methods for evaluation of therapeutic effects, such as cumulative or average scores for symptoms and

medication, QOL, VAS, local symptoms, and days with mild or severe symptoms over periods of days, months, seasons, and years [18, 50, 51]. It will be difficult to score the severity of allergic symptoms using the same grading system because both the pattern and main organ in which symptoms appear may differ among seasonal or perennial allergies in various areas. However, a scoring or grading system for use in scientific reports is needed as a minimum requirement to permit improved understanding by readers.

4. Trials of Adjuvant Therapy with SLIT

Coadministration of an adjuvant with allergens may achieve more efficient and effective SLIT. Many studies in mouse models of asthma or rhinitis have shown increased effects of SLIT with adjuvant therapy. In most cases, the adjuvant is used to enhance development or activation of regulatory T cells (Treg) or increase adherence or permeability of allergens in sublingual mucosa to enhance uptake by antigen-presenting cells (APCs) such as mucosal dendritic cells (DC). Sublingual administration of an antigen conjugated with the nontoxic B subunit of cholera toxin to mice significantly induced antigen-specific Foxp3⁺CD4⁺ T cells in cervical lymph nodes and spleen and suppressed proliferation of cells from cervical lymph nodes after stimulation with antigen to a greater extent than that after treatment with the unmodified antigen. The serum TGF- β level was also higher after administration of the modified antigen compared to the unmodified antigen [52]. Sublingual coadministration of an antigen with either 1,25-dihydroxyvitamin D3 plus dexamethasone (VitD3/DEX) or *Lactobacillus plantarum* suppressed airway hyperresponsiveness (measured as PenH) compared with antigen alone, and coadministration with VitD3/DEX significantly induced Foxp3⁺ cells in mice [53]. Another mouse study supported the adjuvant activity of lactic acid bacteria in enhancing the therapeutic effects of SLIT [54]. A study using polymerized carbohydrate as a mucoadhesive adjuvant showed superior reduction of established airway hyperresponsiveness (PenH) and lung inflammation compared to administration of antigen alone or phosphate-buffered saline [55]. In this study, IL5 and IL10 production from splenocytes was reduced after stimulation with antigen in mice-administered antigen with adjuvant compared with mice-administered PBS or antigen alone. The therapeutic effects of adjuvant SLIT are also under evaluation in humans. In a Phase I/IIa study, coadministration of grass allergens with a high dose of monophosphoryl lipid A, an agonist for toll-like receptor 4, significantly increased the rate of negative findings in a nasal challenge test at two weeks after completion of 8-week treatment [56]. Further large scale studies are needed to evaluate the efficacy of adjuvant SLIT in humans.

5. Recent Findings on Biomarkers for SLIT

Candidate biomarkers for response-monitoring or prognosis have been proposed and evaluated in many studies [4, 57, 58]. IL10 and Treg cells appear to be involved in the therapeutic mechanism of SLIT [59–61]. We reported upregulation of antigen-specific Treg cells (IL10⁺Foxp3⁺ cells)

in CD25⁺CD4⁺ leukocytes from pre- to postpollen season as a response-monitoring biomarker for SLIT [36, 62]. Among patients treated with SLIT, total QOL and QOL-symptom scores after 2 years of treatment significantly improved in a subgroup with increased Treg cells compared with the placebo group, whereas the scores in a subgroup with decreased Treg cells were similar to those in the placebo group (Figure 2(a)). We also proposed that the ratio of antigen-specific IgE to total IgE (sIgE/tIgE) was a candidate as a prognostic biomarker for SLIT in a DBPC trial [36]. SMS in the SLIT group was correlated with the sIgE/tIgE ratio before treatment and was significantly improved in patients with a low sIgE/tIgE ratio compared to that in patients with a high sIgE/tIgE ratio (Figures 2(b) and 2(c)) [36]. The sIgE/tIgE ratio has been found to be significantly higher in responders than in nonresponders following 4-year immunotherapy [63]. In this study, responders to the immunotherapy (42 patients for SCIT and 103 patients for SLIT) showed higher grass- or mite-specific IgE/tIgE ratio than nonresponders (34 patients for SCIT and 100 patients for SLIT) evaluated with VAS score. In our trial, this ratio did not differ significantly between responders and nonresponders [36]. Further validation studies with a large sample size are needed before these biomarkers can be applied in the clinical management of SLIT.

Upregulation of regulatory molecules after SLIT has been reported [57, 64] and programmed cell death ligand 1 (PDL1), IL10, and IgG4 may serve as response-monitoring biomarkers for SLIT [65]. In this report, all patients who received preseasonal, seasonal, and prolonged SLIT had increased percentages of PDL1⁺ and IL10⁺PDL1⁺ cells among CD14⁺ and CD19⁺ cells after stimulation with antigen in pollen season, compared to a placebo group. PDL1 is involved in induction and maintenance of Foxp3⁺CD4⁺ Treg cells in the presence of TGF β in mouse [66, 67], and induction of PDL1 may play an important role in induction of Treg cells by SLIT.

Apolipoprotein is involved in lipid metabolism and lipid transport, and apolipoprotein E has roles in lipid antigen presentation and inhibition of T-cell activation [68, 69]. Upregulation of apolipoprotein A-IV (ApoA-IV) in serum in pollinosis patients from pre- to postpollen season was found to be significantly greater with SLIT than with placebo and was inversely correlated with SMS and QOL scores in the SLIT group [70]. ApoA-IV also significantly reduces histamine release *in vitro* from basophils taken from patients [70], and ApoA-IV induced by SLIT may be involved in downregulation of local or peripheral inflammation during the pollen season.

6. Mechanisms of SLIT

Treg cells play an important role in suppression of Th2 responses and inflammatory cells [4, 71]. However, the cells that induce Treg cells after sublingual administration of allergens and the mechanism of induction remain unclear. DCs that preferentially induce Treg cells are thought to be located in the sublingual mucosa. In a mouse study, three types of DCs with different surface markers were identified

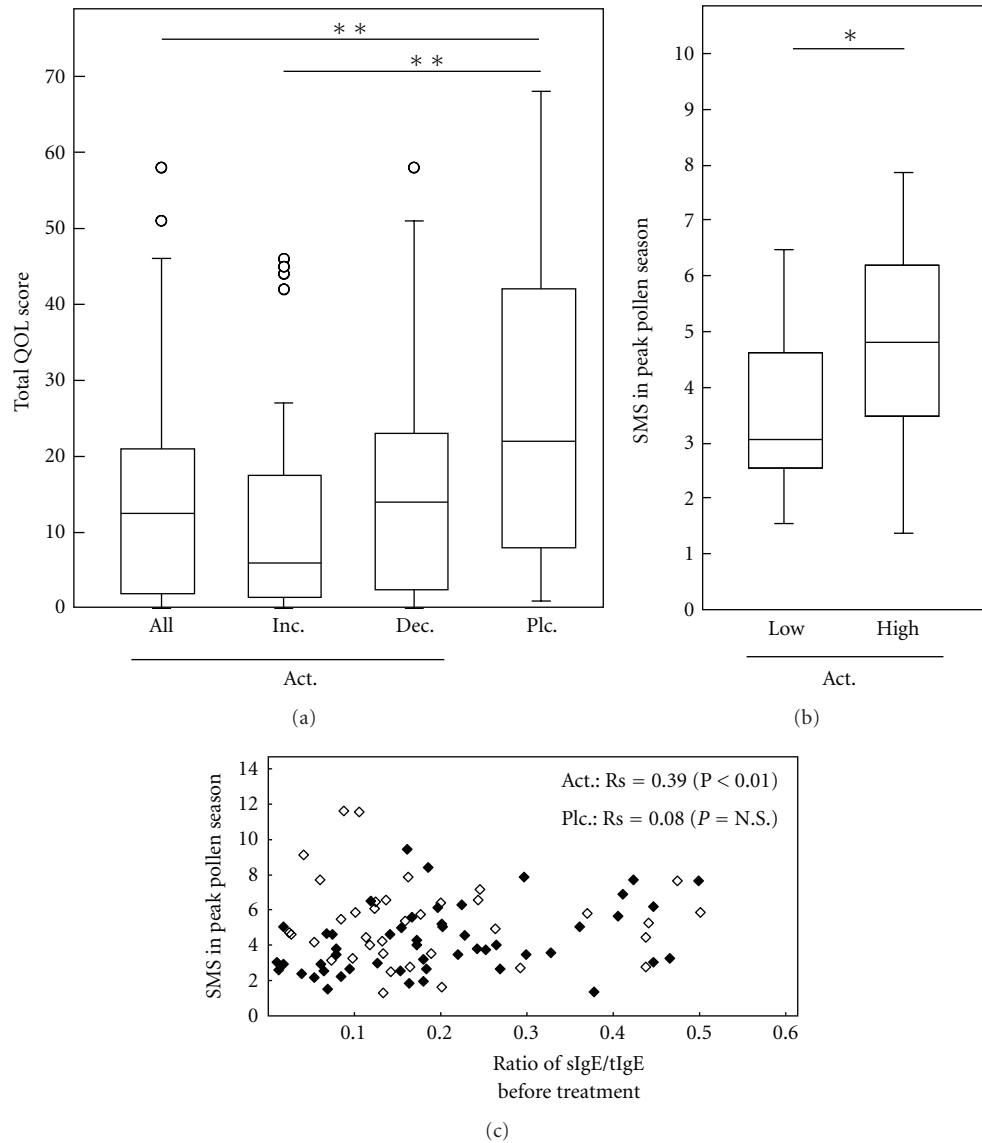


FIGURE 2: Response-monitoring and prognostic biomarkers for SLIT [36]. (a) Total scores from a QOL questionnaire are plotted for the SLIT group (All), a subgroup with increased antigen-specific Treg in the SLIT group (Inc), a subgroup with decreased antigen-specific Treg in the SLIT group (Dec), and the placebo (Plc) group after 2 years of treatment. $**P < 0.01$ (Mann-Whitney U -test). (b) SMSs in the peak pollen season for patients with low and high sIgE/tIgE ratios in the SLIT group (Act.). $*P < 0.05$. (c) Correlation between SMSs after 2 years of SLIT treatment and sIgE/tIgE ratios before treatment in the SLIT (Act, closed diamonds) and placebo (Plc, open diamonds) groups. Statistical data were obtained with Spearman correlation analysis. N.S.: not significant.

within lingual and buccal tissue: CD207⁺ Langerhans cells in the mucosa, CD11b⁺CD11c⁻ and CD11b⁺CD11c⁺ myeloid DCs at the mucosal/submucosal interface, and B220⁺120G8⁺ plasmacytoid DCs [72]. Oral CD11b⁺CD11c⁻ DCs induced IFN- γ production by T cells, and oral CD11b⁺CD11c⁺ DCs and B220⁺120G8⁺ DCs induced IFN- γ and IL10 production by T cells in an antigen-specific manner. These oral DCs may preferentially skew development to antigen specific Th1 or Treg. The function of CD207⁺ Langerhans cells could not be determined because of limited cell numbers. In humans, oral mucosal Langerhans cells (oLCs) that constitutively express Fc ϵ RI on the surface have been found in atopic

and nonatopic subjects [73]. Expression levels of Fc ϵ RI were found to be significantly correlated with serum IgE levels in atopic subjects. oLCs also expressed significantly higher amounts of major histocompatibility complex (MHC) I and II, CD40, CD80, and CD86 compared to skin Langerhans cells [73]. Toll-like receptor 4-ligation of oLCs has also been shown to induce production of IL10, TGF- β , IL2, IFN- γ , and Foxp3 [74], and oLCs might capture allergens within sublingual mucosa and present them to T cells to develop antigen-specific Treg cells [75, 76]. Further studies are needed to determine the importance of oral DCs and oLCs in the therapeutic mechanisms of SLIT.

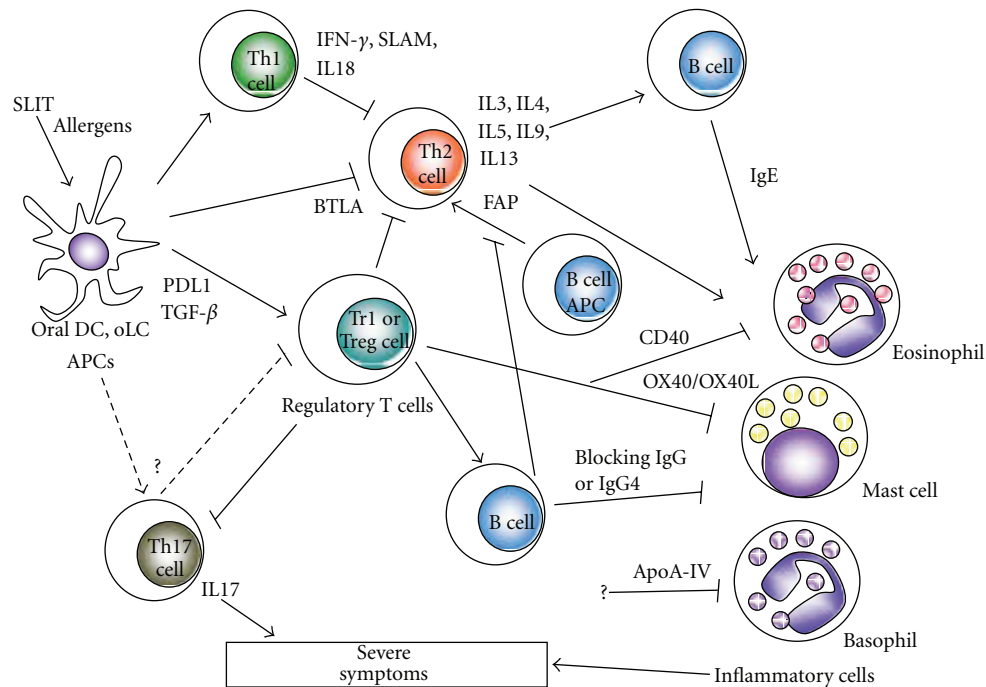


FIGURE 3: Proposed effects of SLIT on T cells, B cells, APC, and inflammatory cells [57]. Oral DCs or oLCs may take up allergens administered sublingually, followed by induction of Th1 or Treg cells to downregulate Th2 cells and inflammatory cells. The Treg cells also activate B cells to produce blocking antibody, which may inhibit binding between allergens and surface IgE on inflammatory cells to prevent secretion of inflammatory mediators and inhibit FAP by APCs or B cells to Th2 cells.

Induction of IgG and IgG4 as blocking antibodies in SLIT is still under debate [77, 78]. IgE enhances uptake and presentation of invading antigens by APCs via CD23, a process known as facilitated antigen presentation (FAP), and transcytosis by human airway epithelial cells in a CD23-dependent manner [79, 80]. The immune complex of IgE with antigen binds to CD23, and this binding leads to enhance antigen presentation by APCs to T cells [79]. There is increasing evidence to show that SLIT inhibits FAP by preventing binding of IgE with antigen or CD23 [81]. Decreased FAP after immunotherapy is correlated with T-cell activation *in vitro* and antigen-specific IgG titer, and FAP activity tends to correlate with IgE/IgG4 ratio and symptom score [82–84]. This inhibition of FAP leads to decrease antigen-specific proliferation and IL4, IL5, IL10, and IFN- γ production from T cells [85]. The inhibition persists over 2 years after discontinuation of 2-year immunotherapy although specific IgG and IgG4 levels decreased to preimmunotherapy levels [86]. Other factors may also be involved in the mechanism of FAP by inhibiting CD23 and IgE binding.

7. Conclusions

One of the aims of immunotherapy is to induce tolerance against invading allergens. The therapeutic effects and efficacy of SLIT vary among allergies with different causal allergen sources. Achievement of a level of tolerance at which drugs are not required and symptoms are absent in the greatest numbers of patients requires further optimization of proto-

cols and modification of SLIT or standardization of allergens as a SLIT vaccine. Adjuvant SLIT and combination with other methods may help to achieve more effective SLIT. The involvement of oral DC, oLCs, Treg, and FAP in the therapeutic mechanisms of SLIT has been proposed in many studies in humans and in mice (Figure 3). To determine the chain of mechanisms of SLIT, more studies are needed using human materials from clinical trials with large sample numbers. Understanding the precise mechanisms of SLIT should facilitate more effective immunotherapy for more patients with allergies.

Abbreviations

APC: antigen-presenting cells
 DBPC: double-blind, placebo-controlled
 DC: dendritic cells
 SCIT: subcutaneous immunotherapy
 SLIT: sublingual immunotherapy
 SMD: standardized mean difference
 SMS: symptom-medication score
 Treg: regulatory T cells
 QOL: quality-of-life
 VAS: Visual Analog Scale.

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Research Article

Quality of Life Improvement with Sublingual Immunotherapy: A Prospective Study of Efficacy

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Due to its excellent safety profile, ease of administration, and economic considerations, sublingual immunotherapy (SLIT) is becoming a preferred form of allergen specific immunotherapy. The efficacy of SLIT is still debated. The purpose of this act of practice trial is to evaluate quality of life outcomes in patients treated with SLIT. Fifty one patients with allergic rhinoconjunctivitis demonstrated by skin testing completed the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) at initiation, at four months and at 10–12 months of SLIT. Significant improvement ($P < 0.05$) on six of seven domain categories of the RQLQ questionnaire was noted. Total RQLQ scores also showed significant improvement. This study supports SLIT as a modality effective in controlling allergic symptoms.

1. Introduction

Allergen-specific immunotherapy in the treatment of IgE-mediated allergy has been used for longer than a century; yet, its major form, subcutaneous immunotherapy (SCIT) has not become a widely accepted routine treatment for allergy. Patients will often suffer from severe symptoms and allergic comorbidities before consulting with an allergist or considering immunotherapy. Children especially are unlikely to adhere to SCIT. Subcutaneous injections for immunotherapy are believed to be tedious and unlikely to lead to sustained improvement. Standardization, safety, and efficacy concerns, along with the inconvenience of injections and frequent office visits, keep the vast majority of allergic patients from receiving SCIT. Recruitment to immunotherapy is poor: less than 5% of all allergic patients receive immunotherapy. Compliance is even poorer: among adult patients who agree to undergo SCIT, adherence is disappointing with more than two thirds dropping out within a year of initiation. One tenth of SCIT candidates fail to show up for their first injection [1]. In some countries, the scope of SCIT has been curtailed

substantially by administrative decisions [2]. At the same time, a wealth of evidence in literature and clinical practice supports the safety, efficacy, feasibility, compliance, and economic profile of sublingual immunotherapy (SLIT) [3–7]. In the United States, however, SLIT remains uncommon and is only offered by few practices with special interest in this method. With this study, we sought to evaluate the subjective symptom responses of patients treated with multiantigen SLIT. The information provided with the present study may lead to better appreciation of the potential of SLIT and may foster the design of large-scale, multicenter studies for its full appraisal.

2. Methods

2.1. Subject Selection and Testing. Subjects were recruited from patients of Allergy Associates of La Crosse, a single specialty practice that has been offering SLIT for 41 years. The study was approved by the Mayo Clinic Health System Franciscan Healthcare-La Crosse, Institutional Review Board. All

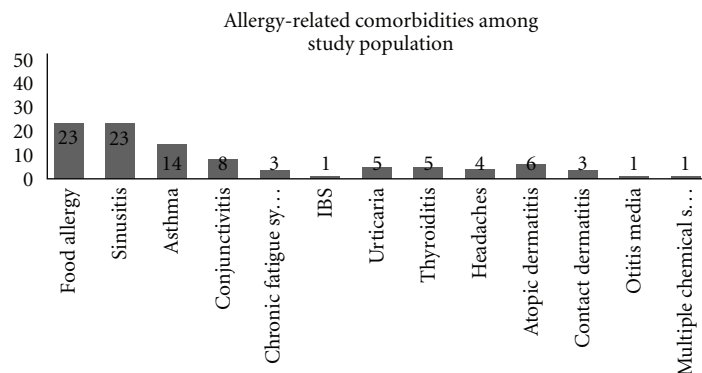


FIGURE 1: Of the 51 study participants, most had one or more comorbid allergic conditions with the average number of 1.9 chronic conditions per participant.

subjects were diagnosed with allergic rhinoconjunctivitis on the basis of their history and positive skin test results. Skin test positivity was assessed by obtaining a response greater than the negative control and greater than two thirds of the histamine control using intradermal dilution testing (IDT) [8]. Antigens selected for testing were determined by a self-administered patient history questionnaire and initial consultation with their physician. Patients with dermatographism or systemic mastocytosis were not included. Figure 1 also shows that the patient population was affected by one or more comorbid allergic condition upon arrival for their first appointment. Skin test panels included 15–30 antigens representing dust mite, weed, tree, grass, and fungal allergens typical of the northern Midwest (see Table 1). The number of allergen extracts varied by patient, as the number of offending allergens ranged from six to 24 with the mean of 15.15. Round one patient enrollment occurred from July through December and round three visits occurred from January through November, thus crossing multiple peak pollen seasons and limiting the influence of allergen season bias.

2.2. Sublingual Immunotherapy Administration. Sublingual immunotherapy based on skin test reactivity was initiated according to the La Crosse Method Practice Protocol [9].

A capital aspect of SLIT, at least as practiced in the United States, is the adjustment of the treating dose to skin reactivity. For this purpose, allergen extracts are serially diluted by decrements of $\times 5$. The purpose of such dilution is to adjust dose to skin reactivity under the premise that adverse reactions (including local reactions) define a level of tolerance. For many patients, skin test reactivity does not necessarily reflect the degree of sensitization. A negative skin test, however, and minimal/absent late-phase responses do establish a de facto threshold of tolerance.

Dosing for each patient was tied to skin test results for each individual antigen and adjusted over the course of treatment (see Figure 2). With ongoing treatment, the need to regularly adjust the degree of testing (and dosing) to the long-term effects of immunotherapy is dictated by the fact that, over time, skin test reactivity tends to decline with immunotherapy. Thus, initiation of SLIT at a strength

TABLE 1: Sensitivities detected by skin testing. Grass mix includes Kentucky Blue/June, Meadow Fescue, Orchard, Perennial Rye, Redtop, Sweet Vernal, and Timothy. Tree mix includes American Beech, American/Eastern Sycamore, American Elm, Black Walnut, Black Willow, Eastern Cottonwood, Red Oak, Red/River Birch, Shagbark Hickory, Sugar/Hard Maple, and White Ash. Weed mix includes Cocklebur, Lamb's Quarter, Common Mugwort, Pigweed (rough/red), and Dock/Sorrel Mix (red/sheep and yellow dock).

| Allergy Associates common environmental allergens and the percentage of participants testing positive to the following. | |
|---|-----------|
| Dust mites | 51 (100%) |
| Ragweed | 51 (100%) |
| Grass mix | 48 (94%) |
| Birch | 42 (82%) |
| Tree mix | 50 (98%) |
| Oak | 37 (73%) |
| Weed mix | 42 (82%) |
| Alternaria | 50 (98%) |
| Cladosporium | 49 (96%) |

corresponding to the highest dilution that produced a near-negative skin test establishes a safe threshold of tolerance; thereafter, upward titration of immunotherapy doses against declining skin reactivity is used for safe build-up and unnecessary local or systemic reactions.

A skin test of greater than 7 mm using dilution number 7 correlates with the highest level of reactivity. Thus, the lowest dose administered of the offending allergen is dilution number 7. As skin test reactivity improves, doses are escalated to the next allergen dilution until the patient has reached dilution number 1 for his/her different allergens. The starting dose of each individual antigen is titrated based on skin test (or in vitro specific IgE testing) level of reactivity, (see Tables 2 and 3). Sublingual immunotherapy with multiantigen treatment addresses multiple allergies that are specific to each individual patient. Each bottle consisted of a 90-day supply that was individually prepared for the patient using Greer Laboratory and ALK-Abello extracts and compounded in the Allergy Associates of La Crosse clinical laboratory.

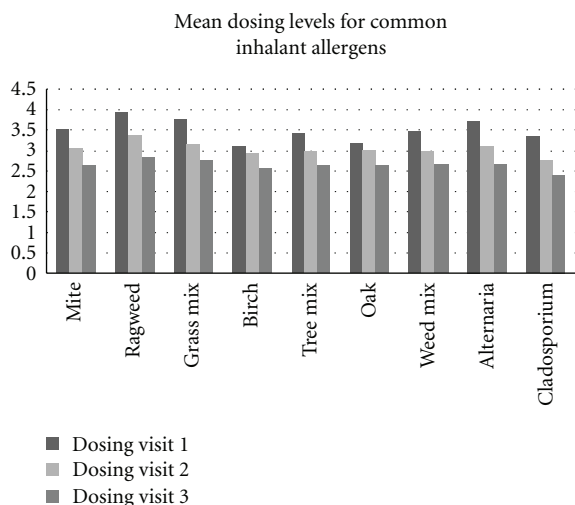


FIGURE 2: Dosing levels with the La Crosse Method are escalated over the course of treatment based on skin test reactivity. Dosing levels are carefully adjusted in an effort to balance therapeutic benefit without creating unnecessary patient side effects.

Over a 1000-fold range of antigen dilutions have been reported to produce clinical improvement with SLIT suggesting that a straight dose-effect does not exist [10]. Other variables such as dosing frequency, extract quality, and length of treatment also need to be considered. Numerous studies have observed and suggested a limited capacity of the sublingual mucosa and have shown clinical improvement with lower, but more frequent doses [11–13]. Patients were advised to take their sublingual immunotherapy drops three times daily. Given that SLIT is retained in the sublingua for up to 48 hours, administering two to three doses per day is reasonably expected to secure unbroken allergen-exposure and overlap generously with antigen uptake by the dendritic cells and migration to lymphoid organs.

2.3. Questionnaire Administration. Symptom severity was evaluated by the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ). This disease-specific validated questionnaire was developed by Professor Elizabeth Juniper and has been used extensively throughout the world in a large number of clinical trials [14]. New clinic patients were asked to complete the RQLQ at their first visit before the onset of sublingual immunotherapy treatment and two subsequent follow-up visits at three- to six-month intervals. The full-version RQLQ encompasses 28 questions in seven domains (activity limitations, sleep problems, non-nose/eye symptoms, practical problems, nose symptoms, eye symptoms, and emotional function). Patients were asked to recall their experiences during the previous seven day period and to give their responses on a 0- to 6-point scale (none of the time to all of the time). A total RQLQ score is also calculated by adding the scores of the individual domains together.

This study was a prospective analysis that compiled and compared collected RQLQ data from patients undergoing sublingual immunotherapy. Collected data for each patient were compared to that particular patient's baseline data and

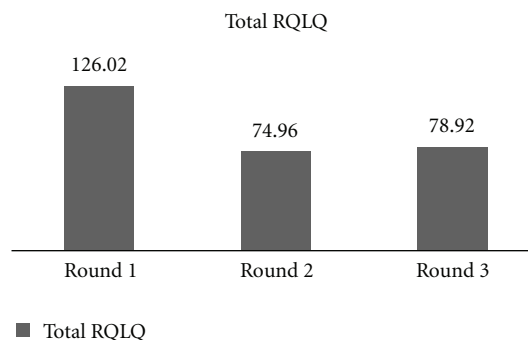


FIGURE 3: Participants' aggregate RQLQ scores were compared from their initial appointment and follow-up visits one and two. Statistical significance was achieved within four months of beginning sublingual immunotherapy and continued through their second return visit (Round 3). $P < 0.05$. Standard error for Round 1 = 7.74, Round 2 = 6.35, and Round 3 = 7.61.

two subsequent patient visits for changes in each RQLQ parameter as well as total RQLQ score. Timing of follow-up for visit two ranged from 1.23 months to 10.94 months, with a mean follow-up time of 4.1 months. Follow-up for visit three ranged from 2.82 months to 17.94 months with a mean follow-up time of 7.06 months. The average duration of treatment during the study was 11.19 months.

2.4. Statistical Analysis. Descriptive and bivariate statistics were performed using the Standard SPSS data package. Statistical significance was designated as $P < 0.05$.

3. Results

3.1. Patient Characteristics. Paired RQLQ data were available for 51 patients who were skin tested and started on SLIT. Participants were comprised of 13 males and 38 females, with ages ranging from 22 to 63, and a mean age at initiation of SLIT of 45.8 years.

3.2. Quality of Life Results. Paired RQLQ results revealed statistically significant ($P < 0.05$) improvement in six of seven domains evaluated by the RQLQ after four months of treatment. Improvements were seen in the activities, non-nose/eye symptoms, practical problems, nasal symptoms, eye symptoms, and emotional categories. Results are presented in Table 4. Statistically significant improvements were noted in 23 of the 28 overall questions. Furthermore, the total RQLQ for the whole cohort declined significantly ($P < 0.5$) from 126.02 to 74.96 within the first four months of treatment (see Figure 3).

Although just shy of achieving statistical significance, participants that adhered to three times daily dosing of sublingual immunotherapy showed better improvement in RQLQ scores than participants who were suboptimally compliant. Advised compliance to treatment declined from round two to three, which may have affected round two to round three overall RQLQ scores (see Figure 4). Further studies with larger study populations are needed to validate

TABLE 2: Serial dilutions are then used to expand La Crosse Method doses from one to seven.

| Antigen | La Crosse method concentrate | Concentration number 1 dilution |
|-------------------------|--|---------------------------------|
| Pollens | 1 mL 1 : 20 w/v | 1 : 100 w/v |
| Mold | 1 : 20 w/v | 1 : 100 w/v |
| Mite mix | 1 mL conc + 2 mL diluents for 10,000 AU/mL | 2000 AU/mL |
| Cat | 1 mL + 4 mL diluents for 2000 BAU/mL | 400 BAU/mL |
| Epithelias (except cat) | 1 : 20 w/v | 1 : 100 w/v |
| Grass mix | 100,000 BAU/mL | 20,000 BAU/mL |
| Bermuda grass | 10,000 BAU/mL | 4000 AU/mL |
| Short ragweed | 100,000 AU/mL | 20,000 AU/mL |

TABLE 3: How 1 : 5 serial dilutions are made: from La Crosse Method number 1 dilution.

| |
|---|
| 1 mL of dilution number 1 + 4 mL of diluent = dilution number 2 |
| 1 mL of dilution number 2 + 4 mL of diluent = dilution number 3 |
| 1 mL of dilution number 3 + 4 mL of diluent = dilution number 4 |
| 1 mL of dilution number 4 + 4 mL of diluent = dilution number 5 |
| 1 mL of dilution number 5 + 4 mL of diluent = dilution number 6 |
| 1 mL of dilution number 6 + 4 mL of diluent = dilution number 7 |

treatment compliance and multiple versus single daily dosing.

4. Discussion

A number of studies have demonstrated statistically significant effects on allergic rhinoconjunctivitis and asthma symptoms in SLIT [15–20]. These studies, however, were all heterogeneous and of small magnitude. More importantly since no two studies used the same protocol, the value of their meta-analysis is questionable. They all used single-allergen monotherapy to evaluate efficacy, an approach which does not reflect the sensitization status of patients with allergic rhinoconjunctivitis, and may in fact have led to undertreatment and subsequent under-appreciation of the efficacy of SLIT. The number of SLIT (or even SCIT) efficacy studies employing multiple allergens in the treatment regimen is so surprisingly small that their low number and insufficient data on efficacy have been addressed unfavorably [21]. These studies were characterized by their sporadic nature. They were not followed by subsequent studies that would have established a continuity of approach which might have made up, to some extent, for methodological defects. Significantly, the ultimate end-point, which is improvement of symptoms, was not assessed by a validated instrument, developed by an independent party, such as the RQLQ [9]. To our knowledge, a modified, shortened version of the RQLQ, the mini-RQLQ, has been used in one study employing SLIT for multiple allergens but this study relied on retrospective selection of subjects and only enrolled fifteen patients, thus raising significant questions as to both its power and freedom of bias [22]. Our study is the first prospective study of SLIT efficacy, employing multiple allergen extracts for treatment, a protocol for SLIT which has been applied for 41 years,

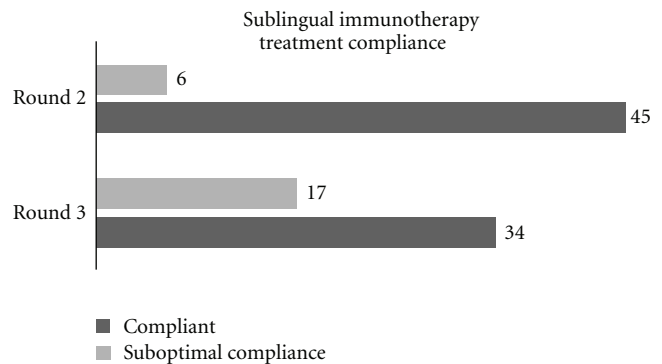


FIGURE 4: Patients were advised by their providers to take sublingual immunotherapy drops three times daily. Patient records showed a greater adherence to three times daily dosing from their initial appointment to second visit than from their second appointment to third.

a validated questionnaire, and a number of subjects large enough to satisfy power requirements.

In the present study, SLIT, as formulated by the La Crosse Method Protocol, is effective in reducing symptoms and improving quality of life after four months of treatment (see Table 4). This improvement was most prominent in activity, non-nose/eye symptoms, nasal symptoms, and emotional domains. Improvement in the sleep domain of the RQLQ was also observed, but did not reach statistical significance. This improvement was sustained and demonstrated again at 10–12 months of treatment. Given the high compliance rates with the La Crosse Method SLIT, it is expected that the improvement achieved is likely to be sustained and possibly expanded with ongoing treatment beyond the first year. Sneezing and irritability, two parameters, which in a previous SLIT efficacy study employing the mini-RQLQ were found unaffected, are demonstrated to decline in the course of the first four months of SLIT [22].

The mechanism underlying SLIT has been reviewed [7]. Although not fully delineated, it appears that a systemic alteration of the Th1/Th2 balance is effected in SLIT by the promotion of tolerogenic T-cell clones. Interaction of dendritic cells with naïve T-cells is necessary for this change to occur. Production of TGF- β , IL-10, and possibly other regulatory cytokines appears to be critical. Ongoing changes may in some cases be reflected in skin reactivity as well as

TABLE 4: Mean RQLQ Scores Presublingual Immunotherapy and at Subsequent Visits.

| Category sum | Round 1 | | Round 2 | | Round 3 | |
|----------------------|-----------|--------|-----------|--------|-----------|--------|
| | Mean (SE) | | Mean (SE) | | Mean (SE) | |
| Activity sum | 8.14 | (.57) | 4.92* | (.44) | 4.63* | (.52) |
| Sleep sum | 5.84 | (.68) | 3.61 | (.45) | 3.92 | (.63) |
| Non-nose/eye sum | 17 | (1.37) | 10.59* | (1.04) | 10.71* | (1.22) |
| Practice problem sum | 8.43 | (.65) | 4.63* | (.50) | 5.06* | (.54) |
| Nasal symptom sum | 11.69 | (.74) | 7.25* | (.60) | 7.63* | (.64) |
| Eye symptom sum | 8.92 | (.82) | 5.49* | (.67) | 6.16* | (.67) |
| Emotional sum | 10.55 | (.87) | 4.27* | (.59) | 4.82* | (.67) |

* Denotes RQLQ domains found to have a statistically significant decrease in symptom scores throughout the duration of sublingual immunotherapy treatment. * $P < 0.05$.

in specific IgG and IgE production changes. The protocol used in the present study may be well suited to effect these changes. It can be summarized in three cardinal points: (i) initial and thereafter regular titration of treating SLIT doses against skin reactivity and symptom response with skin reactivity meant as a biphasic response whose late phase reactions are also taken into account; (ii) frequent administration of SLIT doses to secure continuous, maximal, and uninterrupted saturation of the sublingual dendritic cells' potential for phagocytosis and migration, that is, three doses per 24 hours; and (iii) maintenance of allergens in high glycerin solutions in order to prevent decay and suppress proteolytic activity [9].

In summary, this study represented a preliminary attempt to investigate the effectiveness of multiantigen SLIT in a complex patient base. Experience with this protocol over the years has been rewarding and has shown clinical benefit with a wide variety of allergic conditions including advanced respiratory disease in adults with mold allergy [23], asthma prevention in pediatric patients [24], and contact allergies including nickel [25] and poison ivy [26] while maintaining a remarkable paucity of adverse reactions of any significance. The present study underscores the efficacy of SLIT; however, we recognize that the absence of a placebo group limits the interpretation of results. Given the large number of patients currently treated and high rates of compliance, multicenter, controlled studies are needed of greater magnitude and expanded scope to include morbidities and associations such as recurrent/chronic sinusitis, atopic dermatitis, gastroesophageal reflux, and migraines. Sustained suppression of symptoms after eventual completion of SLIT will also need to be studied.

5. Conclusion

Statistically significant reduction of symptoms and improvement of quality of life are demonstrated during the initial four month period of SLIT. After the first four months, reduction of symptom scores is sustained and continuous. These data support the efficacy of SLIT and need to be followed by controlled trials to evaluate the efficacy of this method.

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Research Article

A Multicenter, Randomized, Parallel-Group Trial Assessing Compliance, Tolerability, Safety, and Efficacy to Treatment with Grass Allergy Tablets in 261 Patients with Grass Pollen Rhinoconjunctivitis

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Background. Allergen-specific sublingual immunotherapy (SLIT) is considered a causal treatment of respiratory allergies. Compliance to the SLIT is an important aspect for a positive clinical outcome. **Study Aim.** To evaluate if compliance with grass Allergy Immunotherapy Tablet (AIT) can be increased by providing an electronic compliance device (CED) (Memozax; a tablet-container with a programmable daily acoustic alarm). **Patients and Methods.** 261 patients with grass allergy were enrolled and randomized (1 : 1) to 1-year treatment with AIT (Grazax) using a CED (group A; $n = 122$) or without (Group B, $n = 139$). Compliance was measured through tablet count at each visit. **Results.** The 12-month compliance, mean (SD), in group A was 83% (21) and 83% (24) in group B. A total of 81% of patients reported a significant clinical improvement of symptoms after treatment in comparison with the previous year. No severe adverse reactions were observed in the study. **Conclusion.** Compliance to the treatment with AIT administered for 12 consecutive months is in general good. The use of CED is not associated with a greater compliance. AIT treatment was associated with a significant clinical improvement in >80% of patients with a good tolerability and safety profile.

1. Introduction

Allergic rhinoconjunctivitis represents a global health problem affecting 10 to 25% of the population [1]. Allergy to grass pollen is one of the most common inhalant allergies in the western world. In an unselected “healthy” population it has been found that 8 to 21% of children and 13% of adults are sensitized to grass pollen [2]. In selected populations of allergic subjects 44% are allergic to grass pollen [3]. Allergic rhinoconjunctivitis has been identified as one of the main reasons for visits to primary care clinics, and although usually not regarded as a severe disease it significantly limits the social life of the subject and affects school learning performance and work productivity [4]. Allergen-specific immunotherapy is the practice of administering to allergic patients increasing amount of allergen in order to obtain

hyposensitization [5]. Allergen-specific immunotherapy is considered the only causal treatment of allergic diseases such as allergic rhinitis asthma and insect venom allergy. The purpose of allergen-specific immunotherapy is to expose the patient to the allergen that causes the allergic symptoms, in order to increase the tolerance to this allergen and reduce symptoms [6]. Mechanisms of action of allergen-specific immunotherapy are not so far clearly identified; however, data are available that allergen-specific immunotherapy can induce an increased production of allergen-specific IgG4 and IL-10 [7]. Alternative mechanisms include immune deviation in favour of TH1 responses and apoptosis and/or anergy of antigen-specific T cells. Allergen-specific immunotherapy exerts its beneficial effects over long periods (i.e., weeks or months), and 3–4 years of treatment are required to obtain a favourable clinical and immunological response [8]. The

treatment with allergen-specific immunotherapy is currently administered as subcutaneous (s.c.) injections by specialists and reduces the allergic symptoms considerably [9]. The “standard” allergy vaccination program requires an uposing period followed by a maintenance period of 3–5 years [10]. This implies that only a fraction of allergic subjects are actually offered allergy vaccination despite the fact that allergen-specific immunotherapy is the only treatment modality that changes the natural cause of the allergic disease and thereby prevents its exacerbation. The treatment modality is well known as clinical application of *Phleum pratense* for s.c. administration has been carried out during the past 30 years in several European countries [11]. Allergen-specific sublingual immunotherapy (SLIT) has gained wide acceptance in many European countries and has raised the level of interest in immunotherapy among practicing allergists and primary care physicians. Large pivotal double-blind, placebo-controlled, randomized clinical trials have confirmed the efficacy and safety of SLIT. Allergen-specific sublingual immunotherapy with grass products has also had a widespread application—especially in Southern Europe throughout the past 30 years [12]. The general recommendation today is to apply a higher than the accumulated dosage applied subcutaneously. Grass allergy immunotherapy tablet (AIT) (Grazax; ALK Denmark) has been developed for allergen-specific immunotherapy [13]. Grazax is formulated as an orodispersible tablet for sublingual use and contains a standardised allergen extract derived from extraction and purification of the source material, *Phleum pratense* Timothy grass pollen [14]. To obtain an optimal therapeutic response with immunotherapy requires patients to be compliant with the recommendations given by the physicians. Maximal compliance can improve the patient's condition and also result in a reduction in drug costs [15]. Conversely, poor compliance may result in the physicians adding in more medications to treat the patient's condition, which may make the problem worse. Compliance to allergen-specific immunotherapy could be negatively influenced by several factors such as: duration of treatment, side effects, especially in the initiation phase, and need to take medication also outside the pollen season period when the patient in general does not have any symptoms. Specific allergen SLIT is a long-lasting home treatment that is directly managed by patients and parents. Therefore, as allergen-specific SLIT is self-managed at home without direct supervision, adequate compliance with this administration route is important.

2. Study Aim

The primary objective of the trial was to evaluate if compliance of once daily dosing with grass AIT in adult subjects with grass-pollen-induced allergic rhinoconjunctivitis could be increased by providing patients with compliance device (Memozax) (Figure 2) given from the beginning of immunotherapy in comparison with patients without the Memozax. Secondary endpoints of the trial were to evaluate safety and tolerability of grass AIT treatment and finally to evaluate the tolerability of the first dose intake of AIT and to evaluate after 48-week treatment with grass allergy

tablet tablets the impact on symptom score and patient's acceptance in comparison with previous pollen seasons.

3. Patients and Methods

3.1. Study Design. This was a 23-centre, single-dose, randomized parallel-group, open-label, controlled trial.

3.2. Patients Selection. A total of 240 subjects were planned for enrolment. Enrolled patients were adult (>18 years), men or women, suffering from mild or moderate/severe grass-pollen-induced allergic rhinoconjunctivitis. A total of 261 patients were screened, enrolled, and randomized to 48-week treatment with AIT using the compliance aid device (Memozax) or 48-week treatment with AIT without the compliance device. The screening phase lasted 1 week. Therefore the total study duration was 49 weeks. All enrolled patients were treated with one tablet of AIT daily for 48 weeks.

3.3. Inclusion and Exclusion Criteria. Subjects were selected from the outpatient population of allergy clinics in Italy. Subject selection was based on the following criteria. Inclusion criteria were as follows: subjects, men and women >18 years of age and <65 years; suffering from mild or moderate/severe grass-pollen-induced rhinoconjunctivitis (according to ARIA Guidelines [16]) and with a positive SPT for *Phleum pratense* extract (≥ 3 mm); every patient should give a written informed consent to participate in the trial. Exclusion criteria at randomization were as follows: current symptoms of, or treatment for, upper respiratory tract infection, acute sinusitis, acute otitis media, or other relevant infectious processes; history of emergency visit or admission for asthma in the previous 12 months; use of an investigational drug within 30 days prior to screening; previous treatment by immunotherapy with grass pollen allergen; previous treatment by immunotherapy with other allergen than grass pollen allergen; within the previous 5 years; history of anaphylaxis, including anaphylactic food allergy, bee venom anaphylaxis, exercise anaphylaxis, or drug-induced anaphylaxis; or history of angioedema.

3.4. Therapeutic Regimen. The treatment used was Grazax oral lyophilisate 75,000 SQ-T tablets (*Phleum pratense* grass pollen allergen extract). The daily dose was one tablet, which should preferably be taken in the morning. The tablet was placed under the tongue and swallowing should be avoided for one minute. Eating and drinking was not allowed within five minutes after trial medication intake. The same drug taking instructions were given to both study groups (randomized to Memozax or not). Concomitant medications were all medications (including rhinoconjunctivitis medications and asthma treatments) being continued by a subject on entry to the trial and all medications given in addition to the treatment during the trial. All concomitant medications should have been documented in the CRF (trade name as appropriate). Further, each change in concomitant treatment (e.g., new treatment, discontinuation of treatment, and change in dosage/routine) during the trial must be

documented in the same way. At each visit, the investigator asked the subject about concomitant medications. Any concomitant medication was recorded in the subject's notes as source data documentation and in the CRF. The investigators were instructed to store the drug in an appropriate, secure area (e.g., locked cabinet) and to store it according to the conditions specified on the labels. The investigator should have to maintain an accurate record of the shipment and dispensing of trial medication in a drug accountability log, a copy of which was given to ALK-Abelló at the end of the trial. An accurate record of the date and amount of trial medication dispensed to each subject was available for inspection at any time. The first dose was taken in the clinic and the subject stayed in the clinic for 60 minutes for observation. The first dose tolerability was recorded in the CRF. The following doses were taken at home. The experimental drug was supplied in blister cards containing 10 tablets each. The blister cards were packed in visit specific boxes.

3.5. Randomization Procedures. Between October 2007 and February 2008 a total of 240 subjects were planned to receive grass AIT as an oral lyophilisate once daily. The subjects were randomized (1:1) using a randomization list with half the subjects planned to receive the compliance device and half not to receive the device. Subjects were identified by ascending 2-digit randomization numbers, plus 2-digit referring to the centre and entered in the Case Report Form. When a subject was randomized in the trial he/she had to be assigned the lowest available randomization number for that centre. The randomization number was a 5-digit number where the two first digits gave a center code. Grass allergen tablet treatment was provided at the screening/randomization visit together with the Memozax, according to the randomization list. In all enrolled patients treatment started at least 3 months before the pollen season of 2008.

3.6. Compliance Evaluation. The primary outcome of the study was the evaluation and comparison of compliance in the two groups (with Memozax and without Memozax) evaluated with pill count at visits 3 (week 2), 4 (week 24), and 5 (week 48) calculated in the following manner: number of pill effectively taken/number of pill to be taken $\times 100$. Subjects were instructed to return all residual and unused trial medications and all empty packaging at every visit. Compliance was assessed by tablet counts.

3.7. Secondary Endpoints of the Trial. Other efficacy assessment was to evaluate after 48 weeks of treatment with Grazax tablets the impact on quality of life, symptom score, and patient's acceptance in comparison with previous pollen seasons. This was evaluated, globally, through a 10 cm VAS scale (0 = big improvement, 5 = not improvement, and 10 = worsening of symptoms). The safety assessments included recording of all adverse events (AEs) and serious adverse events (SAEs) findings from physical examinations and vital signs.

3.8. Conduction of the Trial. This trial was conducted in compliance with the principles of *Good Clinical Practice*. The

trial was monitored according to Sponsor Company standard operating procedures for the monitoring of clinical studies and other trial-specific procedures. The trial was monitored by the sponsor or its delegate by means of on-site visits, telephone calls, and regular inspection of the CRFs with sufficient frequency (every 8–12 weeks) to verify the following: subject enrolment; compliance with the protocol; the completeness and accuracy of data entered in the CRFs by verification against original source documents; compliance in the use of IMP; drug accountability; recording of adverse events.

3.9. Statistical Methods and Sample Size Calculation. All statistical analyses were carried out by SPSS statistical Package software. The following analysis set was defined in the protocol: *full-analysis set (FAS)*, this consists of all subjects randomized following the intent-to-treat (ITT) ICH principle. The FAS was the only analysis set. A scientific publication has shown that compliance to SLIT treatment >90% without any device system was registered in 75% of treated patients. In this study it was hypothesised that the group of patients with the Memozax device should have a better compliance (a relative increase of 15% or more) in comparison with the group without the aid device (86% of patients with a compliance of 90% or more in the Memozax group versus a 75% in the group without the Memozax). A minimum of 120 subjects per group, with an alfa error of 0.05 and a power of 80%, therefore should be enrolled in the trial. Actually a total of 261 patients were enrolled in this trial. The comparison between the two groups was analyzed using the ANOVA test. The comparison of percentage of patients with a compliance <90% and >90% between the two groups was performed with the Fisher exact test.

4. Results

A flowchart of subjects disposition is presented in Figure 1. The subject demographic values, smoking history, and allergy disease history at baseline are summarised in Table 1. There were no major baseline differences between the two groups. It is to note that 73% of the enrolled patients suffered from moderate/severe allergic rhinoconjunctivitis. A total of 50 patients (25 in both groups) also reported asthma (19% of FAS population). Monosensitive patients (subjects with SPT positive only for grass extracts) were 68 (26%) and multisensitive patients (subjects with also at least one positive SPT toward nongrass allergens) were 193 (74%).

4.1. Primary Endpoint: Compliance. The overall mean compliance rate was 91.3% (median 97%) for the subjects with complete compliance data from visit 3 to visit 5. The primary endpoint in this trial was a comparison of the degree of compliance in the two groups (Memozax and non-Memozax). For this purpose compliance was categorised as *excellent* ($\geq 90\%$) or *less excellent* ($< 90\%$). The proportion of subjects with *excellent* compliance in the Memozax group was similar (79%) to that in the non-Memozax group (78%) (Table 2). The difference was not statistically significant ($P = 0.5$).

TABLE 1: Patients demographic characteristics at baseline.

| Treatment Group | Grazax+ Memozax | Grazax– Memozax |
|----------------------------|--------------------|--------------------|
| Number of subjects | 139 | 122 |
| Age (years) # | | |
| N | 139 | 122 |
| Mean (SD) | 32 (9) | 33 (10) |
| Median | 32.0 | 33.0 |
| Min-Max | 19–60 | 18–63 |
| Sex | | |
| N | 139 | 22 |
| Men | 75 (54%) | 74 (60%) |
| Women | 64 (46%) | 48 (40%) |
| Allergic rhinitis | | |
| Mild | 37 (26%) | 33 (27%) |
| Moderate/severe | 102 (74%) | 89 (73%) |
| Monosensitive subjects | 32 (23%) | 36 (29%) |
| Polysensitive subjects | 107 (77%) | 86 (71%) |
| Asthma (Gina class: I–III) | | |
| N | 25 (18%) | 25 (20%) |

N: Number of subjects; (%): Percent of subjects.

TABLE 2: Excellent compliance versus less excellent compliance.

| Treatment Group | Grazax+ Memozax | | Grazax– Memozax | |
|--|--------------------|-------|--------------------|-------|
| | N | (%) | N | (%) |
| Number of subjects | 139 | | 122 | |
| Primary analysis no. | | | | |
| N | 113 | | 99 | |
| Excellent compliance ($\geq 90\%$) | 90 | (79%) | 78 | (78%) |
| Less excellent compliance ($< 90\%$) | 23 | (21%) | 21 | (22%) |

N: Number of subjects; (%): Percent subjects.

4.2. Secondary Endpoints: Clinical Efficacy, Tolerability, and Safety. Other efficacy assessment was to evaluate after 48 weeks of treatment with Grazax tablets the impact on quality of life, symptom score, and patient's acceptance in comparison with previous pollen seasons. This was evaluated, globally, through a 10 cm VAS scale (0 = big improvement, 5 = not improvement, and 10 = worsening of symptoms) which was performed by the patient. At visit 5 the mean VAS score was 2.4 ± 1.8 showing a general clinical improvement. The percentage of patients with a VAS score = or > 5 (no-difference/worsening in comparison with the previous season) was 19%. Therefore 81% of patients reported a clinical improvement of symptoms after treatment with Grazax in comparison with the previous year. Clinical efficacy was comparable both in monosensitized patients (68 out of 261: 26%) and in multisensitized subjects (193 out of 261: 74%). Investigator Global Clinical evaluation of 48-week treatment with Grazax was good/very good: 85%; sufficient: 9%; not

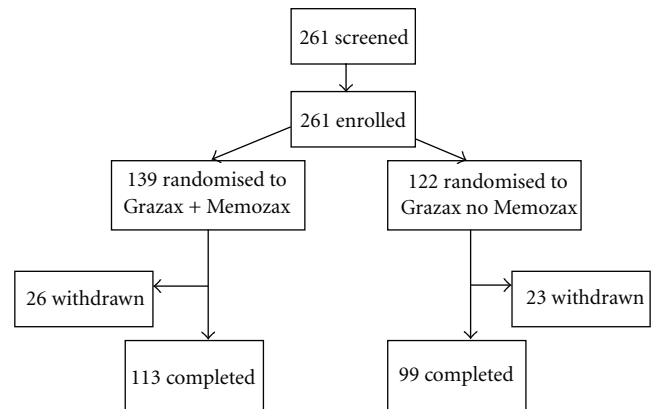


FIGURE 1: GT17 study flow.



FIGURE 2: The compliance device (Memozax) used in the trial.

good: 6%. The percentage of subjects who reported adverse events (AEs) in each of the two groups was almost similar, 14% in the Memozax group and 11% in the non-Memozax group. A total of 63 AEs (78%) reported were judged as probably or possibly related to immunotherapy by the investigator, while 14 reported AEs were judged as unlikely or not related to allergy tablet treatment. For 2 AEs, the investigator did not report the causality. The majority of AEs (71 out of 79: 90%) were either mild or moderate, with only 4% (absolute number: 3) of AE reported as severe AE (mouth itching). No serious adverse events were observed in the study.

5. Discussion

Allergen-specific immunotherapy is the only causative treatment of several allergy diseases. The main feature of this therapeutic approach is its capacity to modify the natural history of the disease, reducing the development of asthma and new sensitizations after 3–4 years of treatment [17]. Adequate compliance to allergen-specific SLIT is, however, mandatory in order to obtain these results.

This study trial investigated the compliance of 48-week grass allergy tablet treatment in two groups of subjects, one issued with the Memozax compliance device and the other not issued with the device. Overall compliance with grass

AIT was high (>90%). The compliance rate in the Memozax group was slightly higher (91.7%) than that in the non-Memozax group (90.3%), but the difference was not statistically significant. A total of 79% of the patients in the Memozax group who completed the trial had a compliance >90%. In the group without Memozax this percentage was 78%. In the global evaluation, a total of 81% of patients reported an improvement of symptoms after treatment with grass AIT, evaluated through a 10 cm VAS in comparison with the previous season. Investigators evaluated the efficacy of treatment as good or very good in 85% of patients. In this trial clinical efficacy was of similar extent both in monosensitive and polysensitive patients. This is a strong indication that treatment with grass is effective in relieving these symptoms, and it is in line with results from previous grass AIT trials. The safety profile seen in this trial reflects the overall good tolerance to grass allergen tablet treatment [18]. In this study no serious adverse events or deaths were observed. In conclusion compliance to the treatment with grass AIT administered every day is in general high. In this specific clinical setting, the use of electronic devices is not associated with a greater compliance. In addition this trial supports the safety, tolerability, and efficacy profile observed in previous trials of this grass allergy immunotherapy tablet in grass allergic patients.

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Authors' Contribution

M. Milani and S. Pecora are employees of ALK Italy. They were involved in the planning and monitoring of the trial and in the final version of the paper.

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Review Article

Development of Mucosal Immunity in Children: A Rationale for Sublingual Immunotherapy?

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The mucosal immune system has bidirectional tasks to mount an effective defense against invading harmful pathogens and to suppress the immune response to alimentary antigens and commensal bacterial flora. Oral tolerance is a suppression of the mucosal immune pathway related to a specific immunophenotype of the dendritic cells and an induction of the regulatory T cells as well as with the silencing of the effector T cell response by anergy and deletion. The physiological dynamic process of the anatomical and functional maturation of the immune system occurring in children during pre- and postnatal periods is a significant factor, having an impact on the fine balance between the activation and the suppression of the immune response. In this paper, mechanisms of mucosal immunity and tolerance induction in terms of maturational issues are discussed with a special emphasis on the implications for a novel therapeutic intervention in allergic diseases via the sublingual route.

1. Introduction

The mucosal immune system comprises the lymphoid-associated structures of the nasal, bronchial, gastrointestinal, and genitourinary tracts as well as lacrimal, salivary, and lactating mammary glands and the synovium of joints. It is composed of a dynamic network of highly specialized components of the innate and adaptive immune responses, which give rise to the functional common mucosal immune system (CMIS) and ensure fine, organ-specific balance between activation and suppression. The fundamental challenge of mucosal immune response is to prevent effectively the entry of invading pathogens and the development and the disseminating of infection, whereas simultaneously its exposition to the external environment and to a high antigenic load elicits immune tolerance. These interrelated processes of active promotion and suppression of immunity provide a defense against microorganisms and neoplasms and protect against inflammatory pathologies such as allergy and autoimmunity as well. To maintain the immune homeostasis in the oral mucosa which represents the entry port to the gastrointesti-

nal tract, protolerogenic mechanisms take place in this tissue and dominate over active immune responses.

The development of mucosal immunity in children is a time-dependent process initiated in the intrauterine growth and is continuous during the postnatal period. Despite the anatomical and functional immaturity of the mucosal immune system and crosstalk between innate and adaptive immune responses, infants and young children are capable of mounting effective immune defense mechanisms. However, during this age, an imperfect regulatory immune response, which is of crucial importance in developing oral mucosal immunity, may pose an increased risk of food allergies. If developing new strategies of immunotherapy which exploit the establishing of an oral mucosal tolerance has a rationale in pediatric patients is here the subject of discussion.

2. Mucosal Defense Mechanisms

2.1. Mucosal Barrier. Extensive noncellular physical barriers and chemical processes as well as cellular components

constitute mucosal barriers to antigen entry in the mucosa-associated lymphoid tissue (MALT). Structural differentiation of the mucosal epithelium and the appearance of intercellular tight junctions lead to the formation of an anatomical basis for an epithelial barrier. A significant protective barrier is constituted by the presence of digestive enzymes starting in the mouth and extending down to the stomach, the small bowel, and the colon, which not only allow the process of digestion, but also modify potentially immunogenic antigens and alter antigen exposure. Mucin glycoproteins, lining the surface epithelium, produce a barrier in which particles and pathogens are trapped and protect the underlying epithelium (the so-called nonimmune exclusion) as well as serving as a reservoir for the secretory IgA [1]. A number of antimicrobial components of saliva contribute to protection against microbial colonization and infection. These include peptides such as salivary peroxidase, lysozyme, lactoferrin, cystatins, SLPI (secretory leukocyte protease inhibitor), agglutinin, peptides of the histatin family, and cathelicidin (LL-37) as well as α - and β -defensins, which are expressed and secreted by salivary glands and/or ducts. In addition to exerting an antimicrobial response, these peptides facilitate and amplify innate and adaptive immune responses [2, 3]. Interestingly, it has been recently demonstrated that the expression and antimicrobial activity of cathelicidin in the oral mucosa is induced by vitamin D [4, 5].

2.2. Innate Mucosal Immune Response. The crucial elements of the innate arm of immunity are pattern recognition receptors (PRR), such as Toll-like receptors (TLR), retinoid acid-inducible gene-I- (RIG-I-) like receptors (RLR) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLR), which recognize pathogen-associated molecular patterns (PAMPs) and molecular structures specific for microbial pathogens. Signaling by pattern-recognition receptors on antigen-presenting cells induces costimulatory molecules and cytokines, and furthermore activating a response in B and T cells. The stimulation of Toll-like receptors by PAMPs initiates signaling cascades that involve a number of proteins, including MyD88 (myeloid differentiation primary response gene 88), IRAK (interleukin- (IL-) receptor associated kinase), Toll/IL-1 receptor (TIR) domain-containing adapter-inducing interferon (IFN)- β (TRIF). Subsequent activation of nuclear factor $\text{NF}\kappa\text{B}$ triggers the production of proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , IL-1, and IL-12, which direct adaptive immune responses. Functional cooperation and cross-regulation between TLRs and the complement components, such as C1q, properdin, and the mannose-binding lectin, using the pattern recognition strategy has been demonstrated. The complement-TLR interplay reinforces innate immunity or regulates excessive inflammation, through synergistic or antagonistic interactions [6]. Moreover, ficolin molecules (L-, M-, and H-ficolin) which recognize pathogen-associated molecular patterns and initiate the lectin pathway of complement activation are thus a further component of mucosal immunity linking innate and adaptive immune responses [7].

The migration of T and B cells from the lymph nodes to the mucosa, which is related to the activation, recirculation, and homing of lymphocytes is controlled by the specific system of integrin-type molecules, selectins [8] and chemokines [9].

2.3. Adaptive Immune Response. The mucosal immune system has generated two arms of an adaptive response, namely, antigen exclusion, performed by different T cell subsets, B cells and secretory antibodies to inhibit or modulate adherence or colonization of microorganisms and prevent penetration of potentially harmful antigens, as well as suppressive mechanisms to avoid overreaction against innocuous substances which are in contact with the mucosal surfaces. A central role in this interrelated network of lymph cell subsets is played by dendritic cells (DCs), which are important initiators of adaptive immunity. DC prime naïve T cells to expand clonally and differentiate into T-cell subsets—T helper Th1, Th2, Th17, or T regulatory (Treg) cells. It has been demonstrated that these cells may have discrete subsets and functions, namely, CX3CR1(+)DC which promote Th1/Th17 cell differentiation, whereas CD103(+)DC induce Treg cell differentiation on an animal model [10]. At the mucosal site, dendritic cells and T lymph cells interact with B cells promoting their differentiation and the production of antibodies. Most immunoglobulin class-switching is T cell dependent; however, it has been demonstrated that T-cell independent process may also occur, whereby DC and mucosal epithelial cells excrete BAFF (B cell activating factor belonging to the TNF family) or APRIL (a proliferation inducing ligand) directly stimulating B cells to become IgA secreting plasma cells [11, 12]. IgA is the major class of antibodies in mucosal secretions and occurs predominantly in a secretory IgA (sIgA) form along with secretory IgM (sIgM). The distribution of IgA subclasses varies at different mucosal sites—in the salivary glands and oral mucosa IgA1, associated with a response to protein antigens predominates, whereas in the distal portion of the gastrointestinal tract mainly IgA2, active in response to polysaccharide antigens is found [13]. The recently characterized Th17 lymphocytes subset is important for the induction of a mucosal adaptive immune response. It has been demonstrated that IL-17 elevates secretory IgA levels by upregulating A polymeric immunoglobulin receptor expression in mucosal epithelia [14] and promotes B cell differentiation in IgA-secreting plasma cells on a T cell-independent manner [15]. Furthermore, IL-17 plays a protective role in infectious diseases at the oral mucosa through the recruitment of neutrophils and extracellular pathogen clearance [16].

3. Maturation of Mucosal Immunity in Children

3.1. Ontogeny of Mucosal Immunity During Prenatal Period. The structures of the mucosal immune system are fully developed by the 28 gestational week, and thus, premature infants older than 28 weeks of gestation are capable of mounting an effective mucosal immune response [13]. Mucosal epithelial barrier formation commences from gestational week 10;

however, the immaturity of intercellular tight junctions results in paracellular permeability, which is advantageous in the intrauterine period by allowing a bidirectional exchange of bioactive molecules between amniotic fluid and fetal serum [17]. Salivary amylase, lysozyme, and lactoferrin concentrations are most prominent in the fetal period as demonstrated by Thrane et al. [18], affording nonspecific protection in the absence of effective specific secretory immunity. Indeed, in the absence of intrauterine infection, the mucosal immune system is essentially devoid of IgA-containing lymphocytes, and until birth, there are no active B cells in the intestinal lymphoid follicles or bronchus-associated lymphoid tissue (BALT). In the salivary glands, IgM positive cells have been reported from 110–140 days of gestation and IgA positive cells with predominance of IgA1 subclass at 180 days of gestation, but no IgD-, IgG-, and IgE-producing cells have been identified by the same authors [18]. The appearance of secretory antibodies in utero can be explained by the possibility that a fetus could have been exposed to bacterial or viral protein antigens or by the induction of a fetal immune response by maternal anti-idiotypic antibodies.

3.2. Postnatal Maturation of Mucosal Immunity. Mucosal permeability is rapidly reduced within the first 48 hours after birth. In the oral mucosa, disappearance of maternally derived IgG reflects this postnatal mucous membrane closure [19]. This maturational process of the gut barrier function is enhanced by human milk [20] as well as by early intestinal colonization with lactobacilli and bifidobacteria [21]. The rapid increase of innate defense factors, such as salivary lysozyme, lactoferrin, and amylase during the first six postnatal months reported by Thrane et al. [18] may provide the infant necessary protection during the period when specific adaptive immunity at mucosal sites is not fully developed. Postnatal maturation of B lymph cell at mucosal surfaces has its peak from birth until the 12 week of age and corresponds with the increase of IgG-producing cells in the parotid salivary glands. Secretory IgM antibodies appear in mucosal secretions only transiently during early infancy. IgA-producing immunocytes, albeit they increase in number during neonatal period and reach an initial peak about 4–6 postnatal weeks, approach the low normal adult level at about 18 months of age, subsequently with small increase throughout early childhood [17]. Qualitative changes in secretory IgA are also seen after birth when a switch from monomeric to polymeric sIgA is observed, indicating maturation of the mucosal secretory immune system. Furthermore, in the perinatal period, IgA1 subclass, associated with responses to protein antigens, predominates in mucosal secretions, but IgA2 subclass increases rapidly after birth by 6 months of age to approach adult proportions. This pattern may also reflect postnatal changes in the type and load of antigenic exposure, in particular to polysaccharide antigens [22]. Interestingly, in preterm infants sIgA appears in secretions at a similar chronological age as in full-term infants although its concentrations may be significantly lower until the eighth month of life, as reported by Kuitonen and Savilahti [23]. However, in contrast to these data, Seidel et al.

[24] demonstrated comparable salivary IgA levels in preterm and full-term infants, suggesting that the development of the oral mucosal immunocompetence in preterm infants is well established within the first 9 months of life. In preschool children, the developmental profile of mucosal immunity depends on the degree of antigenic challenge they experience as well as on the exposure to hazardous environmental agents, such as tobacco smoke [25].

4. The Phenomenon of Mucosal Tolerance

4.1. Induction of Tolerance. In parallel to local defense mechanisms which protect against invading pathogens, the mucosal immune system has developed specialized regulatory and anti-inflammatory mechanisms for eliminating or tolerating harmless food and airborne antigens as well as commensal microorganisms. Mucosal tolerance induction is, therefore, an active process and is seen as preferential the Th2 skewed immune response and the downregulation of Th1 cell-mediated delayed type hypersensitivity and antibody production. These complex regulatory mechanisms include clonal deletion of T cells, clonal anergy, antigen-driven immunosuppression as well as active inhibition by coinhibitory receptors [26]. Many different CD4+ T regulatory (Treg) cell subsets have been identified capable of inhibiting the responses of effector T cells. Thymus-derived CD4+CD25+ Foxp3 (forkhead box protein 3)+Treg cells play a fundamental role in maintaining self-tolerance and preventing autoimmunization as well as contributing to tolerance of nonself antigens by the inhibition of immune responses directed at commensal bacteria in the intestine [27]. Mucosal Foxp3+ cells have been identified in the small and large intestinal mucosa as early as 23 weeks of gestational age, indicating a potential for intestinal immune regulation immediately after birth [28]. In contrast to thymus-derived Treg cells, adaptive Treg cells, which are peripherally induced after feeding protein, are essential for mucosal tolerance. These include TGF- β - (transforming growth factor β -) producing Th3 cells, type 1 T regulatory cells (Tr1) which produce IL-10 as well as Foxp3+Treg cells. The active suppressive mechanisms may also induce a “bystander effect” in that suppressive cytokines released by regulatory T cells in an antigen-specific pattern may also suppress ongoing immune response to an unrelated but anatomically colocalized antigen [29].

It is worth of note that the term “mucosal tolerance” is widely used to describe tolerance induction occurring in the intestinal MALT (mucosa-associated lymphoid tissue), represented by B cell follicles and M cell containing lymphoid epithelium, where the uptaken antigens are passed to APC (antigen-presenting cells), such as dendritic cells, macrophages, and B cells. However, in contrast to the intestine, the oral mucosa lacks inductive site represented by MALT and most likely local organized lymphoid tissue and regional lymph nodes play a role in the induction of oral mucosal tolerance [26].

Dendritic cells, the most important components orchestrating the mucosal tolerance in the gastrointestinal tract,

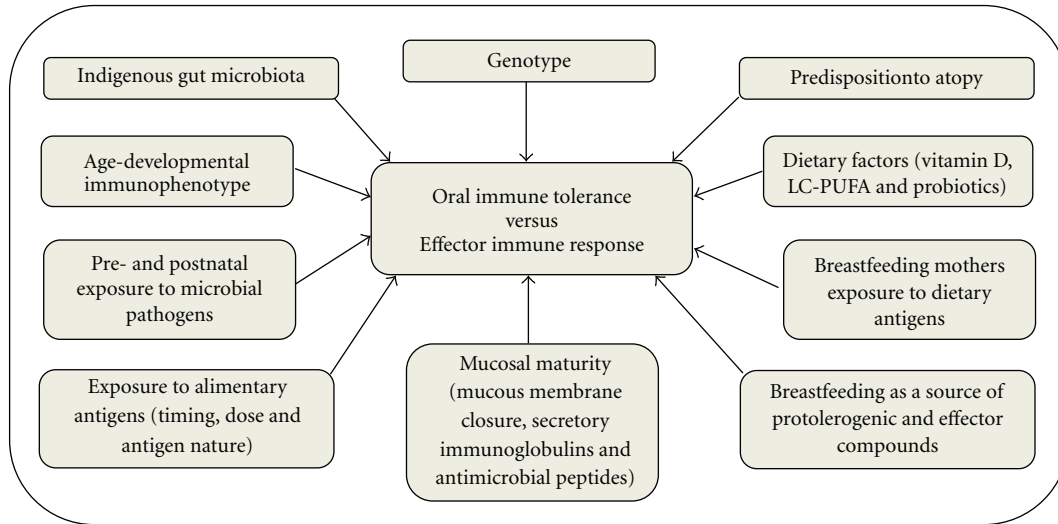


FIGURE 1: Exo- and endogenous biological factors determining mucosal immune response profile in childhood.

have an intrinsic noninflammatory activation state and a rich repertoire of receptors expressed by these cells, such as high-affinity receptor for IgE (FcεRI), high- and low-affinity receptors for IgG (FcγRI and FcγRII, resp.), Toll-like receptors (TLR)2, and TLR4 and LPS (lipopolysaccharide) receptor CD14, are of crucial importance in the induction of antigen-specific regulatory T cells. Furthermore, several factors, such as the nature and dose of antigen, the frequency of its administration, age at first antigen exposure, maternal dietary exposure during pregnancy and breastfeeding, antigen transmission via breast milk, as well as genetic background and immunological status of the child influence the fine balance between tolerance and effector response [29]. Exo- and endogenous biological factors determining mucosal immune response profile in childhood are summarized in Figure 1.

4.2. Role of Breastfeeding. The newborn and infant gut is hypersensitive to proinflammatory stimuli and vulnerable to pathogens. Breastfeeding not only favors the transmission of immunocompetence from the mother to the infant, as reviewed by Chirico et al. [30], but also has immunomodulatory and anti-inflammatory properties. The dietary antigens present in breast milk coupled with immunosuppressive cytokines, such as IL-10 and TGFβ, promote tolerance to food antigens and gut microflora. It has been demonstrated in the study by Field et al. [31] that long chain polyunsaturated fatty acids in human milk alter the infant's ability to produce cytokines enhance the anti-inflammatory effect of IL-10. Soluble TNF-α (tumor necrosis factor) receptors and IL-1RA (interleukin 1 receptor antagonist) in human milk effectively inhibit inflammatory response elicited by TNF-α and IL-1, respectively [32], and IL-10 exhibits a suppressive effect on IL-8 and neutrophilic inflammation [33]. Human milk also contains hormones, such as epidermal growth factor (EGF), insulin-like growth factor (IGF), as well as

adiponectin, which modulate the immune system by the regulation of cytokine expression [20].

5. Mucosal Tolerance: An Implication for Sublingual Immunotherapy

5.1. Oral Mucosal Microenvironment. In the oral mucosa the network of resident dendritic cells (DCs) is mainly composed of the myeloid DC from the Langerhans cell (LC) subtype, expressing CD1a and CD207 antigens (HTA1 and langerin, the LC specific lectin, corresponding with the mannose-containing oligosaccharide receptor, respectively), costimulatory molecules, such as B7.1 (CD80) and B7.2 (CD86) as well as other myeloid markers, eg CD11b (a complement components receptor). These cells are also equipped with a very specific receptor repertoire, such as a high-affinity receptor for IgE (FcεRI) resulting in allergen uptake and IgE binding to specific receptors on their surfaces. Interestingly, cross-linking of FcεRI on dendritic cells results in the induction of both pro- and, most importantly, anti-inflammatory mediators, such as IL-10 [34] and indoleamine 2,3-dioxygenase (IDO) [35], which is involved in the suppression of T cell responses and tolerance. The expression of high- and low-affinity receptors for IgG containing an immunoreceptor tyrosine inhibitory motif (ITIM) enhances the induction of antigen-specific regulatory T cells, as shown by Samsom et al. on an animal model [36]. Furthermore, TLR4 ligation on the oral DC surface leads to a subsequent induction of Foxp3 expressing as well as IL10 and TGFβ producing regulatory T cells [37], which are key players in oral mucosal tolerance. These unique properties of DC to drive Treg cells differentiation relate to their being conditioned by commensal bacteria, TGFβ and IL-10, their expression of αEβ7 integrin (CD103) and retinoid acid [38].

5.2. Immunological Mechanisms of Sublingual Immunotherapy (SLIT). Multidirectional tolerogenic properties of an oral

immune response warrant antigen-specific tolerance induction. Dendritic cells in the oral mucosa, which exhibit the high affinity receptor for IgE Fc fragment, take up allergens administered in SLIT and induce specific immune responses. An increase of serum IgG4 and IgA, noninflammatory and noncomplement binding isotypes as well as reduced allergen specific IgE locally in the target organ have been noted in the occurrence of increased TGF- β and IL-10 in allergen specific peripheral blood mononuclear cells [39]. Similarly, in the clinical study comprising a group of asthmatic/rhinitis pediatric patients, reported by Eifan et al. [40] the immunological mechanisms of SLIT were associated with significant increases of TGF- β and IL-10. Furthermore, T regulatory cell function has also been demonstrated by O'Hehir et al. [41], leading to the suppression of the allergen specific effector T (CD4+CD25-CD127hi) cell proliferation and cytokine production. In a recent study of Angelini et al. [42] the downregulation of the costimulatory molecule CD86 on blood dendritic cells, increased IL-10 and decreased IL-12 production have been demonstrated in a group of ten children with allergic asthma and house dust mites sensitivity after 12 month of SLIT. These significant functional alterations of dendritic cells may contribute to decreased T cell activation and a shift toward regulatory activity.

5.3. Efficacy and Safety of SLIT in Children. In the light of the aforementioned considerations regarding developmental issues of mucosal tolerance in children as well as multiple endo- and exogenous factors which may have an important impact on its outcome, important questions arise with regard to the efficiency and safety of SLIT. In meta-analysis studies performed by several investigators [43–45], comprising of pediatric patients with allergic asthma treated with SLIT, a significant reduction in the symptom scores and the use of rescue medication as well as an improvement in lung function have been demonstrated. It has been well established that SLIT requires a high allergen dose for its efficiency to facilitate a take-up of sufficient amounts of allergens by sentinel dendritic cells within the oral mucosa or due to a lack of adjuvants by sublingual administration [46]. Even though a high dose and long courses of medication are necessary, SLIT is a safe therapeutic option for children, as has been recently reported by Ferrés et al. [47]; although, in this study, the mild and local adverse reaction rate was at 23%; however, none of the cases from the study group showed an anaphylactic reaction. Similarly, in the clinical study by Eifan et al. [40] it was demonstrated that SLIT was associated with clinical improvement and proved to be a safe mode of immunotherapy. Therefore, as has been stated by Wahn [48], it seems likely that the induction of tolerance via the sublingual route to prevent the outset of allergic asthma, even in younger children, will soon be addressed in clinical studies.

6. Concluding Remarks

Mucosal immunity is characterized by a specific maturational pattern initiated in the intrauterine fetal development

and continued during the neonatal period, and in infancy and childhood, dynamically leading to a highly specialized immune response. At mucosal sites, a subtle balance occurs between effective defense mechanisms against the invasion of harmful pathogens and triggers the limitation of effector immune reactions to food antigens and commensal flora. Important factors, such as genetic predisposition and the age of the host, pre- and postnatal exposure to antigens, as well as the properties and the dose of antigen contribute to the development of mucosal tolerance, this being the rationale of critical importance for sublingual immunotherapy. The results of hitherto prevailing clinical studies suggest the efficacy and safety of this treatment option in children, hereby opening new perspectives in pediatric allergology.

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