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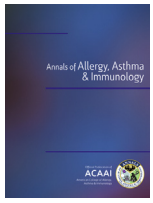

Clinical and cytokine responses to house dust mite sublingual immunotherapy

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ABSTRACT

Background: Cytokine responses accompanying sublingual immunotherapy (SLIT) responder phenotypes have not previously been reported.
Objective: To investigate clinical and cytokine responses of house dust mite (HDM) sensitive patients with allergic rhinitis receiving HDM SLIT or placebo for 2 years.
Methods: Sixty adults were randomized to receive SLIT or placebo. Clinical symptoms were measured using the Total 5 Symptom Score (TSS5) and Juniper Rhinitis Quality of Life Questionnaire. HDM specific IgE, IgG, skin prick tests, and HDM-stimulated release of interleukin (IL) 5 and interferon γ (IFN-γ) in peripheral blood mononuclear cells was studied at 0, 6, 12, and 24 months and IL-13, IL-4, and IL-10 at 0 and 24 months.
Results: A total of 32 of 39 SLIT and 16 of 21 placebo patients completed the study. There was significant clinical improvement in both the SLIT and placebo groups. Median T5SS decreased from 14.75 to 5.25 in the SLIT group ($P < .001$) and 12.7 to 6.0 in the placebo group ($P = .003$). The median quality-of-life score also decreased in the SLIT group ($P < .001$) and the placebo group ($P < .001$). A subgroup analysis of patients found a 60% or greater improvement (on the T5SS and the Juniper Rhinitis Quality of Life Questionnaire) in the good responders group and a 30% to 59% improvement or no improvement in the intermediate responders group. This subgroup analysis also found more good responders in the SLIT group (47%) compared with the placebo group (25%; $P = .07$). Significant decreases in the IL-5/IFN-γ ($P < .001$), IL-13/IFN-γ ($P < .001$), and IL-4/IFN-γ ($P = .03$) ratios were found in the combined good clinical improvement group at 24 months.
Conclusion: A good clinical response (≥60% improvement in both TSS5 and quality of life) is associated with significant decreases in IL-5, IL-13, and IL-4 relative to IFN-γ during 2 years of SLIT therapy for HDMs.

Introduction

Meta-analyses have found sublingual immunotherapy (SLIT) to be effective in adults[1–4] and children[4,5] with allergic rhinitis, but immunologic markers accompanying a responder phenotype after SLIT have not yet been reported. There are more studies of clinical efficacy in patients receiving SLIT for pollen allergies than for house dust mite (HDM) sensitivity[3,6]; thus, recent reviews[2–4] have called for more clinical trials of HDM SLIT vaccines.

SLIT for mites has been reported to be effective for rhinoconjunctivitis.[7] In a study of SLIT in rhinitis, Passalacqua et al[8] observed a significant reduction in the total symptom scores in the first year but not in the second year of treatment, although relief of nasal obstruction was significantly improved in both years.

Little is known about the natural history of disease in patients with HDM-induced persistent allergic rhinitis. It is clear from previous placebo-controlled HDM SLIT studies that some patients improve, even with placebo treatment,[3,8] but other studies have found a lack of improvement.[9] Because patients sensitive to HDM vary with respect to severity, organ involvement, and immune responses to different HDM allergens, the World Allergy Organization[10] has outlined published guidelines for conducting clinical trials using immunotherapy and emphasized the need for the determination of other outcomes of efficacy, such as quality-of-life (QOL) analysis, and the need for more intensive studies of immunologic parameters accompanying immunotherapy, which could be identified as markers of response.

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Our study investigated the association among clinical symptom scores; QOL; allergen HDM specific IgE, IgG, and interleukin (IFN-γ) secretion of IL-5, IL-13, IL-4, IL-10, and IFN-γ by HDM-stimulated peripheral blood mononuclear cells (PBMCs) in vitro in patients with rhinitis receiving double-blind, placebo-controlled, 2-year SLIT treatment with a *Dermatophagoides pteronyssinus* sublingual vaccine.

Methods

Patient Selection

Patients aged 18 to 60 years, male or female, with HDM-sensitive persistent or perennial rhinitis for at least 2 years, who manifested blocked nose, runny nose, itchy throat, and/or itchy eyes or palate were enrolled. Patients with mild asthma symptoms were not excluded. At the screening visit, patients underwent skin prick tests for 6 allergens (Der p, Bermuda grass, cat, dog, *Alternaria,* and a 5-grass mix [Stallergenes, Antony, France]). Only patients who tested positive for Der p sensitivity to HDMs as confirmed by a positive skin prick test result, manifesting as a wheal of 4 mm or greater to a *D pteronyssinus* extract (Der p 1 mix), were randomized. Patients were excluded if they had a history of seasonal allergic rhinitis due to spring or summer allergens (tree or grass pollens), were known to be polysensitive, were pregnant, required daily corticosteroid treatment for asthma, or had cardiovascular disease, hypertension, or treatment with β-blockers. The study was mono-center, double-blind, placebo controlled, and approved by the Ethics and Research Committee of the University of Cape Town.

Study Design

Sixty patients (age range, 18–60 years) were randomized (2:1) into active (39) and placebo (21) groups. The active group received Der p HDM SLIT using Staloral 300 index of reactivity units (IR)/mL (Stallergenes) and stepping up treatment with daily doses during the first 4 weeks (titrating 1 to 100 IR/mL during this period) until the maintenance dose of 10 sprays (0.1 mL per spray) of 300 IR/mL of SLIT was achieved and continued to receive treatment 3 days a week for the rest of the 2-year study period. Study participants were recruited during a 6-month period. They were allowed to continue taking their usual baseline medications of intranasal corticosteroids, antihistamines, or bronchodilators as needed.

Symptoms were scored daily by the patients using a diary card for 2 weeks before the commencement of active and placebo treatment (randomization visit) and taken as baseline. Patients were followed up, and symptom scores assessed at 2 weeks, 4 weeks, 8 weeks, 12 weeks, 6 months, 9 months, 12 months, 18 months, and 24 months The diary cards were completed daily during the first 6 weeks after randomization and on 3 days a week during the 2 weeks before the subsequent follow-up visits (8-week to 24-month visits). All patients were telephoned 2 weeks before the follow-up visits to remind them to complete their diary cards. Adherence for the study population was assessed during the first 6 weeks by summing the daily consumption of IRs.

At each visit, a physical examination was performed including careful examination of the nose and throat. The following were recorded: current rhinitis treatment, review of the diary card recordings of the Total 5 Symptom Score (T5SS; mean daily total of 5 individual graded scores for sneezing; runny nose; itchy ear, nose, or throat; ocular symptoms; and nasal congestion; each scored on a range of 0–5, with 0 indicating absent and 5 indicating intolerable; maximum score, 25), the Total Nonnasal Symptom Score (TNNSS; including headache, fatigue, concentration, and irritability scores; maximum score, 20), adverse events, and concomitant medications. Global evaluation of rhinitis symptoms by the investigator and the patient used a visual analog scoring system on a scale of 0 to 10, with 0 indicating absent and 10 indicating very severe.

The QOL was assessed with the Juniper Rhinitis Quality of Life Questionnaire.[11] The global QOL was the sum of the scores for the individual domains of activity, nasal, non–hay fever, ocular, sleep, emotional, and practical. The QOL was evaluated as part of the source notes at baseline and at 2 years in patients who completed the trial.

Blood samples were taken for lymphocyte and immunoglobulin studies at baseline, 6 months, 12 months, and 24 months. Der p specific IgE and IgG levels, as well as total IgE levels, were measured using the Pharmacia ImmunoCap System (Pharmacia, Uppsala, Sweden). Samples were batched and stored to avoid batch to batch variations in the results. Skin prick tests to Der p 1 extract were performed at the screening, 12-month, and 24-month visits.

HDM Stimulation of PBMCs

PBMCs were isolated and cultured[12] (2×10^5/200 μL of RPMI with 10% AB serum per well for 8 days) with and without HDM extract (200 μg/mL of total protein) at baseline (0), 6, 12, and 24 months. IL-5/IFN-γ release and proliferation were studied at baseline (0), 6, 12, and 24 months. Pilot experiments (1–9 days) revealed that, at this concentration of HDM, proliferation and release of IFN-γ and IL-5 was maximal at 8 days. Cytokines IL-13, IL-4, and IL-10 were also measured at 0 and 24 months. Culture supernatants (pooled from 5–7 wells per individuals) were stored at −80°C.

HDM extract in phosphate-buffered saline was prepared from lyophilized spent Der p culture (Dome/Hollister-Stier, Spokane, WA) and filter sterilized. Protein concentrations were determined using a Pierce BCA Kit (Thermo Fisher Scientific, Waltham, MA).

Proliferation was assessed in parallel cultures pulsed with titrated thymidine (1 μCi per well; 5 Ci/mmol; Amersham Pharmacia, Piscataway, NJ) after 7 days and harvested 18 hours later. Results from quadruplicate cultures are expressed as δ disintegrations per minute (HDM-stimulated proliferation minus spontaneous proliferation).

Cytokines were measured using anti–human monoclonal antibody enzyme-linked immunosorbent assay (ELISA) pairs with recombinant human cytokines as standards (BD Pharmingen, San Jose, CA). Coating and capture antibody was paired with the appropriate biotinylated detection antibody. Assays were performed essentially as described in Pharmingen's ELISA protocol except that AMDEX streptavidin–horseradish peroxidase (Amersham Pharmacia) and tetramethylbenzidine substrate (Kirkegaard & Perry Laboratories, Inc, Gaithersburg, Maryland) were used. Color reactions were amplified by stopping with phosphoric acid and were read at 450 nm (reference filter, 540 nm). The sensitivity of the ELISAs was 15 pg/mL for IL-5; 2 pg/mL for IFN-γ, IL-13, and IL-10; and 0.2 pg/mL for IL-4. Cytokine levels below the sensitivity of the assays were assigned values of 7 pg/mL (IL-5), 1 pg/mL (IFN-γ, IL-13, and IL-10), and 0.1 pg/mL (IL-4) for statistical purposes.

Written informed consent was obtained from each patient according to International Conference on Harmonisation/Good Clinical Practice Declaration of Helsinki guidelines.

Statistical Analysis

Because the clinical data and the PBMC proliferation and cytokine release data were not normally distributed, nonparametric tests were used. The Wilcoxon matched pairs test was used to compare 2 dependent groups and the Mann-Whitney test for 2 independent groups. The Kruskal-Wallis test was used to compare 3 groups (multiple comparisons 2-tailed *P* values). The Spearman rank order test was used to assess correlations. The percentage difference test was use to determine the difference between 2 proportions. A STATISTICA program, release 9 (StatSoft, Inc, Tulsa, OK), was used. $P < .05$ were considered statistically significant.