Intralymphatic allergen-specific immunotherapy: An effective and safe alternative treatment route for pollen-induced allergic rhinitis

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Background: Allergen-specific immunotherapy is the only causative treatment of IgE-mediated allergic disorders. The most common administration route is subcutaneous, which may necessitate more than 50 allergen injections during 3 to 5 years. Recent evidence suggests that direct intralymphatic injections could yield faster beneficial results with considerably lower allergen doses and markedly reduced numbers of injections.

Objective: To evaluate the effects of intralymphatic allergen-specific immunotherapy in pollen-allergic patients.

Methods: In an open pilot investigation followed by a double-blind, placebo-controlled study, patients with allergic rhinitis were treated with 3 intralymphatic inguinal injections of ALK Alutard (containing 1000 SQ-U birch pollen or grass pollen) or placebo (ALK diluent). Clinical pre- and posttreatment parameters were assessed, the inflammatory cell content in nasal lavage fluids estimated, and the activation pattern of peripheral T cells described.

Results: All patients tolerated the intralymphatic immunotherapy (ILIT) treatment well, and the injections did not elicit any severe adverse event. Patients receiving active treatment displayed an initial increase in allergen-specific IgE level and peripheral T-cell activation. A clinical improvement in nasal allergic symptoms upon challenge was recorded along with a decreased inflammatory response in the nose. In addition, these patients reported an improvement in their seasonal allergic disease. No such changes were seen in the placebo group.

Conclusions: Although this study is based on a limited number of patients, ILIT with grass-pollen or birch-pollen extracts appears to reduce nasal allergic symptoms without causing any safety problems. Hence, ILIT might constitute a less time-consuming and more cost-effective alternative to conventional subcutaneous allergen-specific immunotherapy. (J Allergy Clin Immunol 2013;131:412-20.)

Key words: *Allergen-specific immunotherapy, allergic rhinitis, intralymphatic immunotherapy, seasonal allergic rhinitis*

Abbreviations used
ILIT: Intralymphatic immunotherapy
MFI: Mean fluorescence intensity
NAL: Nasal lavage
NPT: Nasal provocation test
SCIT: Subcutaneous immunotherapy
SPT: Skin prick test

Allergic rhinitis is a health problem that causes worldwide disability. It affects social life, sleep, school, and work.[1,2] Allergen-specific immunotherapy is the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate symptoms after exposure to the causative allergen. This is the only treatment that diminishes symptoms, improves quality of life, prevents new sensitizations, and reduces the development of asthma in patients suffering from allergic rhinitis.[3-5] The dominating route for the administration of allergen-specific immunotherapy is subcutaneous injections, and although the maintenance dose can be reached through various regimens, the current treatment protocol requires numerous immunotherapeutic injections and may take several years to complete.[6,7] Despite its proven benefits, only 5% of allergic patients with insufficient symptom control undergo subcutaneous immunotherapy (SCIT)[8,9] and there is a considerable interest in finding alternative routes to shorten the duration of the treatment and to increase the attractiveness of the therapy.

When antigens are administered subcutaneously, only small fractions of the antigenic epitopes reach the lymph nodes.[10,11] As immune responses are initiated in secondary lymphoid organs, it seems reasonable that the direct administration of antigens into the highly immunocompetent environment of the lymph nodes induces greater immunogenicity.[12,13] Animal studies have revealed that vaccines can be given in lower concentrations and with a reduced number of injections when administered directly into the lymph node.[14-16] In mice, intralymphatic immunization has been demonstrated to cause enhanced allergen-specific IgG and T-cell responses when compared with subcutaneous injections. Moreover, only the intralymphatic immunization stimulated the production of the T_H1-dependent subclass IgG2a, which is associated with improved protection against allergen-induced anaphylaxis.[11] Three clinical trials have shown a regime of only 3 intralymphatic injections to be effective in induction of tolerance against grass pollen, cat dander extract, and bee venom.[17-19] The present double-blind placebo-controlled study was designed to further assess the effect of intralymphatic immunotherapy (ILIT) on allergen-related symptoms and nasal inflammation in patients with pollen-induced seasonal allergic rhinitis.

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METHODS

Study population

Study subjects (28 in total) were recruited among patients at the Allergy Department of Skåne University Hospital, Malmö, Sweden. They all had a history of moderate to severe birch-pollen/grass-pollen-induced rhinoconjunctivitis with symptoms including itchy nose and eyes, sneezing, nasal congestion, and secretion. The diagnosis was verified by positive skin prick test (SPT) results, presence of serum-specific IgE antibodies toward birch and/or grass (minimum 0.35 kU/L), and positive nasal provocation test (NPT) results. None of the patients suffered from seasonal asthma, but 7 subjects reported exercise-induced asthma with mild to moderate symptoms.

General contraindications were pregnancy or nursing, wish for pregnancy, autoimmune and collagen disease, cardiovascular disease, current persistent asthma, upper airway disease (nonallergic sinusitis, nasal polyps), chronic obstructive and restrictive lung disease, hepatic and renal disease, cancer, previous immune- or chemotherapy, major metabolic disease, alcohol or drug abuse, mental incapability of coping with the study, or medication with a possible side effect of interfering with the immune response. The local ethics committee approved the study, and all participants, those undergoing ILIT as well as those undergoing SCIT, gave their written informed consent.

Study design

Six patients (3 men and 3 women) and 15 additional patients (10 men and 5 women, Table I) were recruited to participate in an open pilot study and a randomized double-blind, placebo-controlled study of intralymphatic grass-pollen and birch-pollen immunotherapy, respectively (Fig 1).

At the first visit, patient eligibility was determined, SPTs and NPTs were performed, blood was sampled, and nasal lavage (NAL) fluids were collected. After approximately 1 week, patients in the double-blind study were randomly allocated (1:1, based on recruitment order) to receive either placebo (n = 8) or active (n = 7) intralymphatic treatment. At visits 2 to 4, the study subjects received three 0.1-mL injections with either placebo (allergen diluents without aluminum hydroxide; ALK-Abelló, Hørsholm, Denmark) or 1000 SQ-U of a standardized, aluminum hydroxide-adsorbed, depot birch-pollen or grass-pollen vaccine[20] (Alutard; ALK-Abelló) with approximately 4-week intervals. On the basis of the outcome of the allergy tests, patients were challenged and vaccinated with either birch or grass. The vaccines used were packed and blinded by staff with no connection to the study, and thus both patients and physicians were blind. At visit 5 (~4 weeks after the last injection) and at visit 6 (after the next consecutive pollen season), patients were evaluated by means of SPTs and NPTs, NAL fluids, and immunological parameters and the subjects were asked to answer a questionnaire regarding their seasonal allergic symptoms as compared with the previous pollen season. All patients completed the treatment, the emergency envelopes were unbroken, and the study remained double-blind.

Immunization protocol for SCIT patients

As open controls, 7 patients were subcutaneously injected with incremental doses of birch-pollen vaccine (Alutard; ALK-Abelló) over 14 weeks (week 1, 50 SQ-U; week 2, 100 SQ-U; week 3, 200 SQ-U; week 4, 400 SQ-U; week 5, 800 SQ-U; week 6, 1,500 SQ-U; week 7, 3,000 SQ-U; week 8, 6,000 SQ-U; week 9, 10,000 SQ-U; week 10, 20,000 SQ-U; week 11, 40,000 SQ-U; week 12, 60,000 SQ-U; week 13, 80,000 SQ-U; and week 14, 100,000 SQ-U). Thereafter, maintenance injections (100,000 SQ-U) were given every 6 to 8 weeks over 3 years. These patients finished treatment in 2010 and were not part of the initial study. However, they were evaluated by the same parameters as for the patients receiving intralymphatic injections but for the NPTs. In addition, another 10 patients receiving subcutaneous injections were asked to score the discomfort associated with the injection by using an arbitrary scale ranging from 0 to 10 (0 = completely painless and 10 = worst pain ever).

Intralymphatic injections

Using ultrasound guidance and a 25-gauge needle, a superficial inguinal lymph node in either the left or the right groin was aseptically injected for 30 seconds. The same side was targeted during all 3 injections. Aspirations were made before the injections to avoid inadvertent intravascular administration. The peak expiratory flow was measured before and after each injection, and all patients were monitored at the ward for no less than 60 minutes after each injection. All signs of local and/or systemic reactions in conjunction to the injections were recorded by the trial staff, and patients were then asked to record and report all indications of late reactions during the following 24 hours. In addition, patients were requested to estimate the pain associated with the injection by using an arbitrary scale ranging from 0 to 10 (0 = completely painless and 10 = worst pain ever) and relate it to the discomfort associated with a venous puncture (more painful, same pain, less painful, no pain at all).

TABLE I. Baseline characteristics of patients included in the placebo study

	Active ILIT	Placebo ILIT
No. of participants	7	8
Age (y), median (range)	34 (22-51)	29 (19-53)
Gender (male vs female)	4:3	6:2
Allergen-specific IgE (kU/L)	3.5-50	0.35-50
Birch vs grass vaccination	2:5	3:5
No. of monosentisitized subjects	2	3
No. of patients with exercise-induced asthma	2	3

Skin prick tests

SPTs were performed with a standard panel of 11 common airborne allergens (ALK-Abéllo) including pollen (birch, timothy, mugworth, and ragweed), house-dust mite (*Dermatophagoides. pteronyssimus* and *D farinae*), molds (*Cladosporium* and *Alternaria*), and animal allergens (cat, dog, and horse). SPTs were administered on the volar side on forearms, with saline buffer as negative control and histamine chloride (10 mg/mL) as positive control. All patients presented a wheal reaction diameter of more than 3 mm toward birch and/or grass.

Nasal provocations and symptom scores

Before and after the ILIT treatment, all patients were challenged with 10,000 SQ-U birch-pollen or grass-pollen extract per nostril.[21,22] The occurrence and severity of nasal allergic symptoms (itching, secretion, and congestion) were self-recorded during the first 30 minutes after each challenge by using a scale ranging from 0 to 3 (0 = no; 1 = mild; 2 = moderate; 3 = severe symptoms).[23] The combination of these scores is referred to as the total symptom score, which could maximally reach 9 points.

Blood sampling

Venous blood was obtained from participants at baseline, after completion of the treatment, and at the end of the upcoming pollen season. Blood collected in tubes containing EDTA (Vacuette 454209) was used for total and differential leukocyte counts in a Coulter LH750/GenS cell counter (Beckman Coulter, Marseille, France), and allergen-specific IgE and IgG_4 levels were determined by using the Phadia CAP system (Uppsala, Sweden). Blood collected in tubes containing buffered trisodium citrate solution (BD Vacutainer 367704) was used for flow cytometry analyses and *in vitro* experiments.

NAL fluids

Before NPTs were performed, NAL fluids were collected as previously described.[21,22,24] Briefly, after clearing excess mucus by exsufflation, a sterile saline solution was aerosolized into the nostrils and nasal fluids were passively collected in a test tube until 7 mL was recovered. The number of living cells (leukocytes and epithelial cells) was determined in a Burker chamber by using