Immunologic response to administration of standardized dog allergen extract at differing doses

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Background: The immunologic response to immunotherapy with dog extract is not well characterized.
Objective: The purpose of this study was to examine the immunologic response to 3 doses of dog extract expressed as their Can f 1 content.
Methods: Cluster immunotherapy was administered to 28 patients with dog allergy who were randomly assigned to 1 of 4 treatment arms: placebo or acetone-precipitated extract containing 0.6 μg, 3.0 μg, or 15.0 μg Can f 1 per 0.5 mL maintenance dose. Studies included titrated skin prick tests, the late cutaneous response, titrated nasal challenge with dog extract, and serum allergen-specific IgE and IgG_4. Dog allergen-stimulated lymphocyte proliferation was performed with measurement of secreted cytokines by ELISA and of intracellular cytokines by flow cytometry.
Results: There was a significant dose-dependent response in suppression of titrated skin prick tests and suppression of the late cutaneous response. There was a significant increase from baseline in dog-specific IgG_4 in both the high-dose and low-dose groups and a dose-dependent suppression of secreted TNF-α and increase in secreted TGF-β. There was a dose-dependent trend in suppression of secreted IL-4 with a significant decrease from baseline in the high-dose group. There were no significant changes in symptom scores; lymphocyte proliferation; secreted IFN-γ, IL-10, or IL-5; or intracellular cytokine production.
Conclusion: The dose-response in immunologic parameters after immunotherapy with dog extract is similar to that previously demonstrated with cat extract.
Clinical implications: The greatest and most consistent response is seen with a dose containing 15 μg Can f 1.
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Key words: *Immunotherapy, acetone-precipitated dog extract, Can f 1, cytokine, allergen-specific IgE, allergen-specific IgG_4, skin prick test, late cutaneous response*

Allergen immunotherapy is an effective form of treatment for both allergic rhinitis and allergic bronchial asthma.[1] High doses of standardized extracts have been proven effective in treating patients with sensitivity to ragweed, timothy grass, house dust mites, and cat.[1] In 2 previous studies examining the immunologic response to maintenance doses after 5 weeks and after 1 year and 5 weeks of immunotherapy using cat extract, the dose of cat dander extract containing 15 μg Fel d 1 was shown to be most effective.[2,3] The second study demonstrated that the dose-response at 5 weeks is the same as that observed after 5 weeks and 1 year of maintenance immunotherapy, thus providing rationale for a 5-week study examining the immunologic response to dog extract.[3]

To date, few studies have examined the effectiveness of immunotherapy with dog extract. Although dog extracts are not standardized in the United States, the major dog allergen, Can f 1, has been isolated, purified, and expressed.[4] It is therefore now possible to examine the dose-response to dog allergen extracts expressed as potency in content of major allergen despite lack of standardization. Most dog allergen extracts are reported to contain roughly 5 μg Can f 1 per milliliter concentrated extract.[1] However, the acetone-precipitated (AP) dog extract 1:100 wt/vol produced by Hollister-Stier Laboratories (Spokane, Wash) contains more than 100 μg Can f 1 per mL and has been reported to contain as high as 165 μg/mL according the manufacturer. A recent study at National Jewish Medical and Research Center confirmed the greater potency of this extract, comparing it to conventional dog extracts by skin testing.[5] The purpose of this study was to examine those parameters that showed significant changes in the previous cat immunotherapy studies in a cohort of subjects receiving cluster immunotherapy with the AP dog extract. This study addresses the question whether immunotherapy with dog extract follows the same dose-response pattern previously observed with immunotherapy with cat extract.

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Abbreviations used
AP: Acetone-precipitated
cpm: Counts per minute
SPT: Skin prick test

METHODS

Subjects

Adult subjects sensitized to dogs were recruited. Each of the subjects had a history of rhinitis symptoms with or without asthma symptoms on exposure to dogs or had perennial rhinitis symptoms and close exposure to dogs. Skin tests were performed using the prick method with a DuoTip (Lincoln Diagnostics, Decatur, Ill) using AP dog extract 1:100 wt/vol (Hollister-Stier Laboratories). Skin wheals were required to be ≥5 mm in diameter. Each subject had an FEV_1 ≥ 80% predicted, and no subject had a history of persistent asthma or regular use of control medication for asthma. No subject had received immunotherapy with dog or other allergen extracts during the 5 years before the study. Antihistamines were withheld 7 days before skin testing or nasal challenge studies. Corticosteroid nasal sprays were withheld 30 days before and throughout the study. Subjects were excluded if they were pregnant or not using appropriate birth control or if they were taking β-blockers or monoamine oxidase inhibitors. The Institutional Review Board of National Jewish Medical and Research Center approved the study. All subjects signed approved consent forms before participating.

Study design

Twenty-eight subjects were randomly assigned to 1 of 4 treatment arms: placebo or 1 of 3 extracts containing dog antigen prepared from the 1:100 wt/vol extract of AP dog (Hollister-Stier Laboratories), which contained approximately 161 μg/mL Can f 1 (information provided by the extract manufacturer). At the maintenance injection of 0.5 mL of the active extract, subjects received a dose of dog extract containing Can f 1 0.6 μg, 3.0 μg, or 15 μg diluted in albumin saline solution (Hollister-Stier Laboratories). The concentrations of extract in the 3 active treatment groups are shown in Table I. All placebo, low-dose, and medium-dose vials were colored with caramelized sugar (prepared by the National Jewish Medical and Research Center pharmacy) and contained small amounts of histamine to mimic the color and reaction of the equivalent high-dose vial. Each subject received 0.5 mL as a maintenance injection from vial 1.

Injections were administered by a cluster protocol over a period of 4 weeks. Injections were administered twice weekly for 8 visits. Progression was accomplished with 3 injections at 30-minute intervals for the first 3 visits, 2 injections at 30-minute intervals for the next 4 visits, and a single injection at the last visit. An additional maintenance injection was given 1 week later (Table II). Subjects remained in the area for 60 minutes after the last of multiple injections and 30 minutes after single injections. This schedule was altered, if necessary, depending on subject tolerance. All immunotherapy was administered in the Clinical Research Unit by a registered nurse or medical doctor. Subjects received fexofenadine 180 mg and zafirlukast 20 mg approximately 2 hours before each injection visit to reduce the risk of local and systemic reaction. All injections were performed in a double-blind fashion. Each subject underwent immunologic testing including nasal challenge, skin testing, and laboratory analysis before immunotherapy and again within 7 days after receiving the first weekly maintenance injection.

TABLE I. Immunotherapy dosing schedule (μg Can f 1/mL)

Vial #	High dose	Medium dose	Low dose	Placebo
4	0.03	0.006	0.0012	0
3	0.3	0.06	0.012	0
2	3.0	0.6	0.12	0
1	30	6	1.2	0

Titrated nasal challenge

Nasal challenge with dog allergen extract was performed before and after 5 weeks of cluster immunotherapy with AP dog extract (Hollister-Stier Laboratories) using the method previously used.[3] Before starting the allergen challenge, 3 saline lavages were performed to remove accumulated secretions. The nasal challenges were performed by spraying .1 mL solution into both nostrils at 10-minute intervals (metered pump nasal spray bottles; PharmaSource International, Inc, Centennial, Colo). The first dose contained saline, and subsequent doses contained AP dog extract in increasing ½ log concentrations from 1:3,000,000 to 1:100 wt/vol. Patients were asked to score their symptoms on the basis of the scoring system by Bousquet et al[6] 10 minutes after each dose until a score of 5 was reached. Briefly, symptoms such as sneezing, rhinorrhea, nasal congestion, pruritus, and conjunctivitis were assigned a number between 0 and 3. Patients were to be asked to rate their symptoms at each incremental dose until a noncumulative score of 5 was achieved. The same dose at which a score of 5 was produced before immunotherapy was to be administered 1 week after the completion of immunotherapy.

Titrated skin prick tests

Titrated skin prick tests (SPTs) were performed in duplicate on the patient's back using the same dilutions of dog allergen extract as used in the nasal challenges. Testing was conducted with increasing concentrations until a mean wheal of 5 mm was achieved, with at least 1 dilution above and 1 below the dilution that produced the 5-mm wheal.

Late-phase cutaneous response

Intradermal tests with the dog allergen were performed using dilutions of the dog extract used for the nasal challenges, with the starting dose a 1:10 dilution of the dose that produced the 5-mm wheal on skin prick testing. The injected dose was increased until a wheal of ≥15 mm in diameter was achieved. The late cutaneous reaction was read after 6 hours. On subsequent evaluation, the same dose of extract was used that initially produced the 15-mm wheal.

Dog-specific immunoglobulin measurements

Serum was obtained before and after completion of immunotherapy. Undiluted samples were analyzed for allergen-specific IgE by means of Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Dog-specific IgG_4 was assayed via the Pharmacia CAP system-specific IgG_4 FEIA (Pharmacia Diagnostics) using serum diluted 1:1000.

Proliferation assay

PBMCs were isolated by means of Ficoll-Hypaque density gradient centrifugation from heparinized venous blood. Cells were washed and resuspended at 1×10^6 cells/mL in RPMI supplemented (Cellgro, Herndon, Va) with 5% AB human sera, penicillin-streptomycin, and L-glutamine. Triplicate wells containing 1×10^5 PBMCs were incubated with dog extract containing 1, 5, or 10 μg/mL Can f 1 in a 37°C, 5% CO_2 incubator for 6 days. Cultures were then pulsed with tritiated thymidine for 6 hours and harvested onto glass fiber disks. The mean counts per minute (cpm) were determined. Stimulation indices (mean cpm Can f 1/mean cpm media alone) were calculated.

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