

Treatment of Cat Allergy with T-cell Reactive Peptides

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We induced in allergic humans the counterpart of murine experimental T-cell tolerance. T-cell lines from cat-allergic humans were used to map T-cell epitopes for the principal allergen of cat dander, Fel d 1. Two peptides of 27 amino acids each were synthesized to contain the dominant epitopes (ALLERVAX® CAT). After a safety trial, we carried out a blinded study of the dose required for efficacy. We randomly divided 95 cat-sensitive patients into placebo, 7.5 µg, 75 µg, and 750 µg groups. Patients received a subcutaneous injection weekly for 4 wk. Before and after treatment, patients were exposed in a room inhabited by live cats and scored by nose and lung symptoms. Baseline nasal and lung scores ($\pm$ SEM) were 6.2 ± 0.56 and 5.4 ± 0.73 in the 750 µg group; 7.8 ± 0.53 and 4.7 ± 0.68 in the placebo group. Six weeks after treatment, scores adjusted for baseline differences were reduced in the 750 µg group: -2.3 ± 4.9 and -2.3 ± 0.59 compared with -0.84 ± 0.50 and -0.85 ± 0.62 in the placebo group. The 75 µg group showed intermediate effects and the 7.5 µg group no effect. Linear trend analysis indicated a significant dose response effect: $p = 0.05$ for nose and 0.03 for lung symptoms. Allergic side effects occurred an hour or more after the first 750 µg dose in 16 of 24 patients but required little or no treatment with one exception. T-cell reactive treatment peptides safely improved allergic responses to cats. **Norman PS, Ohman JL, Jr., Long AA, Creticos PS, Gefter MA, Shaked Z, Wood RA, Eggleston PA, Hafner KB, Rao P, Lichtenstein LM, Jones NH, Nicodemus CF. Treatment of cat allergy with T-cell reactive peptides.**

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In hay fever and asthma, T cells direct the immunologic responses that cause symptoms after exposure to allergenic proteins in pollens and animal danders. Allergen-specific Th2 cells are essential to the development of the IgE antibodies that fix to the mast cells that mediate rapidly occurring responses (1). The same T cells also secrete cytokines that help initiate a later eosinophilic inflammation (2).

T cells and antibodies interact with different ligands on allergens. IgE antibodies attach to complex B cell epitopes that require intact tertiary structures. T cell receptors, on the other hand, respond to short peptides from the allergen imbedded in surface mixed histocompatibility molecules on antigen presenting cells (APC). T cell activation occurs when presentation is accompanied by pro inflammatory second signals (3). These events usually engender immunity against foreign antigens, but can cause pathologic immune reactions such as allergies or autoimmune diseases.

Human T cells presented receptor specific peptides on APC in the absence of costimulatory signals become non-responsive

(4). Furthermore, mice administered peptides that represent major T cell epitopes of cat allergen become tolerant to the whole antigen as well (5). The effect persists for three to five months (G. Sunshine, personal communication). Tolerized mice show less of the eosinophilic inflammation of the lung that occurs when immunized control mice are exposed to aerosolized cat antigen (M. Lugman, personal communication). In allergic patients, T-cell non-responsiveness might therefore be induced by peptides representing T-cell epitopes of allergens. Selecting peptides to avoid protein tertiary structures recognized by IgE antibodies minimizes the risk of immediate hypersensitivity.

To test the hypothesis, cat allergy was chosen because it is caused mainly by the single protein, Fel d 1, found in cat epithelium and saliva. Fel d 1 was cloned and sequenced, and regions recognized by T-cell lines from more than 100 cat-sensitive patients were mapped. Two 27 amino acid sequences from chain I were selected as containing multiple dominant T-cell epitopes and called IPC-1 and IPC-2 (Figure 1) (5, 6). Synthetic peptides were produced and formulated in equal proportions (ALLERVAX® CAT; Immunologic Pharmaceutical Corporation, Waltham, MA). Unlike intact allergen, the peptides caused histamine release from basophils in $< 1\%$ of cat allergic patients. In an *in vitro* study, a human T-cell clone specific for IPC-2 was exposed to a high dose of IPC-2. Upon subsequent exposure to antigen in the presence of APC, these cells were found to be unresponsive (R. Garman, personal communication).

A low-risk immunologic treatment that requires few injections and that has effects lasting weeks or months would be a distinct

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Primary Sequence of Fel d 1

Fel d 1 Chain 1

10 20 30 40 50 60 70
E I C P A V K R D V D L F L T G T P D E Y V E Q V A Q Y K A L P V V L E N A R I L K N C V D A K M T E E D K E N A L S L L D K I Y T S P L C

Fel d 1 Chain 2

10 20 30 40 50 60 70 80 90
V K M A E T C P I F Y D V F F A V A N G N E L L D L S L T K V N A T E P E R T A M K K I Q D C Y V E N G L I S R V L D G L V M I A I N E * * Y C M G E A V Q N T V E D L K L N T L G R

Figure 1. Primary sequence of Fel d 1 showing sequences of trial tolerogenic peptides.

step forward in the treatment of diseases caused by unwanted immunologic responses. For treatment of allergies, peptides have the advantage of being defined chemical substances that do not require the cumbersome biological standardization required of crude extracts currently in use for immunotherapy.

In a preliminary safety study of 16 cat-sensitive patients, we administered 5 increasing doses (one each week) up to 1,500 µg of each peptide. The final dose represents 500 nmoles of each peptide, which is 100-fold the equivalent of the usual maintenance dose of Fel d 1 in traditional allergen immunotherapy (7, 8). Immediate allergic side effects were not observed. Following this experience, we organized a study exploring the efficacy of the treatment in a range of doses.

METHODS

Treatment Peptides

The treatment material was lyophilized in vials that, when reconstituted with water for injection, contained 750 µg/mL of each peptide with sodium phosphate and mannitol. Vials were reconstituted within an hour before administration and diluted with saline to give the proper dose in 1.0 mL. To preserve blinding, vials were prepared and labeled with a reference number by a technician who never saw the patients. The placebo consisted of lyophilized excipients.

Study Design

We studied cat-allergic patients in a double-blind, placebo-controlled parallel study at two centers. After skin tests and baseline exposure in a cat room to establish eligibility, four groups received at random 7.5

µg, 75 µg, or 750 µg of treatment peptides or placebo. We gave a subcutaneous injection of the designated dose on each of 4 wk. After each dose, the patient kept a diary of any symptoms that occurred post injection and any medication taken. The primary efficacy variables were nose, lung, and symptom scores during 60-min exposure in a room inhabited by live cats. Additional variables were immediate responses to skin tests with cat extract, serum IgE and IgG antibodies to Fel d 1 and to treatment peptides, and proliferation assays on cultures of T cells from peripheral blood. Evaluations were done at baseline and repeated 1 and 6 wk after the last dose. Adverse experiences were recorded.

Patients

Subjects for study responded to advertisements for cat-allergic individuals. If volunteers reported asthma or rhinitis symptoms on exposure to cats in the last year and showed a positive prick test reaction to cat hair and dander extract containing 3.3 or less FDA Units/mL of Fel d 1, they received a cat room challenge. The response in the cat room had to consist of an increase in at least two symptoms by two points or more, or an increase in one symptom by two points accompanied by a 15% drop in FEV₁ from baseline. Exclusion criteria were: (1) any contraindication to immunotherapy (such as beta blocker therapy, major medical illness); (2) use of a tricyclic antidepressants, doxepin, astemizole, or monoamine oxidase inhibitors within 6 wk of pre-treatment evaluations; (3) women of child bearing potential unless using effective continuous birth control; (4) FEV₁ of less than 70% predicted after bronchodilation; (5) prior immunotherapy with cat extract or house dust extract; (6) severe or chronic unstable asthma; (7) previous administration of treatment peptides; (8) cats present in the household or as part of a regular environmental exposure.

Patients were stratified into those with ≥ 15% fall in FEV₁ and those with < 15% fall in FEV₁ during the control cat room exposure (Table

TABLE 1
DEMOGRAPHICS

	Placebo	Treatment Peptides			p Value
		7.5 µg	75 µg	750 µg	
n (Intent to treat)	26	23	22	24	
≥ 15% Δ FEV ₁ in cat room	14	12	11	13	
Male/Female	12/14	11/12	10/12	9/15	0.9
White/Black/Asian	22/2/2	20/3/0	19/2/1	23/1/0	0.6
Age	31.1 ± 7.7*	29.9 ± 5.4	33.0 ± 6.9	35.1 ± 8.0	0.08
Height, cm	170.6 ± 7.8	168.1 ± 10.5	170.4 ± 7.5	169.9 ± 11.2	0.7
Weight, kg	73.2 ± 17.0	74.2 ± 15.7	74.1 ± 16.3	70.9 ± 13.0	0.9
Cat Ext. Prick Test, Σ _w	1.19 ± 2.3	12.5 ± 4.3	12.4 ± 3.5	1.19 ± 2.8	
IgE to Fel d 1†	1.19 ± 0.70 (25/1)	1.14 ± 0.74 (23/0)	1.31 ± 0.80 (21/1)	1.18 ± 0.86 (21/3)	
IgG to Fel d 1	0.89 ± 0.38 (26/0)	1.00 ± 0.42 (23/0)	0.86 ± 0.35 (22/0)	0.91 ± 0.4 (24/0)	
IgE to IPC-1	(0/26)	(0/22)	(0/22)	(0/24)	
IgG to IPC-1	0.38 ± 0.52 (11/15)	0.32 ± 0.36 (6/17)	0.20 ± 0.39 (2/20)	0.18 ± 0.27 (4/20)	
IgE to IPC-2	(0/26)	(1/22)	(0/22)	(0/24)‡	
IgG to IPC-2	0.07 ± 0.22 (2/24)	(0/23)	(0/22)	(0/24)	

* Mean ± SD.

† Antibody determinations are listed as mean OD ± SD at sample dilutions of 1/3 for IgE and 1/50 for IgG. The numbers in parentheses list numbers of patients with/without measurable antibody responses pretreatment.

‡ One person had IgE to peptide in a preliminary test that could not be confirmed in a second test.

1). Equal numbers were assigned to each treatment group. None of the patients were on continuing medication for cat allergy, because they preferred to avoid cats.

Skin Tests

We carried out skin prick tests and intradermal tests with cat hair and dander extract (ALK Laboratories, Inc., Milford CT). The skin prick test concentration was 10 FDA Units/ml of Fel d 1. An intradermal titration with threefold dilutions of the extract was carried out to establish a dose response curve in order to calculate an endpoint. Wheal and erythema responses were measured in two diameters and summed. The intradermal end point was a sum erythema of 50 mm. Each patient was given a prick test with his randomized study medication of day of the first injection. Post treatment, skin prick tests were carried out with 10-fold dilutions of the peptide mixture to detect sensitization. Prick tests were considered positive if they induced a wheal diameter 3 mm greater than diluent.

Antibody Measurements

We measured serum IgE and IgG antibodies to Fel d 1, IPC-1, and IPC-2 with an enzyme linked immunosorbent assay (ELISA) (9). We recorded a positive response to peptide if the mean optical density (O.D.) of duplicate wells was greater than 2 SD above the mean value obtained from non-cat allergic plasma.

T Cell Proliferation

Lymphocytes were separated by Ficoll Hypaque gradient and cultured in the presence of recombinant chain 1 and chain 2 of Fel d 1. At 6 days, viable cells were separated and further cultured in the presence of human IL-2 and recombinant human IL-4. The cells were expanded with addition of cytokines, rested, and then assayed for peptide specific proliferation to a panel of Fel d 1 recombinant chain 1 and chain 2 and overlapping peptides of the entire sequence of Fel d 1. Proliferation in individual microtiter wells was assessed by ^3H thymidine incorporation on the third day.

Cat Room Exposure

We exposed patients to two live cats who continuously occupied the exposure room for at least 5 months. Similar rooms were used in Boston (634 cu ft) and Baltimore (489 cu ft). Each room was furnished with a 4 × 5 carpet, a single bed, a wooden chair, a scratching post, and a wire cage for the cats during a patient exposure. The rooms were cleaned only by gently sweeping the rug lightly every week and lightly mopping the room surfaces to remove excess cat hair. The outer sheet on the bed was replaced weekly. Litter boxes were changed every 2 days.

Prior to an exposure, the room ventilation was turned off, the chair wiped down, the cats moved to the wire cage, and the bed sheet was shaken. The subject sat in the chair and recorded symptoms every 5 minutes. The planned exposure was 1 hour, but the patient could leave earlier if symptoms became intolerable or if the monitoring physician chose to end the exposure. At each challenge, Fel d 1 in the air was measured using a personal monitor (Gilian Instrument Corporation, Wayne NJ) containing a glass fiber filter (Millipore Corp., Bedford, MA). Filters were extracted by the method of Wood and colleagues and Fel d 1 assayed by ELISA (10).

Symptoms scores were collected just prior to entering the cat room (Time 0), and then every 5 minutes until the end of cat room exposure at Minute 60. The 10 symptoms were: (1) itchy and/or watery eyes; (2) itchy nose or throat; (3) runny watery nose; (4) sneezing; (5) nasal congestion or stuffiness; (6) wheezing in chest; (7) tightness of chest and/or constrictive sensation in throat; (8) cough; (9) shortness of breath; (10) itching of skin. The intensity rating scale for each of the 10 symptoms was: 0 = no symptoms apparent; 1 = barely noticeable; 2 = definitely present; 3 = annoying; 4 = bothersome; 5 = very severe—incapacitating.

Clinical Data Analysis

Three summary symptom scores were computed from the individual symptom scores of each patient: Nasal Symptom Score defined as the sum of symptoms 2, 3, and 5; Lung Symptom Score defined as the sum of symptoms 6, 7, and 9; Total Symptom Score defined as the sum of all 10 symptoms. Overall symptom scores during the 60 min cat room exposure were computed by averaging the symptoms across all 5 min

evaluations; the Time 0 (pre-exposure) value was subtracted from the averages to adjust for background symptoms. In advance of the study, the nasal and lung scores were named as the primary measures of efficacy. Patients who prematurely exited the cat room had all missing observations replaced by their last non-missing symptom measurement.

The primary efficacy measures were the symptom scores in the cat room at baseline (pre-treatment), 1 wk post-treatment and 6 wk post-treatment. An analysis of covariance model was used to compare the average symptom scores. The model contained the change in the patient's average symptom score from baseline as the response variable, and treatment group, investigative site and baseline symptom score as predictors. The baseline symptom score was included as a covariable to adjust for moderate baseline symptom differences among the treatment groups. A linear trend contrast was used to provide a test for overall dose-response (11). As many patients found that symptoms became intolerable for 60 min in the cat room, before and after treatment "survivals" for the full 60 min were also compared.

RESULTS

Ninety-five patients were randomized; 49 had a history of asthma in the past. Patients who had severe enough asthma to require continuing treatment were not admitted to the study (*see exclusion criteria*). None exhibited prick test skin sensitivity to the treatment material he or she was about to receive the day of the first injection. Two patients, one placebo and one at the 7.5 μg dose, requested discontinuance after the first dose for personal reasons. Two patients receiving the 750 μg dose requested discontinuance after the first injection because of suspected adverse events (*described below*), leaving 91 well-matched patients for evaluation (Table 1).

At 6 wk post-treatment, the average nasal, lung and total symptom scores following cat exposures are displayed in Fig. 2. Patients who exited the cat room before 60 min because of intolerable symptoms had all missing observations replaced by their last non-missing symptom measurement. This approach produces a conservative estimate of the treatment effect because symptoms were almost always increasing in severity before exiting the cat room, and because patients on placebo left the cat room earlier than patients on active treatment. Therefore, the estimated symptom scores for patients who exited early are likely less than they would have been had the patients remained for the full 60 min. In general, all groups showed increasing symptoms during the first 40 min of exposure, but then stabilized. This plateauing of symptoms occurred in part because the majority of patients with more severe symptoms chose to leave during the first 40 min.

Measurement of Fel d 1 in the cat room air showed no significant differences either across treatments or across days of exposure. Table 2 shows median levels with the 25th and 75th percentile values (the data were not normally distributed) across treatment and exposures.

After treatment, the 750 μg group exhibited the least severe symptoms among the treatment groups. Furthermore, 6 wk after treatment, the 750 μg group had a somewhat larger number of patients who stayed in the cat room the full 60 min. (Table 3).

The average change from baseline symptom scores during the 60 min exposure was used to compare the treatment groups statistically (Table 4). A dose-response relationship was demonstrated for all three symptom scores at 6 weeks post-treatment. The 750 μg dose provided the best protection (at 6 wk post-treatment $p = 0.06$ for nasal symptoms, $p = 0.03$ for lung symptoms, and $p = 0.08$ for total symptoms). An alternate analysis that used only data where there were pairs of pre- and post-treatment data and did not impute values for data missing because of departure from the cat room gives smaller p values (Table 4). By the analysis for total symptoms at the 750 μg dose, $p = 0.03$; and at the 75 μg dose, $p = 0.04$.

We measured serum IgE and IgG antibody to Fel d 1, IPC-1,

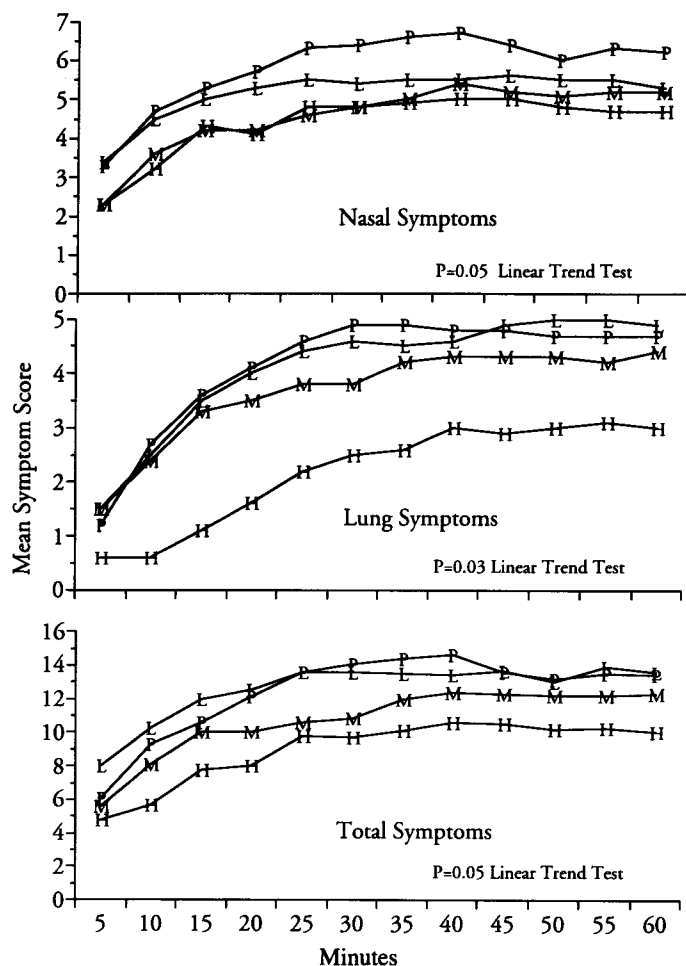


Figure 2. Mean symptom scores recorded every five minutes in a cat room six weeks after completion of treatment for four weeks of an injection once a week of treatment peptides. P = placebo; L = 7.5 µg per dose; M = 75 µg; H = 750 µg.

and IPC-2 before treatment and at one and six weeks after treatment. Before treatment, 94% of patients demonstrated IgE to Fel d 1. After treatment, no group showed a significant change in IgE and IgG antibody to Fel d 1. Only one patient demonstrated preexisting IgE antibody to IPC-2 (Table 1). After treatment, in patients receiving 7.5, 75, and 750 µg respectively, 3 of 23, 3 of 22, and 5 of 24 demonstrated increased IgE to a peptide. For increased IgG to peptides these numbers were 0 to 23, 1 of 23, and 7 of 24, respectively.

We demonstrated no diminution in immediate skin test reactivity to cat hair and dander extract after treatment (data not shown). Five patients had positive skin prick tests to study medication after treatment as follows: 7.5 µg, 1 of 17, 75 µg, 1 of

TABLE 3
NUMBER OF PATIENTS WHO REMAINED IN THE CAT ROOM FOR THE FULL 60 MINUTE CHALLENGE

	Placebo	7.5 µg	75 µg	750 µg	p Value*
n	25	22	22	22	
Baseline	12	12	15	11	0.7
1 wk after treatment	13	11	12	13	0.9
6 wk after treatment	10	10	13	15	0.09

* p Value is from Mantel-Haenszel test of association between dose and outcome, adjusting for site and baseline FEV₁ category.

17, 750 µg, 3 of 20, placebo, none. Four of the five skin test positive patients had IgE to a peptide.

T cell proliferation assays to peptides and recombinant Fel d 1 chain 1 and chain 2 protein did not reveal any consistent alterations attributable to treatment (Table 5). The distribution of specific responses to Fel d 1 peptides did not shift during the treatment (data not shown).

Although a full dose, whether 7.5, 75, or 750 µg, was given on the first injection, peptide treatment was well tolerated. Nevertheless, a number reported on the diary forms that had been furnished that they had later experienced some allergic symptoms. There was no local redness or swelling at the site of injection, but respiratory symptoms, most often chest tightness or runny nose, occurred one hour or more after the injection. The onset of these symptoms could be as late as three or more hours after injection. Itching skin without hives occurred in a few. Several described the symptoms as being similar to, but less severe than, those induced by cat exposure. The incidence and severity of symptoms changed little from that observed on the first dose in the placebo, 7.5 µg and 75 µg groups (Table 6). In the 750 µg group, on the other hand, incidence and severity (by several criteria) decreased progressively with each subsequent dose and were somewhat less by the fourth dose than in the 7.5 and 75 µg groups.

Two high dose patients discontinued treatment because of post-treatment symptoms. One experienced chest tightness, runny nose and itchy eyes six hours following the first dose. She had noted onset of spring seasonal allergies and preferred not to continue. Another patient experienced chest tightness, nasal congestion and non-itching facial flushing ten minutes after the first injection. She had a 2 cm area of redness at the injection site without swelling or itching. She was normotensive but peak flow decreased from 450 L/min to 330 L/min. Two hours after treatment with epinephrine, antihistamine, decongestant, and steroid she improved and went home.

DISCUSSION

We limited ourselves to peptides representing a small but reactive proportion of the T cell epitopes in Fel d 1. Similar treatment in animal models has down regulated the immune response to a whole protein (12). Experiments suggest that anergic T cells are actively suppressive of an inflammatory response.

Diminution of the allergic symptoms that occur shortly after exposure to cats was our most striking finding. Treatment effects appeared to be present at one week but failed to meet tests of significance. At 6 wk, a significant treatment effect progressive with increasing doses was evident.

Neither primary or secondary cultures of T cells in the presence of recombinant chains of Fel d 1 demonstrated a consistent change in proliferation due to treatment. Proliferative responses occurred in primary cultures infrequently and the positive responses obtained were in most cases too low to draw conclusions

TABLE 2
FEL D 1 LEVELS (ng/M³) IN CAT ROOM AIR

	Baseline	1 wk After Treatment	6 wk After Treatment
Placebo	666 (181–2,808)	651 (125–3,733)	512 (236–2,433)
7.5 µg	736 (87–2,066)	510 (230–2,210)	616 (148–2,582)
75 µg	678 (102–1,209)	830 (137–2,710)	251 (194–2,623)
750 µg	466 (237–1,550)	562 (143–1,418)	286 (189–942)

Data shown are medians (25th percentile–75th percentile).

TABLE 4
MEAN SYMPTOM SCORES FROM CAT ROOM CHALLENGES

	Symptom Score Mean $\pm$ Standard Error (Mean and Standard Errors are adjusted for investigative site and baseline.)				Linear Trend Test
	Placebo	7.5 μ g	75 μ g	750 μ g	
Nasal score					
Baseline	7.8 $\pm$ 0.53	6.5 $\pm$ 0.56	5.8 $\pm$ 0.56	6.2 $\pm$ 0.56	N/A
Change 1 wk post treatment	-1.5 $\pm$ 0.47	-1.1 $\pm$ 0.49 $p = 0.6^*$	-1.9 $\pm$ 0.50 $p = 0.6$	-2.3 $\pm$ 0.49 $p = 0.3$	$p = 0.2$
Change 6 wk post treatment	-0.84 $\pm$ 0.50	-1.5 $\pm$ 0.52 $p = 0.4$	-2.0 $\pm$ 0.54 $p = 0.1^\dagger$	-2.2 $\pm$ 0.52 $p = 0.06^\ddagger$	$p = 0.05^\ddagger$
Lung score					
Baseline	4.7 $\pm$ 0.68	5.9 $\pm$ 0.73	3.9 $\pm$ 0.73	5.4 $\pm$ 0.73	N/A
Change 1 wk post treatment	-0.26 $\pm$ 0.55	-0.64 $\pm$ 0.6 $p = 0.7$	-0.71 $\pm$ 0.6 $p = 0.6$	-2.3 $\pm$ 0.59 $p = 0.01$	$p = 0.02$
Change 6 wk post treatment	-0.85 $\pm$ 0.62	-0.93 $\pm$ 0.66 $p = 0.9$	-1.3 $\pm$ 0.68 $p = 0.6$	-2.8 $\pm$ 0.66 $p = 0.03$	$p = 0.03$
Total score					
Baseline	17.7 $\pm$ 1.4	17.0 $\pm$ 1.5	13.3 $\pm$ 1.5	15.7 $\pm$ 1.5	N/A
Change 1 wk post treatment	-3.5 $\pm$ 1.2	-2.8 $\pm$ 1.2 $p = 0.7$	-3.8 $\pm$ 1.3 $p = 0.8$	-6.2 $\pm$ 1.2 $p = 0.1$	$p = 0.09^\dagger$
Change 6 wk post treatment	-3.7 $\pm$ 1.3	-3.4 $\pm$ 1.3 $p = 0.8$	-5.1 $\pm$ 1.4 $p = 0.5^\dagger$	-7.0 $\pm$ 1.3 $p = 0.08^\ddagger$	$p = 0.05^\ddagger$

* Significance of the difference between the change in the placebo group and the change in the treatment group. One patient completed the one week post-treatment challenge but not the 6 wk challenge but is included.

† An alternate analysis that used only data where there were pairs of pre- and post-treatment data and did not impute values for data missing because of departure from the cat room have $p < 0.05$ for these comparisons.

‡ $p < 0.02$ by alternate analysis.

about treatment induced changes. Although the T cell lines in secondary cultures show higher proliferative responses they may not be appropriate for demonstrating anergy induction. After exposure to Fel d 1, IL-2, and IL-4 for several weeks any modulation of response may be overcome. In a new set of treated patients, we are using short term cultures, and examining cytokine mRNA expression and protein production in the presence of Fel d 1.

Although treatment designed to induce T cell tolerance might eventually lower specific IgE antibody, serum antibodies to Fel d 1 were not altered during the brief time we observed the patients here. If T cell cytokine secretion was changed without altering antibody synthesis one must suspect either a T cell influence on mast cell and/or basophil releasability of mediators or some hitherto unsuspected direct role for T lymphocytes in the earliest phase of the allergic response.

The suppression of symptoms on cat exposure observed here was quantitatively similar to the effects of antiinflammatory

drugs. When evaluated by a cat room challenge using a different scoring scale, nasal triamcinolone produced similar changes in nose and lung symptoms. Median nasal symptom scores were 1.0 on placebo and 0.65 on drug; pulmonary symptom scores were 0.9 on placebo and 0.45 on drug (13). Studies of immunotherapy with cat extracts also show at best partial suppression of symptoms on exposure in a cat room (7, 8, 14).

Peptides were given in four injections, whereas standard immunotherapy commonly requires 25 or more injections to reach a full dose. The peptides, as expected, were poorly immunogenic even though the patients already have antibodies to Fel d 1. A minority of patients in our study developed any detectable IgE or IgG antibody to either peptide (most at the lower limit of the sensitivity of the assay), whereas in immunotherapy with cat extract, most patients have early rises in IgE and IgG antibodies to the extract (probably mainly to Fel d 1) at a time when the dose in the gradual buildup is less than 1 μ g Fel d 1 equivalent (8). Low immunogenicity should be accompanied by improved

TABLE 5
STIMULATION INDICES IN SECONDARY CULTURES OF T CELLS*

	Placebo	7.5 μ g	75 μ g	750 μ g
Antigen = Recombinant Chain 1 of Fel d 1				
Pretreatment	n = 12 13.5 (4.6-79)	n = 12 17 (4.4-147)	n = 13 18 (3.2-375)	n = 13 17 (2.9-123)
1 wk after treatment	n = 9 17 (1.0-112)	n = 9 5.0 (1.3-71)	n = 9 5.8 (1.0-219)	n = 10 15 (1.0-423)
6 wks after treatment	n = 12 6.0 (1.0-61)	n = 10 8.5 (1.0-36)	n = 9 12 (1.0-130)	n = 11 14 (1.0-256)
Antigen-Recombinant Chain 2 of Fel d 1				
Pretreatment	n = 12 12.2 (4.0-123)	n = 12 27 (2.3-318)	n = 13 12.2 (2.8-99)	n = 13 16 (5.9-150)
1 wk after treatment	n = 9 32 (1.0-87)	n = 9 6.6 (1.0-39)	n = 9 8.5 (1.0-24)	n = 10 14 (1.3-65)
6 wk after treatment	n = 12 17 (1.0-85)	n = 10 3.0 (1.0-29)	n = 9 18 (1.0-35)	n = 11 11 (1.0-77)

* Values = median SI(low-high)

TABLE 6
ALLERGIC SYMPTOMS REPORTED AFTER INJECTIONS

Dose	First	Second	Third	Fourth
Placebo, n	26	25	25	25
Symptoms	4	7	5	3
Severe	0	0	0	0
Chest symptoms	2	2	1	1
Medications	0	0	0	0
7.5 µg, n	23	23	22	22
Symptoms	10	11	9	7
Severe	1	1	1	1
Chest symptoms	7	9	6	6
Medications	6	5	4	3
75 µg, n	22	22	22	22
Symptoms	10	8	8	10
Severe	3	1	0	1
Chest symptoms	7	7	7	5
Medications	5	5	6	3
750 µg, n	24	22	22	22
Symptoms	16	12	11	6
Severe	6	2	2	0
Chest symptoms	15	10	7	3
Medications	6	5	4	1

safety. Nevertheless, the possibility of an occasional patient developing anaphylactic sensitivity to one or the other peptide can not be discounted entirely. If there were polymerization or aggregation of peptides in solution they might become more immunogenic. During the development of the formulation used here, light scattering studies of solutions of lyophilized peptides after reconstitution showed no evidence of aggregation of peptides.

Most patients who formed or developed an increase in IgE or IgG antibody to peptides post treatment experienced post injection symptoms but many patients without such antibodies had similar symptoms. Furthermore, the symptoms were most common on the first injection before antibodies could have developed. These symptoms are therefore unlikely to be antibody mediated. That a progressive decline in incidence and severity of symptoms occurred more often in the 750 µg group (Table 6) may indicate that the larger dose was needed to develop tolerance regularly. The larger dose also was the more effective in modifying symptoms during cat exposure. Comedication with antihistamines is being explored to minimize these post injection allergic like symptoms.

To describe the mechanism of treatment induced symptoms we took blood from eight patients on thirteen occasions before injection and during post injection symptoms. There was no increase in mast cell tryptase or in serum free histamine; both have been used as indicators of systemic anaphylaxis. On four occasions in two patients there was a disappearance of the histamine normally resident in peripheral blood basophils. This suggests that circulating basophils had been signaled to migrate to the respiratory tract where they might have released mediators. O'Hehir and colleagues showed that when clones of T cells from an allergic patient were exposed *in vitro* to high concentrations of specific peptide in the absence of APC there was considerable production of IL-2, IL-4, and IFN- γ . The cells became tolerized and no longer produced IL-4 when subsequently exposed to APC and peptide but still produced IFN- γ (15). The clinical results in the study are consistent with similar events occurring *in vivo*, i.e., after injection of peptides, allergen specific T cells concentrated in sensitized respiratory mucosa could first attract basophils and eosinophils through pro inflammatory cytokines, but then become down-regulated.

In mice with a preexisting IgG (not IgE) immune response

induced by Fel d 1 in incomplete Freund's adjuvant, IPC-2 subcutaneously resulted in tolerization of T cells as evidenced by decreased IL-2 production when spleen cells were cultured with IPC-2. In another experiment, a combination of IPC-1 and IPC-2 produced as good a tolerance to subsequent immunization with chain 1 as did chain 1 itself (5). Although this is good evidence that the peptides are specific for murine T cells, the model is in several ways different from human allergy with its Th2 driven IgE antibody synthesis induced after mucosal exposure.

The data reported here are necessarily preliminary. More work to define the risk of untoward reactions, the duration of the treatment effect, and the optimum dosage regimen are needed. The mechanism of action in man needs to be defined. We are currently studying the effects of the peptides on mediator release in the respiratory tract, and the production of Th1/Th2 cytokines by T cells.

Although induced specific immune tolerance has been well described in animal models since 1946 (16), this is the first demonstration that peptides designed to induce T cell tolerance can alter the manifestations of a human immunologic disease. The use of molecular species directed specifically toward T cell receptors is novel.

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