

Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects

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Abstract

We tested peptide immunotherapy in cat-allergic humans, using a formulation of two synthetic peptides, IPC-1 and IPC-2, each of which is 27 amino acids long and contains T cell-reactive regions of Fel d 1, the major cat allergen. In this exploratory, randomized, double-blind, parallel-group study, 42 subjects received s.c. injections of treatment peptides 250 μ g or placebo weekly for four consecutive weeks. Changes in immediate- and late-phase skin test reactivity, and in antigen-driven cytokine synthesis were assessed. Epicutaneous (end-point titration) and intradermal tests were performed with cat extract (ALK SQ Cat Hair) containing Fel d 1, before the first injection, then 2, 6 and 24 weeks after the fourth and last injection of peptides or placebo.

IL-4, IL-10 and IFN- γ expression by circulating peripheral blood mononuclear cells (PBMC) in response to cat extract was measured using short-term bulk culture of PBMC and short-term limiting dilution analysis. Subjects who received peptide immunotherapy did not tolerate significantly more cat extract containing Fel d 1 in the skin tests 2, 6 or 24 weeks after the last injection than they did at baseline, and their late-phase responses did not decrease significantly compared to baseline. Substantial IL-4, IL-10 and IFN- γ responses were observed following primary culture of cat antigen-stimulated PBMC; however, the intensity of cytokine synthesis and the IFN- γ :IL-4 ratio were unchanged in peptide- and placebo-treated groups 6 and 24 weeks after the last injection. A few hours after the injections, subjects receiving peptides reported more allergic rhinitis and asthma symptoms and more pruritus than those receiving placebo. We conclude that under the conditions tested, peptide immunotherapy did not reduce immediate- or late-phase skin reactivity to cat extract containing Fel d 1 or modify cat antigen-specific cytokine production significantly.

Introduction

A novel and promising approach to management of ongoing allergic (1) or autoimmune disease (2,3) is that of peptide immunotherapy. Identification of defined T cell epitopes containing peptides, based on the primary structure of major allergens, may provide an effective tool for modification of human immediate hypersensitivity to allergens (4). The definition of human T cell epitope maps for a number of clinically important allergens, including cat dander, house dust mite, birch pollen, honey bee Api m 1 and others, has greatly stimulated research in this area.

Here, immediate hypersensitivity to domestic cats was selected for human *in vivo* evaluation of peptide immunother-

apy for several reasons. Cat allergy is common, affecting ~10% of individuals, as confirmed by positive epicutaneous tests or elevated specific IgE measurements to cat dander (5,6). Sensitized persons cannot completely avoid exposure to cat dander, which is ubiquitous in the environment (7,8). Prevention or treatment of cat-induced symptoms in disorders such as rhinoconjunctivitis, asthma or urticaria using conventional pharmacologic management provides only a short-term solution to the problem (9). Long-term modification of the cat-allergic state including decreased skin sensitivity and decreased T cell reactivity to cat dander can potentially be achieved after months or years of traditional immunotherapy,

but this is associated with a risk of immediate hypersensitivity reactions and its effects are often temporary (10–16).

The major cat allergen, Fel d 1, is recognized by ~80% of cat-allergic subjects. It is well characterized in terms of physicochemical properties (17) and T cell epitopes have been defined for it (18,19). A formulation of two Fel d 1 chain 1 homologous synthetic peptides, termed IPC-1 and IPC-2, is now available for investigation in human subjects. Each peptide is 27 amino acids long and contains T cell-reactive regions of Fel d 1, the major cat allergen, but no B cell epitopes recognized by endogenous IgE in sera of most subjects with cat allergy and elevated specific IgE to Fel d 1 (20).

In a murine model, both naive and sensitized animals developed tolerance to allergen and to peptides after s.c. or intranasal administration of peptides (21–24); specifically, tolerance to Fel d 1 and peptides derived from it can be induced by multiple s.c. injections of Fel d 1 peptides (21). In initial human studies with synthetic Fel d 1 peptides (20), dose-related reduction of symptoms induced by cat exposure, decreased bronchial reactivity and decreased skin responses to cat extract have been reported (25–28).

We hypothesized that treatment of cat-allergic subjects with these peptides would render them tolerant to Fel d 1 and reduce their cat-induced immunologic responses as evidenced by decreased immediate skin test reactivity, decreased late-phase response and modified cat antigen-specific cytokine responses.

Methods

Subjects

The study protocol was approved by the University of Manitoba Faculty Committee on the Use of Human Subjects in Research. All participants gave written informed consent before enrollment.

We recruited 44 subjects with a history of rhinitis and/or asthma symptoms on exposure to cats and a positive epicutaneous test to an extract containing ≤ 3.3 FDA units/ml Fel d 1, defined as the sum (Σ) of the longest and orthogonal diameters of the wheal at least 6 mm greater than the diluent control.

We excluded subjects who currently had a cat in their home, those who were of child-bearing potential or pregnant, those with medical disorders other than allergy-related problems and those who had received immunotherapy within the preceding year or had ever received peptide injections. Subjects requiring β -adrenergic blockers, oral glucocorticoids, ketotifen, astemizole, doxepin, tricyclic antidepressants or MAO inhibitors were also excluded; other medications were withheld for an appropriate length of time before each study visit.

Study design

In a randomized, double-blind, placebo-controlled, parallel-group study, subjects received either treatment peptides or placebo s.c. once weekly for four consecutive weeks. They were assessed 10 times during the study: at a baseline visit, at four visits for treatment injections and at five follow-up

visits, including two brief visits for evaluation of the late-phase skin response 24 h after intradermal testing (Table 1).

Tests for efficacy were performed at baseline, and at 2, 6 and 24 weeks after the last injection. Outcome variables, including epicutaneous reactivity to several dilutions of cat extract (ALK SQ Cat Hair; ALK Laboratories, Milford, CT) containing Fel d 1, intradermal test reactivity and late-phase response and modification of cytokine production after stimulation with cat extract containing Fel d 1 are described below.

Peptide administration

Single-dose vials containing Fel d 1 peptides, 750 μ g IPC-1, 750 μ g IPC-2 buffered in sodium phosphate and mannitol, and were provided in the form of a sterile, injectable, freeze-dried powder (Allervax® Cat; ImmuLogic Pharmaceutical, Waltham, MA). The placebo powder appeared identical and contained the same excipients. Within 1 h of each treatment injection in each subject, the dose was reconstituted with 1 ml water for injection and further diluted with 2 ml sterile 0.9% sodium chloride.

Using a computer code, subjects were randomized to receive a s.c. injection of treatment peptides 250 μ g or placebo at weekly intervals. Before the first injection, an epicutaneous test was performed with treatment peptides or placebo. If negative, it was followed by an intradermal test with the peptides or placebo. Subjects who had a positive epicutaneous test (wheal diameter 3 mm > diluent control) or intradermal test (erythema > wheal and wheal 3 mm > diluent control) were treated with a split dose of treatment peptides or placebo as follows: 0.1 ml was administered s.c. followed by a 40 min observation period. If the test dose was tolerated well, the remaining 0.9 ml of the dose was administered, followed by a 120–240 min observation period. If epicutaneous and intradermal tests were negative, the subject received the full initial 1 ml dose of treatment peptides or placebo as a single subcutaneous injection, followed by a 120–240 minute observation period.

Epicutaneous and intradermal tests with cat extract

Epicutaneous reactivity to cat extract containing Fel d 1 was evaluated before the first injection, and at 2, 6 and 24 weeks after the last injection. The same lyophilized commercial preparation of cat extract (ALK SQ Cat Hair) was used for *in vivo* and *in vitro* testing. The most concentrated solution contained 10 FDA units/ml of Fel d 1. Fifteen full dilutions were made using 0.9% saline containing 0.03% human serum albumin and 0.4% phenol, to the weakest concentration of 3×10^{-7} units/ml. The positive control was histamine base 1.0 mg/ml and the negative control was the diluent. All skin tests were performed at the same time of day ± 2 h and measured 15 min later by the same two study nurses. The end-point titration method was used. Dilutions that produced a Σ_E of 20 mm and a Σ_W of 6 mm were interpolated. The change from baseline to post-treatment of the resulting interpolations referred to as baseline epicutaneous test dilution for Σ_E and Σ_W constituted the primary outcome measure for the statistical analysis (29).

Before the first injection and 2 weeks after the last injection, the late-phase skin response was evaluated 24 $\pm$ 2 h after an intradermal test on a marked portion of the upper arm

Table 1. Study design

Week	Visit	Procedure
-1	1	skin prick/intradermal test(s) to cat extract containing Fel d 1, cat antigen-driven cytokine synthesis
	2	late phase response
0	3	skin test with treatment peptides or placebo; injection of peptides or placebo
1	4	injection of peptides or placebo
2	5	injection of peptides or placebo
3	6	injection of peptides or placebo
5	7	skin prick/intradermal test(s) to cat extract containing Fel d 1
	8	late-phase response
9	9	skin test with treatment peptides or placebo; skin prick/intradermal test(s) to cat extract containing Fel d 1, cat antigen-driven cytokine synthesis; IPC-1 and IPC-2
27	10	skin prick/intradermal test(s) to cat extract containing Fel d 1, cat antigen-driven cytokine synthesis

Table 2. Demographic characteristics (all treated subjects): descriptive statistics by treatment group

	Placebo × 4 (n = 21)	250 µg peptides × 4 (n = 21)
Age ± SD	29.5 ± 10.1	34.8 ± 7.6 ^a
Gender		
female	3 (14%)	12 (57%) ^a
male	18 (86%)	9 (43%)
Race		
Caucasian	19 (90%)	18 (86%)
other	2 (10%)	3 (14%)
Previous immunotherapy	4 (19%)	3 (14%)

^a*P* < 0.05.

unused in previous tests. A concentration of cat extract containing Fel d 1 that produced an Σ_W of at least 30 mm was injected. No Fel d 1 concentration higher than 0.12 FDA units/ml was used. The change from baseline to post-treatment and the resulting interpolations of the parallel lines were analyzed. The shift in curves or distance between the two lines from baseline to post-treatment, and the change from baseline and the diameter of the erythema resulting from the intradermal skin test producing an immediate Σ_W of 30 mm were also analyzed.

In vitro tests with cat extract

Cell and antigen preparation. All cytokine studies were performed in a double-blind manner. Blood (25 ml) was diluted with 1 ml sterile 2.7% EDTA immediately after collection. Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation with Histopaque-1077 (Sigma, St Louis, MO), carried out within 30 min of blood collection. Cells collected from the interface were washed twice in serum-free PBS, counted (>99% viability as determined by Trypan blue exclusion) and used for bulk culture or limiting dilution analysis.

Primary bulk culture. Cells were cultured at 300 000 cells/well in 96-well plates (Corning Science Products, Rochester, NY) using RPMI 1640 supplemented with 10% FCS 10 mM L-glutamine, 2×10^{-5} M 2 mercaptoethanol and antibiotic-

antimycotic (Gibco BRL, Burlington, Ontario, Canada). Three short-term cultures were set up in triplicate for each subject in the absence and presence of Fel d 1 (500 BAU/ml). As recombinant Fel d 1 was not available to us, we used cat extract (ALK SQ Cat Hair, the same allergen used for skin testing. Cytokine production in culture supernatants was evaluated by ELISA for IFN- γ , IL-10 or bioassay (CT.h4S $\pm$ neutralizing anti-IL-4 antibody) for IL-4 as previously described (30–32). Cytokine levels were measured following duplicate titrations of four to eight dilutions falling on the linear part of the curve for the cytokine-specific assays employed.

PBMC populations were used in preference to highly enriched CD4 T cells to avoid an excessively reductionist approach and allow evaluation of the collective cytokine response to cat antigens. Initial experiments carried out with PBMC from eight cat-allergic donors identified the optimal cell concentration, period of culture and antigen concentration for cat antigen-stimulated cytokine synthesis in primary culture (data not shown). Using these conditions, we evaluated IFN- γ , IL-10 and IL-4 synthesis before and 6 weeks after the fourth and last peptide or placebo injection; at 24 weeks, we studied only the first 20 consecutive subjects returning for reevaluation.

Safety

Before and after each treatment injection, subjects recorded allergy-related symptoms and peak expiratory flows every 30 min for 3 h, then every 3 h while awake, also at bedtime and on arising the next morning. Symptoms were graded from 0 (none) to 3 (annoying) to 5 (very severe or incapacitating). Itchy and/or watery eyes, nasal discharge, sneezing, or congestion, itchy nose or throat, constricted sensation in the throat, wheezing, chest tightness, cough, or shortness of breath, itchy skin or hives, were specifically monitored and treated appropriately.

The laboratory parameters assessed were: hemoglobin, hematocrit and red cell indices, platelets, white blood cells and differential (hematology); sodium chloride, calcium, phosphorous, potassium, lactate dehydrogenase, aspartate transaminase, gamma glutamyl transferase, alanine transaminase, bilirubin, alkaline phosphatase, cholesterol, triglycerides, total protein, globulin, albumin, blood urea nitrogen, creatinine, uric acid and glucose (biochemistry); and urinalysis (pH, specific gravity, glucose, protein, and ketones).

Table 3. Skin prick test results: change in mean dilution from baseline to visits 7, 9 and 10 by treatment, assuming parallel assays^a

Number of weeks after last injection	Placebo × 4 (n = 21)	250 µg peptides × 4 (n = 20)	P value ^b
2 weeks			
dilution so that wheal = 6 mm	0.12 ± 0.18	0.02 ± 0.18	0.72
dilution so that erythema = 20 mm	0.20 ± 0.19	0.53 ± 0.22	0.26
6 weeks			
dilution so that wheal = 6 mm	0.34 ± 0.20	0.08 ± 0.20	0.34
dilution so that erythema = 20 mm	0.61 ± 0.19	0.42 ± 0.20	0.49
24 weeks			
dilution so that wheal = 6 mm	0.27 ± 0.22	0.07 ± 0.22	0.53
dilution so that erythema = 20 mm	0.69 ± 0.20	0.83 ± 0.22	0.63

^aAdjusted means ± SE of interpolated dilutions to produce $\Sigma_W = 6$ and $\Sigma_E = 20$.^bP value from analysis of covariance comparison between placebo and peptides in adjusted mean interpolated dilutions.

Statistics

For the epicutaneous and intradermal tests, least-squares regression lines for each patient were fit to predict the dilutions that would have produced an Σ_W of 6 mm and an Σ_E of 20 mm. The changes from baseline in these interpolated values were modeled with an analysis of covariance with terms for treatment group, baseline values and their interaction. Treatment means were adjusted to the overall mean baseline values. Separate analyses of the epicutaneous tests were done assuming parallel assay lines across visits.

An intent-to-treat approach was used in which all subjects with baseline and post-treatment data were included in the analyses. Subjects with missing data had their last observation carried forward. One of the two subjects who left the study during the treatment period had post-treatment data and so was included in the intent-to-treat analyses.

Non-parametric statistics were used for cytokine data analysis.

Results

Forty-four subjects were enrolled. For personal reasons, two subjects dropped out before treatment injections began and two dropped out after the first and second injections had been given; 42 subjects were available for safety analysis and 41 subjects for efficacy analysis. Following unblinding, it was found that the peptide-treated group was older and contained more females than the placebo-treated group. There was no difference between the groups with regard to documented allergies or previous immunotherapy (Table 2).

Skin tests

Before the first injection and after the last injection of peptides or placebo, epicutaneous and intradermal tests with peptides or placebo were generally negative, and did not differ significantly between the treatment groups (Fisher's exact test $P \gg 0.05$).

Epicutaneous test reactivity to cat extract containing Fel d 1 did not differ significantly from baseline, i.e. the same size wheal or erythema was produced at the same dilution of Fel

d 1 before and after treatment with peptides or placebo (Table 3).

Before treatment with peptides or placebo, intradermal test reactivity to cat extract containing Fel d 1 was noted in 42% of subjects receiving peptides and 38% of subjects receiving placebo. After treatment, intradermal test reactivity and late-phase responses did not differ significantly from baseline (Tables 4 and 5).

Antigen-driven cytokine synthesis

Before treatment, all but two subjects generated significant IFN- γ responses *in vitro* following antigen stimulation (Fig. 1). In the absence of antigen, IFN- γ synthesis was very low, usually ranging from undetectable to 5 U/ml. No significant difference was evident between the two groups. After treatment, comparison of median cat antigen-driven IFN- γ production in the two groups, or, alternatively, longitudinal analysis of changes in individuals treated with peptides or placebo, failed to indicate significant changes in the frequency of individuals capable of cat antigen stimulated cytokine synthesis (Fisher's exact test $P \gg 0.05$) or in the intensity of IFN γ synthesis (Wilcoxon $P \gg 0.05$).

IL-10 and IL-4 synthesis in these cultures was substantial before treatment (Figs 2 and 3), in contrast to normal subjects (S. Fitzpatrick-Wong *et al.*, in preparation) who did not exhibit detectable (~1–2 pg/ml) IL-4 responses to cat antigen stimulation. Neither IL-4 nor IL-10 synthesis was significantly altered by injection of peptides or placebo. As previously observed in related studies, IL-10 levels typically ranged between 15 and 250 pg/ml in the absence of antigen. Interestingly, IL-4 production was substantial in the absence of cat antigen-mediated *in vitro* re-stimulation among both placebo and peptide-treated groups (data not shown). This is in marked contrast to normal subjects or grass pollen-allergic subjects (30–32).

As an alternative approach to evaluating cat antigen-driven cytokine responsiveness, a subset of randomly selected subjects was examined by limiting dilution analyses 6 weeks after the last peptide or placebo injection, to obtain minimal estimates of the frequency of antigen-specific IFN- γ -producing PBMC. The frequency of cells producing IFN- γ in primary

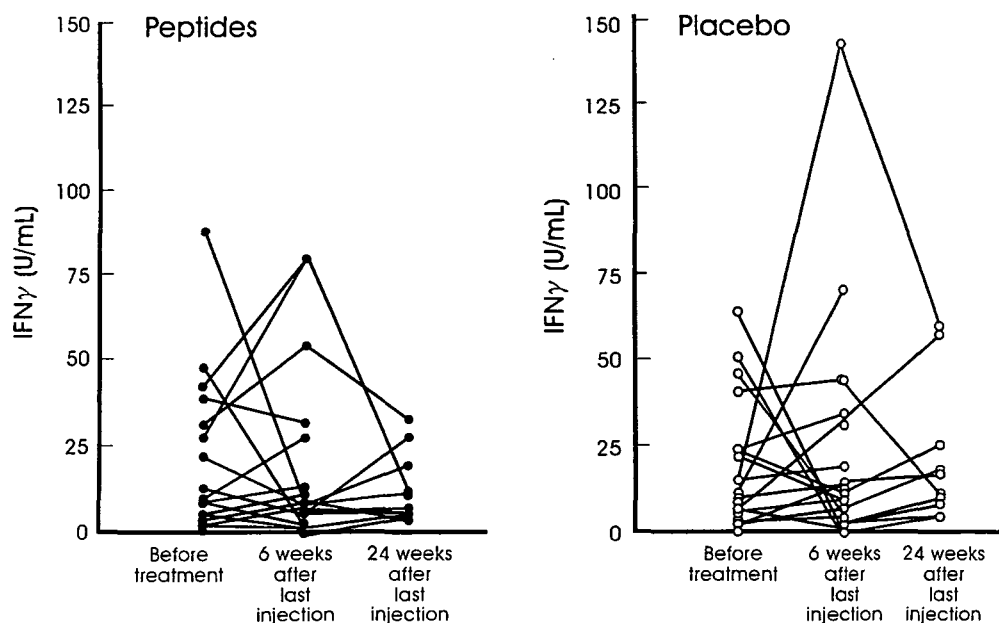


Fig. 1. IFN γ production in culture supernatants after short-term bulk culture of PBMC in the presence of cat extract (ALK SQ Cat Hair) containing Fel d 1 (500 BAU/ml), before the first injection, and 6 and 24 weeks after the fourth injection of peptides or placebo. Tests were performed in a double-blind manner.

Table 4. Intradermal test reactivity: change in mean dilution from baseline to visits 7, 9 and 10 by treatment, assuming parallel assays^a

Number of weeks after last injection	Placebo $\times$ 4 ($n = 21$)	250 μ g peptides $\times$ 4 ($n = 20$)	P value ^b
2 weeks dilution so that erythema = 50 mm	0.32 ± 0.12	0.49 ± 0.12	0.33
6 weeks dilution so that erythema = 50 mm	0.62 ± 0.14	0.53 ± 0.15	0.65
24 weeks dilution so that erythema = 50 mm	0.70 ± 0.15	0.50 ± 0.15	0.51

^aAdjusted means $\pm$ SE of interpolated dilutions to produce $\Sigma E = 50$.

^b P value from analysis of covariance comparison between placebo and peptides in adjusted mean interpolated dilutions.

Table 5. Late-phase response: descriptive statistics of erythema area from baseline to visit 8 by treatment

	Statistic	Placebo $\times$ 4 ($n = 21$)	250 μ g peptides $\times$ 4 ($n = 19$)
Before first injection	mean $\pm$ SD	15.50 ± 2.88	9.63 ± 4.66
	range	12, 19	4, 15
	no. with late-phase response	8 (38%)	8 (42%)
Two weeks after last injection	mean $\pm$ SD	13.00 ± 6.02	11.00 ± 3.52
	range	6, 22	8, 20
	no. with late-phase response	8 (38%)	11 (58%)

culture in response to antigen-mediated activation was readily determined (Table 6). Neither peptide- nor placebo-treated subjects exhibited a substantive change in the frequency of such cells during the study.

Safety

During the first hour after injection of peptides or placebo, no subjects had a problem with swelling at the injection site or with systemic symptoms. Within 24 h, and generally within

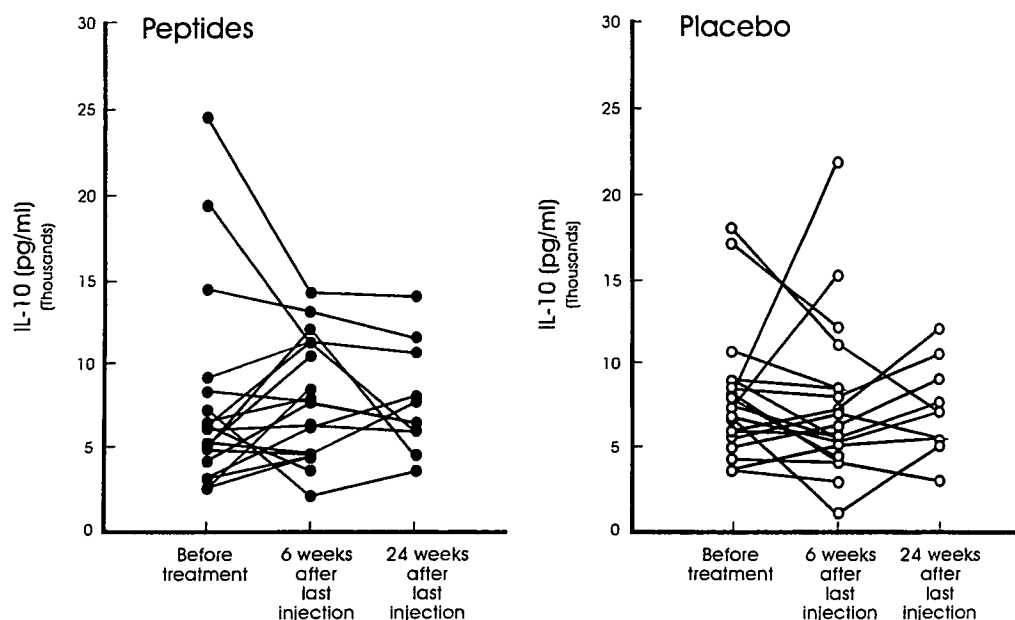


Fig. 2. IL-10 production in culture supernatants after short-term bulk culture of PBMC in the presence of cat extract (ALK SQ Cat Hair) containing Fel d 1, before the first injection, and 6 and 24 weeks after the fourth injection of peptides or placebo. Tests were performed in a double-blind manner.

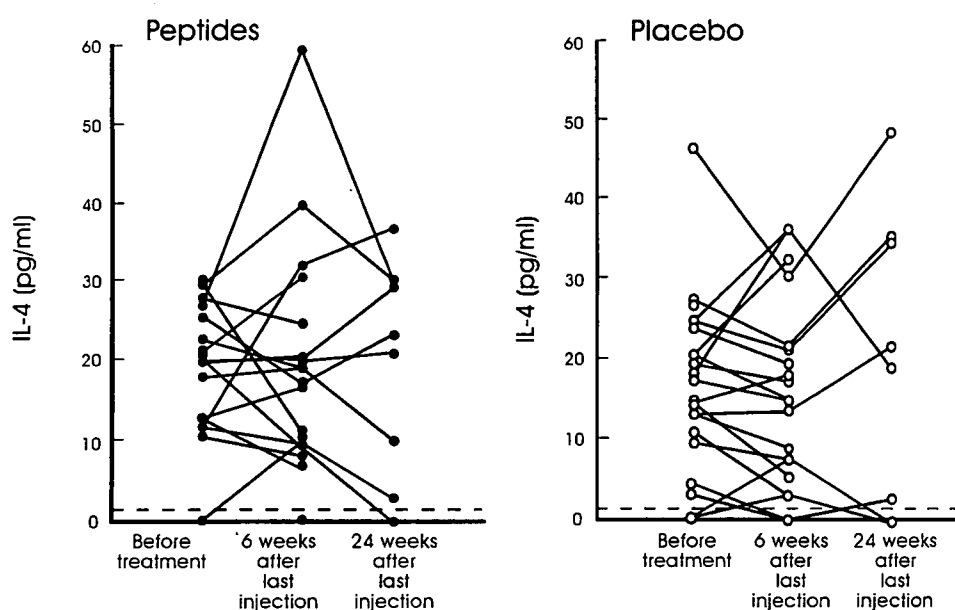


Fig. 3. IL-4 production in culture supernatants after short-term bulk culture of PBMC in the presence of cat extract (ALK SQ Cat Hair) containing Fel d 1, before the first injection, and 6 and 24 weeks after the fourth injection of peptides or placebo. Tests were performed in a double-blind manner.

1–3 h after injection, peptide-treated subjects had more adverse events, especially allergic rhinitis symptoms, chest tightness or other asthma symptoms, or pruritus, than placebo-treated subjects (Table 7). These symptoms were more likely to occur after the first injection of peptides than after subsequent injections. The peptides did not cause any significant abnormalities in laboratory parameters.

Discussion

Successful immunotherapy with peptides optimized for T cell recognition and MHC class II binding has been reported, although some issues remain to be resolved (21–28,33–36). In rodent models, treatment with synthetic peptides containing T cell reactive regions of *Dermatophagoides*, ragweed or Fel d 1/Fel d 1 synthetic peptides has been highly successful in

Table 6. Limiting dilution analysis of IFN- γ -producing PBMC

Treatment	Subject no. ^a	IFN- γ -producing cells/10 ⁷ PBMC		Statistical significance	Change
		Before first injection	6 weeks after last injection		
Placebo	4	377	298	no	—
	5	1448	2062	no	—
	14	558	762	no	—
	15	1398	3763	yes	2.7-fold increase
	7	1417	1101	no	—
	10	750	890	no	—
	9	1150	630	no	—
Peptides	6	3053	953	yes	3.2-fold decrease
	28	766	1009	no	—
	1	2368	4373	no	—
	18	1190	7225	yes	6.0-fold increase
	22	1560	779	no	—
	20	1030	914	no	—

^aRandomly selected.**Table 7.** Adverse events within 24 h of dose (all treated subjects)

	Placebo (n = 21)		Peptides (n = 21)	
	n	%	n	%
Rhinitis	1	4.7	3	14.3
Asthma worse	1	4.7	3	14.3
Pulmonary function test abnormal	0	0.0	2	9.5
Pruritus	0	0.0	5	23.0

suppressing development of immune responses in naive animals and, most importantly, in down-regulating ongoing T_H2-type responses to these antigens (21–24).

In human studies, allergen-reactive CD4 T cells have been anergized *in vitro* by exposing them to supraoptimal concentrations of their complementary peptide (33). Pre-treatment of T cell clones restricted by HLA-DP with the Der p I peptide rendered them non-responsive to an immunogenic challenge with house dust mite allergen. This loss of antigen-dependent proliferation was associated with down-regulation of membrane expression of the TCR. In a subsequent report, polyclonal *in vitro* T cell responses to house dust mite allergens were inhibited by a TCR peptide. The magnitude of modulation varied considerably among donors (9–92%), presumably reflecting differences in the relative contribution of TCR β chain variable region CDR2 peptide to the house dust mite response among individuals. However, >50% inhibition of the polyclonal *in vitro* response to antigen was observed in 16 of 21 donors tested and only two of the 16 failed to show substantial inhibition of house dust mite responses by the CDR2 peptide (34).

Little detailed information has been published on the capacity of peptide immunotherapy to interfere with ongoing allergic responses in humans. T cell reactivity to purified Amb a I, the major ragweed antigen, and peptides containing Amb a 1 epitopes was analyzed in 10 ragweed-allergic subjects

after completion of 10 years of conventional ragweed allergen immunotherapy. In this study, peripheral blood lymphocytes were isolated from subjects and controls, and the T cells were exposed to Amb a I or peptides derived from Amb a I in culture (35). Compared to the T cell responses in untreated ragweed-allergic control subjects, the subjects who had undergone successful immunotherapy exhibited decreases in T cell activation to Amb a I and Amb a I-specific peptides, and this finding correlated with decreased symptoms. More recently, T cell responses to Fel d 1 and Fel d 1 synthetic peptides were analyzed in 18 cat-allergic subjects before, during and after a 52 week course of conventional immunotherapy with cat pelt extract. In this study, which was not placebo-controlled, only subjects with positive assays at baseline were included. As immunotherapy progressed, Fel d 1-specific T cell responses decreased, paralleled by a decrease in skin test sensitivity. Serum levels of Fel d 1 specific IgE and IgG antibodies were unchanged (15).

Experience in rodents and humans to date therefore suggests that it may be possible to intervene in T cell activation during antigen presentation and produce antigen-specific inactivation of T cells and down-regulation of the cytokine response. In the study reported here, however, weekly subcutaneous injection of 250 μ g Fel d 1 synthetic peptides for 4 weeks to cat-allergic humans did not produce detectable alterations in immediate- or late-phase skin reactivity to cat extract containing Fel d 1. Also, the peptides did not appear to modify IFN- γ , IL-10 or IL-4 expression significantly in response to stimulation with cat extract containing Fel d 1, 6 and 24 weeks after the last treatment injection. Collectively, bulk culture and limiting dilution analysis data indicated that the capacity to mount antigen-driven cytokine responses to cat antigen *in vitro* was not quantitatively decreased.

To test the alternative hypothesis that the treatment peptides may have qualitatively altered cytokine synthesis patterns, we evaluated IFN- γ : IL-4 ratios in normal and atopic subjects. The relative balance of IL-4 and IFN- γ production is likely to be more important than the absolute amounts of any single cytokine in determining clinical hypersensitivity versus clinical

unresponsiveness. In previous reports, the ratio of grass pollen stimulated IFN- γ :IL-4 synthesis revealed a pronounced shift towards increased T_H2-like activity in the circulating immune repertoire of grass pollen sensitive individuals ($P = 0.000002$) versus that of individuals not allergic to grass pollens (30–32). In the present study, peptide- and placebo-treated subjects did not differ significantly in their IFN- γ :IL-4 ratio prior to or following treatment (data not shown). In this respect, the cytokine analyses correlate strongly with the *in vivo* measurements of skin test reactivity which also fail to indicate a difference between peptide-treated and placebo-treated groups.

The finding that IL-4 synthesis is readily demonstrable among these subjects in the absence of *in vitro* re-stimulation with cat antigen is of particular interest. We speculate that this may reflect the higher degree of sensitization among the subjects in this study compared to previously studied normal subjects or those with allergic rhinitis and grass pollen sensitization, in whom this effect was not observed (30–32). Alternatively, the propensity of cat allergic subjects to produce IL-4 (median 14 pg/ml, range <1–79 pg/ml, $n = 40$ subjects) independent of *in vitro* re-stimulation may be attributable to the fact that exposure to grass pollen is seasonal, but exposure to Fel d 1 is perennial and ubiquitous (7,8). Thus, one could speculate that cat allergic subjects may exhibit ongoing *in vivo* activation by environmental antigen, obviating the need for *in vitro* re-stimulation to observe IL-4. Further experiments to test this hypothesis and better characterize the nature of the cell(s) responsible for cytokine production are needed.

The synthetic peptides IPC-1 and IPC-2 used in this study have been selected to represent a significant proportion of human T cell epitopes, mapped employing T cell lines from cat-allergic subjects. Failure to reduce skin test reactivity to cat extract or to detectably modify the frequency, intensity or nature of cytokine expression by circulating peripheral blood mononuclear cells in response to re-stimulation with cat extract could potentially be attributed to a number of factors, such as inactivity of the peptides, unsuitable route of administration, low doses or infrequent dosing, or selection of sub-optimal times for efficacy measurements. However, it should be noted that in previous studies, injections of these peptides resulted in significantly improved ability to tolerate exposure to live cats, with 86% of peptide-treated subjects reporting improvement, compared to 63% of placebo-treated subjects (26). Symptom improvement was dose-related, being evident after four injections of 75 or 750 μ g at weekly intervals.

Intervention with T cell reactive tolerogenic peptides potentially represents a major advance in our ability to modify ongoing allergic responses in humans, using a short course of treatment and giving a dose of defined dominant antigen several orders of magnitude greater than can be administered during conventional immunotherapy (36). In the context of early-phase peptide development, the reason for the apparent discrepancy between our results and those reported in other preliminary studies remains unresolved and awaits publication of data from additional investigations as they are completed.

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Abbreviation

PBMC peripheral blood mononuclear cells

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