Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats

Jérôme Pène, PhD,[a] Anne Desroches, MD,[b] Louis Paradis, MD,[b] Bernard Lebel, PhD,[a] Martine Farce,[a] Christopher F. Nicodemus, PhD,[c] Hans Yssel, PhD,[a] and Jean Bousquet, MD, PhD[a,b] *Montpellier, France, and Waltham, Mass*

Background: Cells producing a T_{H2}-cytokine profile play an important role in the onset and maintenance of atopic diseases, and therefore specific immunotherapy is aimed to induce a switch to cells producing a T_{H1}- or T_{H0}-cytokine profile. Recently, a novel form of immunotherapy making use of synthetic peptides from the major cat allergen Fel d 1 has been developed, but its mechanisms of action are unknown.

Objectives: We examined the effects of immunotherapy with Fel d 1 peptides on the response to bronchial provocation tests ($PD_{20}FEV_1$) with a standardized Fel d 1 cat extract on Fel d 1–specific serum IgE and IgG levels and in vitro IL-4 and IFN-γ production.

Methods: Patients allergic to cats received 6 weekly injections of 7.5 μg (low dose), 75 μg (medium dose), or 750 μg (high dose) of Fel d 1 peptides (25 patients) or a placebo (6 patients).

Results: Six weeks after ending immunotherapy, posttreatment $PD_{20}FEV_1$ was not significantly different between the treated and placebo groups. However, in the medium- and high-dose groups there was a significant improvement between baseline and posttreatment days. IL-4 release was significantly reduced in the high dose–treated group ($P < .005$, Wilcoxon W test), whereas it was unchanged in the low or medium dose– and in the placebo-treated groups. In all groups, IFN-γ, IgE, and IgG levels remained unchanged.

Conclusion: There was no correlation between the improvement of $PD_{20}FEV_1$ and the decrease in IL-4 production. These data suggest that peptide immunotherapy may act by shifting the Fel d 1–induced response of PBMCs in vitro from the T_{H2}-like to the T_{H0}-like phenotype. (J Allergy Clin Immunol 1998;102:571-8.)

Key words: *T_{H1}/T_{H2}, allergy, T-cell cytokines, allergens, peptides, T-cell epitopes*

The regulation of IgE synthesis is controlled by several factors, among which cytokines play a central role. As in the mouse, 3 subsets of $CD4^+$ T helper/inducer cells have been identified in humans on the basis of their cytokine production profiles. T_{H1} cells release predominantly IFN-γ and IL-2, whereas T_{H2} cells secrete predominantly IL-4 and IL-5, but low amounts of IL-2 and IFN-γ. A third group, called T_{H0} cells, secrete the cytokines of both subsets on activation.[1,2] The T_{H2} cytokine IL-4 plays a critical role in allergy by inducing IgE synthesis, whereas IFN-γ has inhibitory effects.[3-7] Although in humans a strict dichotomy between T-cell subsets is not as clear as in the murine system,[8] an imbalance of T_{H1} and T_{H2} cells has been proposed to be involved in IgE-mediated allergic diseases and asthma as a result of the regulation of IgE synthesis and cellular recruitment at the sites of inflammation.[2,9-11]

Abbreviations used
A23187: Calcium ionophore A23187
BPT: Bronchial provocation test
PMA: Phorbol myristate acetate

Allergen-specific immunotherapy, widely used in the treatment of allergic rhinitis and asthma, is effective by using standardized allergenic extracts.[12] The cloning and characterization of the structure of major allergens has made it possible to generate allergen-derived synthetic peptides. Two peptides of 27 amino acids that contain some, but not all, of the T-cell epitopes from the *Felis domesticus* cat allergen Fel d 1 chain I have been synthesized (ALLERVAX CAT).[13] In mice subcutaneous tolerization with the peptides prevented the T-cell response to nasal challenge with cat allergen by decreasing the T-cell proliferation to the entire recombinant Fel d 1 chain I.[14] In humans immunotherapy with Fel d 1 peptides resulted in a decrease of clinical symptoms of patients allergic to cats and bronchial reactivity after nasal challenge with Fel d 1 in a cat room.[15] Several studies reporting the modulation of IL-4 and/or IFN-γ release by allergen-specific T cells have suggested a correlation between successful immunotherapy and the induction of a phenotypic switch from a T_{H2} to a T_{H0}[16] or a T_{H1} cytokine phenotype.[17-19] However, the mechanisms of action of immunotherapy with peptides such as those derived from Fel d 1 are still unknown.

Here we describe the results from a double-blind, placebo-controlled study carried out in 31 patients allergic to cats making use of Fel d 1 peptides to evaluate the clinical (bronchial challenge with a standardized cat extract) and immunologic (IL-4 and IFN-γ release by

From [a]the INSERM U. 454 and [b]the Clinique des Maladies Respiratoires, Hôpital Arnaud de Villeneuve, Montpellier; and [c]ImmuLogic, Waltham.
Supported by Hoechst Marion Roussel, Kansas City, Mo.
Received for publication May 27, 1997; revised May 26, 1998; accepted for publication June 4, 1998.
Reprint requests: Jérôme Pène, PhD, INSERM U.454, Hôpital Arnaud de Villeneuve, 375, avenue du Doyen G. Giraud, 34295 Montpellier cedex 5, France.

0091-6749/98 $5.00 + 0 **1/1/92394**

TABLE I. Treatment schedule with Fel d 1 peptides

Treatment*	n	Weeks 1†	2	3	4	5	6
Placebo	6	0	0	0	0	0	0
Low dose	2	0	0	0	0	7.5	7.5
Low dose	2	0	0	7.5	7.5	7.5	7.5
Low dose	4	7.5	7.5	7.5	7.5	7.5	7.5
Medium dose	1	0	0	0	0	75	75
Medium dose	1	0	0	75	75	75	75
Medium dose	4	75	75	75	75	75	75
High dose	3	0	0	0	0	750	750
High dose	4	0	0	750	750	750	750
High dose	4	750	750	750	750	750	750

*The patients were clustered in 4 groups according to the treatment they received: placebo or low, medium, or high doses of Fel d 1 peptides.
†Values are amount of ALLERVAX CAT received (μg/mL).

PBMCs, as well as serum Fel d 1–specific IgE and IgG) effects of the treatment.

METHODS

Patients

Nineteen men and 12 women, ranging in age from 20 to 37 years (mean ± SD: 27.7 ± 5.1 years), were recruited in the Allergy Department of the Montpellier Hospital on the basis of a clinical history of allergy to cats and enrolled in the present double-blind, placebo-controlled study of cytokine release by PBMC in vitro. All patients were first seen with (1) a positive skin prick test response performed as previously described[20] to the Aquagen extract, (2) a positive bronchial challenge to the same extract, and (3) the presence of cat dander-specific serum IgE. All patients had respiratory symptoms during cat exposure. Moreover, they had positive methacholine and cat allergen challenge results. Many patients were also sensitive to other allergens. Patients, matched for asthma severity, had mild asthma, the definition of asthma being based on recent guidelines.[21] Those with an FEV_1 below 70% of predicted value, with more than 3 asthma attacks per week, and those having a cat in their household or being routinely exposed to a cat were excluded from the study. None of the patients had received immunotherapy of any form during the 5 years preceding the study. None of the patients had been treated with systemic or inhaled corticosteroids, sodium cromoglycate, nedocromil sodium, theophylline, or ketotifen for the previous 3 months in view of the possible immunomodulatory effects of these drugs.[22,23]

The study was approved by the Ethics Committee of the Montpellier University, and written consent was obtained from each patient.

Reagents

Purified recombinant human IL-4 (1.0×10^7 U/mg) and IFN-γ (1.0×10^6 IU/mg) were purchased from Genzyme S.A. (Paris, France). The rat anti-human IL-4 mAb 11B4 and the 2 anti-IFN-γ mAbs A35 and B27 were kind gifts from Dr. F. Rousset (Schering-Plough France, Dardilly, France). The 11B4 mAb was used as ascite fluid, whereas for mAbs A35 and B27, the Ig fraction was purified from ascite fluids by adsorption onto a G column (Pierce Europe B.V., Oud-Beijerland, The Netherlands) according to the instructions of the manufacturer. The mAb B27 was biotinylated by coupling LC-biotin (Pierce) as previously described.[24] A lyophilized and standardized cat extract (Aquagen; ALK, Laboratories, Horsholm, Denmark) prepared according to the guidelines of the Allergen Subcommittee of the International Unions of Immunological Societies and reconstituted in isotonic saline was used for bronchial provocation tests (BPTs) and for T-cell stimulations in vitro. These extracts contained 150 μg of Fel d 1/mL.

Specific immunotherapy

This study was part of a large European multicentric, double-blind, placebo-controlled study on clinical efficacy of Fel d 1 peptides that included 140 patients receiving multiple doses of placebo or 7.5, 75, or 750 μg of ALLERVAX CAT. Patients were placed in 3 groups depending on the cumulative dose of ALLERVAX CAT received: 6 patients received placebo, 8 patients received 15 to 45 μg and were placed in the "low dose" group, 6 patients received 150 to 450 μg and were placed in the "medium dose" group, and 11 patients received 1500 to 4500 μg and were placed in the "high dose" group. Fel d 1 peptides or placebo were administrated by subcutaneous injection once a week for 6 weeks. Assignment of subjects to study medication was at random. Patients who received the treatment for less than 6 weeks were given placebo on the other weeks. The protocol of immunotherapy is given in Table I.

Fel d 1 peptides (ImmuLogic Pharmaceutical, Waltham, Mass) were provided as sterile, injectable, freeze-dried powder. Each vial contained 7.5, 75, or 750 μg of the 2 T-cell epitope-containing peptides IPC-1 and IPC-2 in sodium phosphate and mannitol excipient.[13] For injection, the peptides were reconstituted with water and diluted in isotonic saline the day of the administration. Placebo vials were of identical appearance and contained the same excipients.

All subjects underwent the exact same investigations, including BPTs to cat extract and immunologic tests carried out before immunotherapy and 6 weeks after the last injection of study medication. More specifically, methacholine challenge was carried out 2 weeks before the first allergen challenge. Bronchial challenges were carried out 2 weeks before immunotherapy and 6 weeks after immunotherapy, which consisted of 6 weekly injections of peptide or placebo for every patient. Skin tests were done just before allergen provocation, 2 weeks before immunotherapy, and 6 weeks after immunotherapy. Blood was drawn at 8 AM the day of the bronchial challenge.

BPTs

BPTs were performed by using the Aquagen extract that was freshly reconstituted at each test day. Dilutions from 10^8 to 10^6 SQU/mL were used. Pulmonary function tests were carried out with the same equipment (Pneumoscreen; E. Jaeger Laboratories, Würzburg, Germany). Administration of all drugs that might interfere with performance of BPTs, in particular β_2-agonists, was interrupted for at least 12 hours. The baseline FEV_1 was always over 70% of predicted value. Diluent or allergen extracts were delivered by using an automatic, inhalation-synchronized dosimeter jet nebulizer (Spira Elektro 2; Respiratory Care Center, Hameenlinna, Finland).[25] The reproducibility of the generation of the aerosols was controlled by a constant aerosolization pressure of 2 bar. The dosimeter recorded the number of inhalations taken by each patient. The bronchial response to aerosol challenge was assessed by measuring serial FEV_1. Inhalations were continued until $PD_{20}FEV_1$ was obtained or until the maximal concentration had been administered.

Allergen-specific IgE and IgG

Fel d 1–specific serum IgE and IgG levels were measured with an ELISA as previously described.[13]

Cytokine release by PBMCs stimulated by cat extract

Twenty milliliters of peripheral venous blood from each patient was drawn into a tube containing heparin, and PBMCs were isolat-

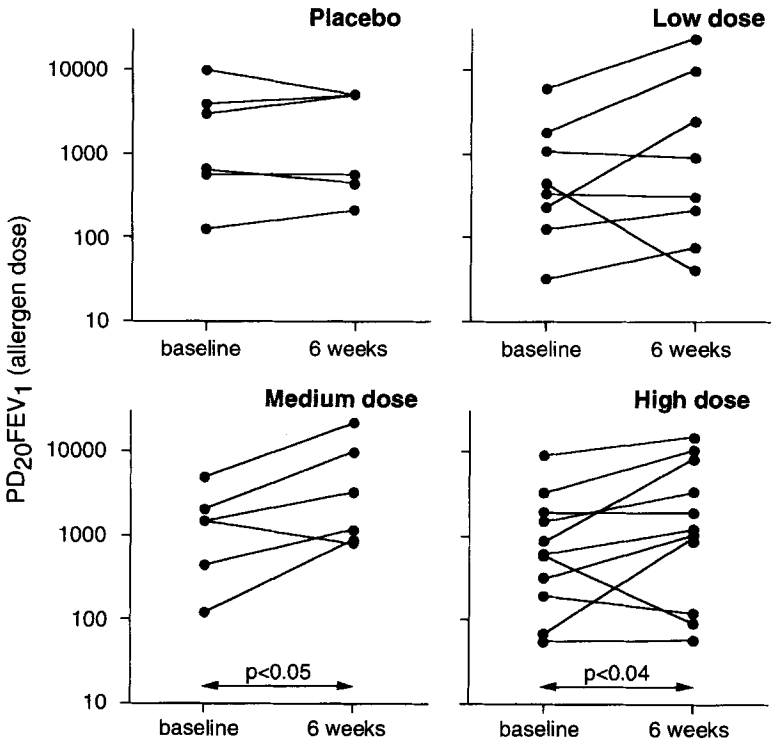

FIG 1. Effect of Fel d 1 peptides on cat allergen $PD_{20}FEV_1$. Patients were divided into 4 groups according to the treatment they received as indicated in Methods section. Bronchial challenge of patients was performed as described in Methods section before (baseline) and after the specific immunotherapy with Fel d 1 peptides (6 weeks). Statistical analysis is according to the Wilcoxon signed-rank test.

ed as previously described.[11] Then the PBMCs were resuspended in Iscove's modified Dulbecco's culture medium (TechGen International, Les Ulis, France) supplemented as described by Yssel et al,[26] with 5% autologous patient's serum.

For testing the cytokine release by PBMCs, the cells were plated at 2.0×10^6 cells/mL in 48-well cluster plates (Costar Co, Cambridge, Mass) in a volume of 0.5 mL/well.[11] Cells were stimulated at 37° C in a humidified atmosphere containing 5% CO_2 in the presence of increasing concentrations of the Aquagen extract (2.4×10^{-3} to 15.0 μg/mL of Fel d 1) for 24 hours. The cultures were carried out in duplicate. A positive control activation was performed simultaneously by stimulating the cells with phorbol myristate acetate (PMA) (10 ng/mL) and calcium ionophore A23187 (100 ng/mL), both purchased from Sigma Chimie (St-Quentin Fallavier, France). Culture supernatants were then harvested, centrifuged for 10 minutes at 4° C, and stored at –20° C until cytokine measurement. The cell viability, assessed by trypan blue exclusion, was always over 95%.

Cytokines released by PBMCs were measured by cytokine-specific ELISA in 96-well, flat-bottomed microtiter plates (Nunc-Immuno Plate Maxisorp, Rosekilde, Denmark) as described previously,[11,27,28] with slight modifications. For IL-4, the capture antibody was made of a polyclonal goat anti-IL-4 antiserum (R & D Systems Europe Ltd, Abingdon, UK) 5 μg/mL and the tracer antibody of the anti-IL-4 rat mAb 11B4 diluted at 2 μg/mL. Calibration curves consisted of serial dilutions of purified recombinant IL-4 (Genzyme). A horseradish peroxidase–conjugated goat anti-rat Ig (Tago Inc. Burlingame, Calif) diluted 1/5000 was used for labeling. ELISA for IFN-γ used the mAb A35 2.0 μg/mL and the biotinylated mAb B27 1.0 μg/mL as capture and tracer antibodies, respectively. Calibration curves were made with serial dilutions of purified recombinant IFN-γ (Genzyme). A streptavidin-alkaline phosphatase conjugate (Tago) diluted 1/5000 was used for labelling. The sensitivity of the assay was 40 pg/mL for IL-4 and 20 pg/mL for IFN-γ.

For each patient, cytokine release in vitro was compared before and after immunotherapy at an optimal concentration of allergen extract that was determined as the one resulting in the highest release of cytokine.

Histamine release by PBMCs stimulated by Fel d 1 extract

Before immunotherapy, histamine release was determined in parallel to the cytokine production in 10 donors. Unstimulated PBMCs or PBMCs activated with PMA + A23187 or Fel d 1 extract were studied. Histamine was measured by using a highly specific and sensitive enzyme-immunoassay (Immunotech, Luminy, France) with a mAb against acylated histamine.[29] The sensitivity of the assay was 0.2 nmol/L.

Statistical analysis

Statistical analysis was performed by means of nonparametric tests. The overall interpatient analysis was performed with the Kruskall-Wallis test. The Mann-Whitney U test was used for intergroup analysis, and the Wilcoxon signed-rank test was used for the intragroup analysis of the subjects studied before and after the end of immunotherapy.

TABLE II. Effect of immunotherapy on serum-specific IgE and IgG titers

	IgE (U/mL)*		IgG (U/mL)*	
Treatment†	Baseline‡	6 weeks§	Baseline‡	6 weeks§
Placebo	263.7 ± 622.3	175.9 ± 395.0	11.6 ± 4.3	11.2 ± 3.0
Low dose	218.4 ± 441.7	288.0 ± 598.8	18.1 ± 15.3	25.9 ± 31.6
Medium dose	40.2 ± 56.4	31.3 ± 47.4	13.6 ± 7.1	12.4 ± 7.5
High dose	186.1 ± 398.5	131.5 ± 238.5	13.7 ± 9.1	13.9 ± 7.4

Values are expressed as mean ± SD.

*Serum cat allergen–specific IgE and IgG were titrated as described in Methods section.

†Patients received weekly injections of either placebo or 7.5 μg (low dose), 75 μg (medium dose), or 750 μg (high dose of Fel d 1 peptides as described in the Methods section.

‡IgE and IgG titers before immunotherapy.

§IgE and IgG titers after the end of immunotherapy.

RESULTS

Clinical efficacy of peptide immunotherapy

$PD_{20}FEV_1$ varied widely between the different groups of patients. As shown in Fig 1, 6 weeks after ending immunotherapy, posttreatment $PD_{20}FEV_1$ was not significantly different between the treated and placebo groups. However, in the medium and high groups there was a significant improvement between baseline and posttreatment days. The effects of administration of low doses of peptide on the $PD_{20}FEV_1$ were less pronounced, whereas 3 of 8 patients in these group had a decrease in $PD_{20}FEV_1$. Bronchial challenge in patients treated with placebo was similar before and after treatment (Fig 1).

Immunotherapy does not modify serum allergen–specific IgE and IgG titers

Concentration of cat allergen–specific IgE and IgG in the serum of the patients 6 weeks after the end of immunotherapy were compared with the serum levels obtained before its initiation (Table II). There were no significant changes in IgE and IgG levels after administration of either placebo or Fel d 1 peptides whatever the dose of peptide received and the number of injections, indicating that immunotherapy with various doses of Fel d 1 peptides had no effect on the serum levels of Fel d 1–specific IgE and IgG antibodies.

In vitro activation of PBMCs by allergen extract induces cytokine production

The cytokine release by PBMCs was tested before the immunotherapy protocol had been initiated and 6 weeks after its end. PBMCs from 17 of the 31 patients allergic to cats spontaneously released IL-4 when cultured in vitro for 24 hours, whereas those of the 14 other patients did not produce detectable levels of IL-4 (mean ± SD for the entire group: 77 ± 55 pg/mL). PBMCs from 20 patients produced IFN-γ spontaneously, whereas for the 11 other patients, the IFN-γ levels were under the limit of sensitivity of the assay (mean ± SD for the entire group: 230 ± 430 pg/mL).

As shown in Fig 2, stimulation of the PBMCs for 24 hours with the cat allergen extract resulted in an increased release of IL-4 and IFN-γ before immunotherapy, and this effect was found to be statistically dose dependent for the production of IL-4 only ($P < .0001$, Kruskall-Wallis test). The maximal stimulatory dose was different for the 2 cytokines because the maximal production of IFN-γ was observed at a concentration of allergen that was about 25-fold greater than that for IL-4. Depending on the patients, optimal allergen concentration for induction of IL-4 secretion ranged from 0.0024 to 0.3 μg/mL and peaked generally at 0.06 μg/mL (mean ± SD of net maximal allergen-induced IL-4 production: 89 ± 80 pg/mL; $P < .0001$, Mann-Whitney U test). At the highest allergen concentrations, there was a strong decrease in IL-4 production. After immunotherapy, the dose-response curve was lowered by comparison with that before immunotherapy, and IL-4 production was found not to be statistically dose dependent, although the doses causing the maximal IL-4 response were of the same order as those obtained before treatment (from 0.0024 to 0.3 μg/mL). The optimal allergen concentration for inducing IFN-γ secretion ranged from 0.06 to 4.5 μg/mL depending on the patients and peaked generally at 1.5 μg/mL (mean ± SD of net maximal IFN-γ production: 75 ± 144 pg/mL; $P < .04$, Wilcoxon W test). Doses of allergen greater than 1.5 μg/mL induced a decrease of IFN-γ production.

Stimulation of the PBMCs with the combination of PMA and A23187, which served as the positive control, induced the production of IL-4 (mean ± SD of net induced IL-4 production: 494 ± 1317 pg/mL; $P < .0001$, Wilcoxon *W* test) in all but 1 patient. PMA/A23187-induced stimulation almost always resulted in a greater release of IL-4 as compared with Fel d 1–specific stimulation ($P < .02$, Wilcoxon *W* test). In all but 1 patient, IFN-γ was also significantly higher on PMA + A23187 activation as compared with spontaneous levels (mean ± SD of net IFN-γ production: 4400 ± 8170 pg/mL; $P < .0001$, Wilcoxon *W* test). This nonspecific stimulation almost always resulted in a greater release of IFN-γ than cat allergen ($P < .0001$, Wilcoxon *W* test).

Cat allergen–induced IL-4 and IFN-γ release is not due to basophil activation

To determine whether the IL-4 detected in the culture supernatants was produced by basophils or T cells, the

cytokine release by the PBMCs of 10 of the 31 donors allergic to cats was compared with that of histamine. First, we regularly found less than 0.1% of basophils in the PBMC preparations of all patients. As shown in Fig 3, PBMCs of all patients spontaneously released histamine, and histamine levels were not significantly increased after stimulation for 24 hours with Fel d 1 or PMA and A23187. However, the spontaneous IL-4 release by the PBMCs was increased significantly on Fel d 1– or PMA/A23187-induced activation (both $P < .008$, Wilcoxon W test). IFN-γ release by the PBMCs was also significantly increased by the Fel d 1 activation ($P < .02$, Wilcoxon W test) and by the PMA + A23187 activation ($P < .006$, Wilcoxon W test). Finally, a 4-hour activation resulted in the secretion of similar levels of histamine (not shown), and histamine levels detected in the culture supernatants were stable because measurements were found constant until 72 hours in culture (not shown). These results suggest that the IL-4 release 24 hours after allergen stimulation is not due to the production of basophils.

Cat allergen–induced IL-4, but not IFN-γ, release by activated PBMCs is suppressed

The maximal in vitro release of IL-4 by PBMCs 6 weeks after the end of the treatment with Fel d 1 peptides was compared with that obtained before its initiation as determined in the allergen concentration range defined in Fig 2. The spontaneous in vitro release of IL-4 by PBMCs was not modified by Fel d 1 peptide injections or by placebo (not shown).

It was found that PBMCs from most patients treated with Fel d 1 peptides produced lower levels of IL-4 after in vitro stimulation with Fel d 1 as compared with IL-4 production by these cells before immunotherapy ($P < .0007$, Wilcoxon W test). However, as shown in Fig 4, only immunotherapy with the high doses of peptide resulted in a significant decrease in Fel d 1–induced IL-4 production ($P < .005$, Wilcoxon W test), whereas in the other groups the decrease in IL-4 production was statistically nonsignificant. There was no correlation between ΔIL-4 release and $\Delta PD_{20}FEV_1$. Treatment with Fel d 1 peptides had no effect on the polyclonally induced production of IL-4 after stimulation with PMA and A23187 (results not shown), indicating that the intrinsic capacity of the cells to produce this cytokine was not affected. IL-4 production after stimulation with Fel d 1 peptides or with PMA and A23187 was unaltered in the placebo-treated patient group. Interestingly, Fel d 1 treatment at any of the doses administered did not significantly modulate the in vitro Fel d 1–induced production of IFN-γ by PBMCs 6 weeks after the end of the treatment (not shown).

DISCUSSION

The results presented in this study show that immunotherapy with Fel d 1 peptides (ALLERVAX CAT) of individuals allergic to cats results in an increase of $PD_{20}FEV_1$ and is accompanied by a suppression of the

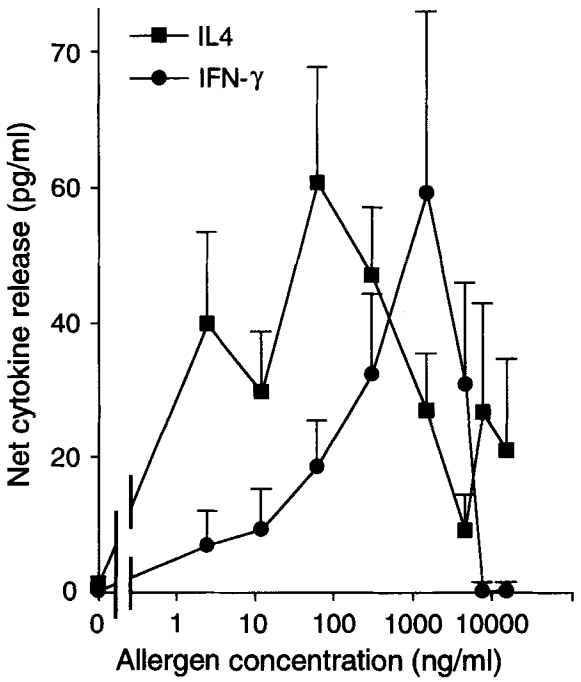

FIG 2. Allergen dose-response curves. Before immunotherapy, PBMCs of 31 patients were stimulated in vitro by increasing amounts of cat allergen extract as described in the Methods section. Cytokine release in culture supernatants was determined by ELISA methods as described in Methods section. Values represent net cytokine release ± SEM. Net cytokine release = cytokine amount – cytokine release in absence of allergen.

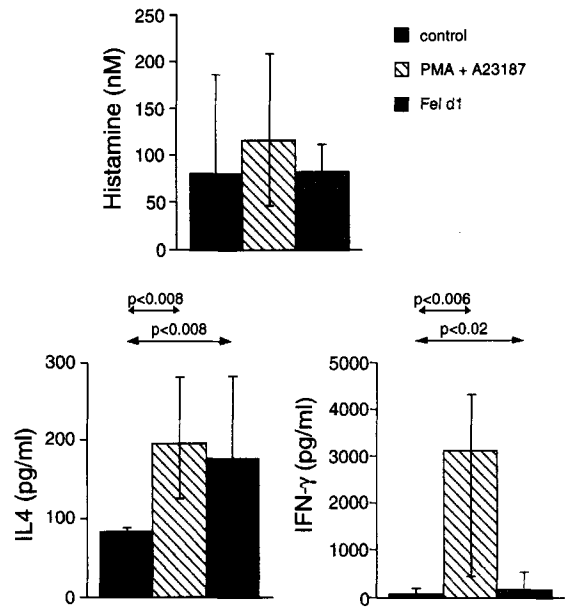

FIG 3. Comparison of histamine and cytokine release by PBMCs. PBMCs of 10 patients were activated with Fel d 1 or PMA + A23187, and level of histamine, IL-4, and IFN-γ was determined in culture supernatants as indicated in Methods section. Results are expressed in means ± SD. Statistical analysis is according to Wilcoxon signed-rank test.

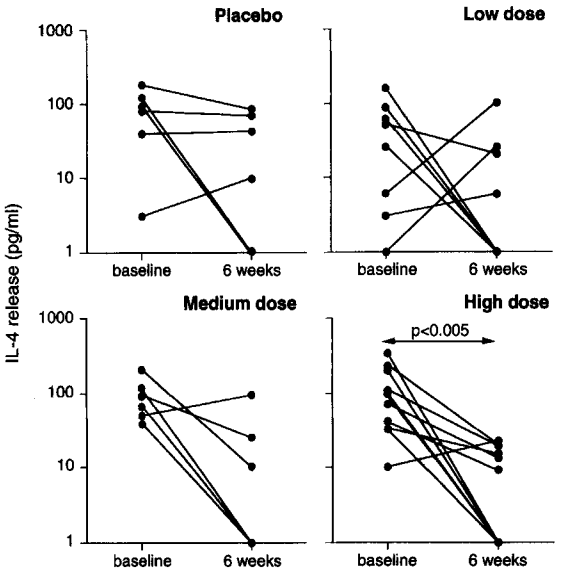

FIG 4. Effect of Fel d 1 peptides on cat allergen–induced IL-4 release. Patients were divided into 4 groups according to treatment they received as indicated in Methods section. PBMCs collected from patients before (baseline) and after specific immunotherapy with Fel d 1 peptides (6 weeks) were stimulated in vitro by various amounts of cat allergen extract as described in Methods section. IL-4 release in culture supernatants was determined by ELISA as described in Methods section. Only optimal IL-4 response is presented. Statistical analysis is according to Wilcoxon signed-rank test.

in vitro IL-4 release by PBMCs stimulated with a cat allergen extract, whereas the production of IFN-γ was not affected. These data suggest that peptide immunotherapy of allergic patients may be acting by altering the cytokine profile of the in vitro cat allergen–induced response of PBMCs from the T_{H2}-like to the T_{H0}-like phenotype.

It has not been possible to have the same number of patients in each group of donors, which complicates the statistical analysis of the data. The difference between the numbers of patients in each group was related to the fact that the study was randomized across the centers, and some patients had systemic reactions such that we had to stop the protocol before the second evaluation. This was particularly the case for the medium dose in the present center.

BPTs are widely used to assess the effectiveness of specific immunotherapy, but they should be performed very carefully. Because the quality of the allergen extract is critical, in this study we used a standardized extract with known potency and validity. The results of our study show that immunotherapy with medium and high doses of Fel d 1 peptides significantly increase $PD_{20}FEV_1$, confirming previous studies carried out with cat room challenges.[15] However, it should be noted that the improvement in $PD_{20}FEV_1$ does not necessarily imply that patients are protected during exposure to a living cat.[12]

We did not find a correlation between IL-4 suppression induced by immunotherapy with Fel d 1 peptides and the clinical improvement measured by BPTs with cat allergen. It is possible that the cytokines measured may not be correlated with the effects of bronchial challenge because IL-4 and IFN-γ are related to IgE production. In addition, because the frequency of allergen-specific T cells has been shown to be extremely low,[30] although a change in cytokines was observed in peripheral blood, confirmation of the results should be obtained by studying nasal or bronchial tissues. Recent studies have investigated the role of immunotherapy on the production of IL-5.[19,31] However, IL-5 is more related to the occurrence of the late-phase allergic reaction than to the early-phase allergic reaction. The late-phase reaction could not be examined according to the study design of the present multicentric study.

This study was part of a large European multicentric, double-blind, placebo-controlled study on the clinical efficacy of ALLERVAX CAT, which included 140 patients. In the study set-up, individual patients in each of the groups received either the relevant peptide dose during 6 subsequent injections or a combination of the peptide and the placebo (patients who received treatment for less than 6 weeks were given placebo on the other weeks). Although the data analysis should have taken into consideration this classification, it would have yielded results that are not statistically significant because of the low number of patients studied. Therefore to have a sufficient number of patients, we combined patients into 4 groups (placebo and low-, medium-, and high-dose groups). Depending on the final clinical results of the whole study, it appears that the selection was correct. These results accord with previously published data, showing significant improvement irrespective of the injection protocol.[15]

In vitro activation of the patients' PBMCs by a cat allergen extract induced the release of IL-4 and, to a lesser extent, IFN-γ. Although the IL-4 production induced by Fel d 1 stimulation was low compared with the spontaneous release, it was detectable, and a strong statistical difference was found ($P < .0001$). In addition, the effects of the cat allergen extract on IL-4 production were dose dependent, indicating the stimulation of T cells. The production of IL-4 is, however, not restricted to T cells. Preformed IL-4 is expressed in tissue human mast cells and released on cell activation[32,33] and is found in the cytoplasm of in vitro–activated human peripheral blood basophils from normal donors.[34] On IgE-dependent activation, the IL-4 mRNA is transcribed in human basophils that produce IL-4 in addition to various mediators.[35,36] Although basophil numbers are low in peripheral blood, these cells produce higher levels of IL-4 on a per cell basis than T cells. The very low frequency of allergen-specific T cells in PBMCs[30,37,38] and the fact that IL-4 is a key cytokine required for the development of T_{H2}-type responses support the hypothesis that basophils may contribute to the initiation and maintenance of allergic reactions as one of the principal source of early IL-4 production.[39] Our results suggest that, using our model and time course of mediator release, it is unlikely that the

observed IL-4 release was caused by the activation of basophils instead of T cells. This is in contrast to the results of Kasaian et al[39] who reported that basophils in Fel d 1–stimulated PBMC cultures may represent the major source of early IL-4 production. In comparison with our results, the percentage of basophils in the PBMCs were higher than those we found, and the histamine release was also higher, whereas the amounts of IL-4 released were much lower than in our experiments. It is possible that these discrepancies may be related to the differences in culture conditions, origin of the Fel d 1 allergen, and ELISA used in both studies. The IL-4 release that we obtained is more similar to that reported by McHugh et al[40] and Simons et al.[41] The possibility that T cells, rather than basophils, are among the targets of immunotherapy is supported by the results of Akoum et al[42] who reported, using in situ hybridization, that venom immunotherapy decreased IL-4 and increased IFN-γ mRNA-expressing peripheral blood T cells.

In this study Fel d 1 peptide immunotherapy, by decreasing IL-4 release without affecting IFN-γ release, may indicate the induction of a shift from a T_{H2}-like memory T-cell response to a T_{H0} response. This shift of the response rapidly occurs after immunotherapy because it was observed 6 weeks after the end of the treatment. However, a similar investigation of the effects of immunotherapy with the Fel d 1 peptides has been performed by Simons et al[41] who reported, in contrast to our study, that immunotherapy did not modify IL-4, IL-10, and IFN-γ production in vitro. These differences might be related to the fact that in this study the Fel d 1 peptides were administered as 4 weekly injections of 250 μg, whereas we found that IL-4 was only significantly reduced in the group of patients receiving injections of 750 μg. In addition, the optimal dose of Fel d 1 used for the stimulation of the PBMCs was higher (500 BAU/mL, which represents 2.9 μg/mL of Fel d 1) than in our study (0.06 μg/mL), and we show that for Fel d 1 concentrations greater than 0.3 μg/mL, there was strong decrease in IL-4 production. The mechanisms of successful allergen immunotherapy with inhalant allergens are not fully understood, but several other recent studies, reporting a modulation of the cytokine profile by allergen-specific T cells, have suggested a switch from a T_{H2} to a T_{H0} phenotype[16,43] or a T_{H1} phenotype.[17-19] Recently, a decrease of serum IL-4 levels in patients with allergic rhinitis after immunotherapy has been reported.[44] Moreover, immunotherapy was reported to alter cytokine mRNA patterns in peripheral blood T cells by decreasing IL-4 mRNA-expressing cells and increasing IFN-γ mRNA-expressing cells.[42] Similarly, Jutel et al[19] observed that bee venom immunotherapy decreased IL-4 and IL-5 production and increased IFN-γ production. Durham et al[43] also reported an increase in the number of cells expressing transcripts for IFN-γ after successful grass pollen immunotherapy and proposed that its mechanisms of action might be the result of the inhibition of allergen-induced T-cell and eosinophil recruitment, as well as eosinophil activation at the sites of allergic inflammation. However, our results observed for IFN-γ release are in contrast with these studies reporting an increase of IFN-γ release after specific immunotherapy with various allergens,[17-19] as well as an increase of mRNA expression.[42,43] One of the possible explanations is that the amounts of peptide administered are far greater than those of allergens in conventional immunotherapy regimens. Moreover, peptides were administered as a single dose in contrast to conventional immunotherapy in which allergens are administered in increasing doses.

We found that cat allergen–specific IgE and IgG levels in the serum were not affected by Fel d 1 peptide immunotherapy. It is tempting to associate the immunotherapy-induced decrease of IL-4 secretion by T cells with a decrease in IgE concentrations. However, such an effect is rarely observed in the short term for immunotherapy with inhalant or venom allergens. Our results are in accordance with those of Jutel et al[19] who showed that the decrease in IL-4 and IL-5 secretion after bee venom immunotherapy was not accompanied by a significant decrease of specific serum IgE antibodies, whereas increased IgG concentrations became only significant after 9 months. On the other hand, our result suggest that peptides may induce an immune response different than that induced by native allergens because in most studies allergen-specific IgG antibodies were increased during treatment with a native aeroallergen or bee venom. However, except for the early effects of venom immunotherapy,[45,46] a significant correlation between serum antibody levels and the clinical benefits of immunotherapy has been rarely observed.[47]

We thank Drs François Rousset and Jacques Banchereau for providing the mAbs anti-IFN-γ, and Dr Alison Campbell for revising the manuscript.

REFERENCES

1. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989;7:145-73.
2. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994;12:227-57.
3. Pène J, Rousset F, Brière F, Chrétien I, Bonnefoy JY, Spits JH, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E2. Proc Natl Acad Sci USA 1988;85:6880-4.
4. Pène J, Rousset F, Brière F, Chrétien I, Paliard X, Bauchereau J, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-γ. J Immunol 1988;141:1218-24.
5. Del Prete G, Maggi E, Parronchi P, Chrétien I, Tiri A, Macchia D, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 1988;140:4193-8.
6. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989;169:1295-307.
7. Gascan H, Gauchat JF, Aversa G, Van Vlasselaer P, De Vries JE. Anti-CD40 monoclonal antibodies or CD4+ T cell clones and IL-4 induce IgG4 and IgE switching in purified human B cells via different signaling pathways. J Immunol 1991;147:8-13.
8. Kelso A. Th1 and Th2 subsets: Paradigms lost? Immunol Today 1995;16:374-9.
9. Kapsenberg ML, Wierenga EA, Bos JD, Jansen HM. Functional subsets of allergen-reactive human CD4+ T cells. Immunol Today 1991;12:392-5.

10. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326:298-304.
11. Pène J, Rivier A, Lagier B, Becker WM, Michel F-B, Bousquet J. Differences in IL-4 release by PBMC are related with heterogeneity of atopy. Immunology 1994;81:58-64.
12. Bousquet J, Michel F-B. Specific immunotherapy in asthma: Is it effective? J Allergy Clin Immunol 1994;94:1-11.
13. Bond JF, Brauer AW, Segal DB, Nault AK, Rogers BL, Kuo MC. Native and recombinant Fel dI as probes into the relationship of allergen structure to human IgE immunoreactivity. Mol Immunol 1993;30:1529-41.
14. Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. Proc Natl Acad Sci USA 1993;90:7608-12.
15. Norman PS, Ohman JL Jr, Long AA, Creticos P, Gefter MA, Shaked Z, et al. Treatment of cat allergy with T-cell reactive peptides. Am J Respir Crit Care Med 1996;154:1623-8.
16. Varney VA, Hamid QA, Gaga M, Ying S, Jacobson M, Frew AJ, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen induced late phase cutaneous responses. J Clin Invest 1993;92:644-51.
17. Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. J Exp Med 1993;178:2123-30.
18. McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine responses from a TH-2 to a TH-1 dominant pattern: comparison of rush and conventional immunotherapy. Clin Exp Allergy 1995;25:828-38.
19. Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-γ secretion in specific allergen-stimulated T cell cultures. J Immunol 1995;154:4187-94.
20. Bousquet J, Calvayrac P, Buerin B, Hejjaoui A, Dhivert H, Hewitt B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. J Allergy Clin Immunol 1985;76:734-44.
21. Global strategy for asthma management and prevention. WHO/NHLBI report. National Institutes of Health, National Heart, Lung and Blood Institute; 1995. Publication number 95-3659.
22. Schmidt J, Fleissner S, Heimann-Weitschat I, Lindstaedt R, Pomberg B, Werner U, et al. Effect of corticosteroids, cyclosporin A, and methotrexate on cytokine release from monocytes and T cell subsets. Immunopharmacology 1994;27:173-9.
23. Schmidt J, Fleissner S, Heimann-Weitschat I, Lindstaedt R, Szelenyi I. The effect of different corticosteroids and cyclosporin A on interleukin 4 and interleukin 5 release from murine TH2 type T cells. Eur J Pharmacol 1994;260:247-50.
24. Chrétien I, Pène J, Brière F, De Waal Malefijt R, Rousset F, De Vries JE. Regulation of human IgE synthesis. I. Human IgE synthesis in vitro is determined by the reciprocal antagonistic effects of interleukin 4 and interferonγ. Eur J Immunol 1990;20:243-51.
25. Nieminen MM, Lahdensuo A, Kellomaeki L, Karvonen J, Muittari A. Methacholine bronchial challenge using a dosimeter with controlled tidal breathing. Thorax 1988;43:896-900.
26. Yssel H, De Vries JE, Koken M, Van Blitterswijk, Spits H. A serum-free medium for the generation and propagation of functional human cytotoxic and helper T cell clones. J Immunol Methods 1984;72:219-25.
27. Pène J, Lagier B, Rivier A, Chanez P, Vendrell JP, Bousquet J. Phenotype of T cell clones obtained from bronchial biopsies and peripheral blood from three asthmatics. Cell Biol Int 1993;17:353-7.
28. Lagier B, Pons N, Rivier A, Chanal I, Chanez P, Bousquet J, et al. Seasonal variations of interleukin-4 and interferon-γ release by peripheral blood mononuclear cells from atopic subjects stimulated by polyclonal activators. J Allergy Clin Immunol 1995;96:932-40.
29. Morel A, Delaage M. Radioimmunoassay for histamine, application to histamine release. J Allergy Clin Immunol 1988;82:646-54.
30. Li Y, Simons FER, Jay FT, Hayglass KT. Allergen-driven limiting dilution analysis of human IL-4 and IFN-γ production in allergic rhinitis and clinically tolerant individuals. Int Immunol 1996;8:897-904.
31. Akdis CA, Akdis M, Blesken T, Wymann D, Alkan SS, Möller U, et al. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. J Clin Invest 1996;98:1676-83.
32. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, et al. Interleukin-4 is localized to and released by human mast cells. J Exp Med 1992;176:1381-6.
33. Bradding P, Feather IH, Wilson S, Barding PG, Meusser CH, Molgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects: the mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. J Immunol 1993;151:3853-65.
34. Mueller R, Heusser CH, Rihs S, Brunner T, Bullock GR, Dahinden CA. Immunolocalisation of intracellular interleukin-4 in normal human peripheral blood basophils. Eur J Immunol 1994;24:2935-40.
35. Brunner T, Heusser CH, Dahinden CA. Human peripheral blood basophils primed by interleukin-3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med 1993;177:605-11.
36. McGlashan DWJ, White JM, Huang S-K, Ono S, Schroeder JT, Lichtenstein LM. Secretion of IL-4 from human basophils. The relationship between IL-4 mRNA and protein in resting and stimulated basophils. J Immunol 1994;152:3006-16.
37. Sager N, Feldman A, Schilling G, Kreitsch P, Neumann C. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: frequency and lymphokine profile in the allergen patch test. J Allergy Clin Immunol 1992;89:801-10.
38. Burastero SE, Fenoglio D, Crimi E, Brusasco V, Rossi GA. Frequency of allergen-specific T lymphocytes in blood and bronchial response to allergen in asthma. J Allergy Clin Immunol 1993;91:1075-81.
39. Kasaian MT, Clay MJ, Happ MP, Garman RD, Hirani S, Luqman M. IL-4 production by allergen-stimulated primary cultures: identification of basophils as the major IL-4-producing cell type. Int Immunol 1996;8:1287-97.
40. McHugh S, Deighton J, Rifkin I, Ewan P. Kinetics and functional implications of Th1 and Th2 cytokine production following activation of peripheral blood mononuclear cells in primary culture. Eur J Immunol 1996;26:1260-5.
41. Simons FER, Imada M, Li Y, Watson WTA, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. Int Immunol 1996;8:1937-45.
42. Akoum H, Tsicopoulos A, Vorng H, Wallaert B, Dessaint JP, Joseph M, et al. Venom immunotherapy modulates interleukin-4 and interferon-γ messenger RNA expression of peripheral T lymphocytes. Immunology 1996;87:593-8.
43. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RW, McKay IS, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4(+) T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-γ. J Allergy Clin Immunol 1996;97:1356-65.
44. Ohashi Y, Nakai Y, Okamoto H, Ohno Y, Sakamoto H, Sugiura Y, et al. Serum level of interleukin-4 in patients with perennial allergic rhinitis during allergen-specific immunotherapy. Scand J Immunol 1996;43:680-6.
45. Golden DB, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. J Allergy Clin Immunol 1982;69:489-93.
46. Golden DB, Lawrence ID, Hamilton RH, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. J Allergy Clin Immunol 1992;90:386-93.
47. Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. II. Comparison between parameters assessing the efficacy of immunotherapy. J Allergy Clin Immunol 1988;82:439-46.