

Original article

The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects

Background: We previously showed that overlapping Fel d 1-derived T-cell peptides inhibited surrogate markers of allergy (i.e. early and late-phase skin reactions and T-cell function) in cat allergic subjects. The present pilot study was designed to determine whether this treatment affected clinically relevant outcome measurements such as the allergen-induced nasal and bronchial reactions, and asthma/rhinitis quality of life (QOL).

Methods: Sixteen cat-allergic asthmatic subjects who gave a dual (early and late) asthmatic response (DAR) to inhaled cat allergen were randomly assigned to receive either Fel d 1 peptides (approximately 300 µg in increasing, divided doses) or placebo (8 active : 8 placebo). Twelve single early responders (SER) were also studied in an open fashion design. Allergen-induced bronchial and nasal measurements as well as the QOL was measured at baseline, 4–8 weeks (follow-up 1 (FU1)) and 3–4 months (FU2).

Results: In the active, but not placebo, group there were significant decreases in the late asthmatic reaction (LAR) to whole cat dander ($P = 0.03$) at FU2 but with no between group difference. There were also significant improvements in asthma quality of life (QOL) scores [asthma-activity limitation ($P = 0.014$); rhinitis-sleep ($P = 0.024$), non-nose/non-eye symptoms ($P = 0.031$), nasal problems ($P = 0.015$)]. In the open study Fel d 1 peptide treatment resulted in significant decreases in number of sneezes ($P = 0.05$), weight of nasal secretions ($P = 0.04$) and nasal blockage ($P = 0.01$) following allergen challenge.

Conclusions: Multiple, short, overlapping Fel d 1 T-cell peptides have potential for inhibiting upper and lower airway outcome measurements in cat allergic patients. Larger, dose-ranging, studies are required before firm conclusions on clinical efficacy of peptide allergen therapy can be made.

C. Alexander, M. Tarzi, M. Larché, A. B. Kay

Department of Allergy and Clinical Immunology, Faculty of Medicine, Imperial College, National Heart & Lung Institute, London, UK

Key words: asthma; epitope; immunological tolerance; rhinitis; T lymphocyte.

A. B. Kay
Leukocyte Biology
Division of Biomedical Sciences
Imperial College
Room 374, Sir Alexander Fleming Building
South Kensington Campus
London
SW7 2AZ
UK

Accepted for publication 6 November 2004

It is well established that unpredictable, immunoglobulin E (IgE)-mediated, anaphylactic reactions limit the usefulness of conventional, whole allergen-specific immunotherapy. In an attempt to circumvent this problem, short, allergen-derived peptides have been evaluated in the treatment of atopic allergic disease (1–3). Short peptides have been shown to inhibit T-cell function whilst having only very limited ability to cause anaphylaxis due to the loss of three-dimensional B-cell epitopes.

In a previous study we prepared 12 short, synthetic, overlapping peptides encompassing most of the T-cell epitopes of the major cat allergen Fel d 1, for use as a potential T-cell allergy vaccine (1). In a double-blind, placebo-controlled study, the peptides inhibited the whole allergen-induced early- and late-phase allergic skin reactions and production of interleukin (IL-4, IL-13) and interferon- γ by peripheral blood cells in culture (2). There were also increases in IL-10, consistent with the concept that therapy may involve T-cell-associated regulatory pathways. However, there was no significant change in the allergen PD₂₀ (as a measure of the early bronchial

reaction to inhalation of cat allergen), or in the methacholine PC₂₀ (as an index of airway hyperresponsiveness) and nasal outcomes were not measured. As the cumulative amount of peptide given in the study was relatively small (approximately 90 µg of each peptide), we hypothesized that higher doses would be more efficacious (as has been shown with conventional whole allergen immunotherapy). Accordingly we have conducted a pilot study in which we gave cat-allergic asthmatic subjects approximately 300 µg of Fel d 1 peptides (intradermal injection in saline) in escalating, divided doses, specifically to determine their effects on allergen-induced rhinitis and asthma. Subjects also completed validated rhinitis and asthma quality of life (QOL) questionnaires (4, 5).

Materials and methods

The study received prior approval from the Ethics Committee of the Royal Brompton and Harefield Hospitals NHS Trust. Subjects were recruited by advertisement. Written, witnessed informed consent

was obtained. The age range was 18–55 years. During the previous 12 months all subjects had a clear history of asthma, e.g. cough, wheeze, dyspnoea and a history of sneezing, rhinorrhoea, or nasal blockage (rhinitic) symptoms on exposure to cats. Subjects had a positive skin prick test to cat dander allergen extract (ALK, Hørsholm, Denmark) and a cutaneous late-phase response to intradermal challenge with cat dander allergen extract resulting in induration of at least 3 cm in diameter. Subjects had a forced expiratory flow rate in 1 s (FEV₁) of >70% of predicted normal and had not had previous allergen injection immunotherapy. Subjects were nonsmokers and had no history of current illness or clinically significant abnormalities in routine haematology, biochemistry or urinalysis. Subjects took inhaled bronchodilators as required. No subject had received oral corticosteroids within the previous 6 months, or inhaled steroid within the previous 2 months, or nasal corticosteroids within the previous 7 days. Cat ownership was not associated with differences in clinical outcomes or prevalence of peptide-induced late asthmatic reactions (LARs). All noncat owners had intermittent contact with cats.

Study design

The study was conducted in 2001–2002. Subjects were divided into ‘single early’, or ‘dual’ (early and late), responders on the basis of their response (change in FEV₁) to inhalation of whole cat dander. The methods for eliciting early- and late-phase asthmatic reactions have previously been described (6). A positive early asthmatic reaction (EAR) was defined as a 20% reduction (or greater) in FEV₁ and an LAR as 15% reduction or greater. Dual responders were randomly assigned to receive either Fel d 1 peptides, or placebo in a randomized, placebo-controlled and double-blind fashion (8 received active, 8 placebo) using a power calculation based on a previous study (2). The single early responders (12 subjects) took part in an open-study. All subjects (open study and placebo-controlled study) were scheduled to be given 1, 5, 10, 25, 50, 100 and 100 µg (total 291 µg), of each of 12 peptides as a mixture, at 14-day intervals by intradermal injection, followed by observation for 6 h. Individuals who developed a late-phase asthmatic reaction following peptides, had the same dose given on the next or subsequent occasion and were not up-dosed until the peptide-induced LAR was less than 20% of baseline FEV₁. As a result of dose alterations in certain subjects, individuals in each treatment group received a median (with range) cumulative dose of peptides as follows: DBPC active arm: 294 µg (216–341) Open Study: 274 µg (131–316).

The study comprised a 6-week enrolment and run-in period for baseline measurements, a minimum of 12 weeks of intradermal administrations of Fel d 1 peptides or placebo: a 4–8 -week follow-up period (FU1) with repeat baseline measurements, a 3–4 -month follow-up period (FU2) with repeat baseline measurements. Subjects who were skin test positive to grass or trees were studied outside of the pollen season.

Outcome measures were the early-phase and late-phase asthmatic reaction to whole cat dander extract, nasal symptoms after challenge (sneezing, weight of secretions, nasal inspiratory flow rate (NIFR) and nasal blockage) and validated asthma/rhinitis QOL scores as previously described in detail (4, 5).

A flow chart of the study design is shown in Fig. 1.

Peptide synthesis and validation

Overlapping peptides from chains 1 and 2 of Fel d 1 were synthesized by F-moc chemistry at the Advanced Biotechnology Centre, Imperial College, London, UK. Mixing and dispensing of the peptides under sterile conditions for single patient

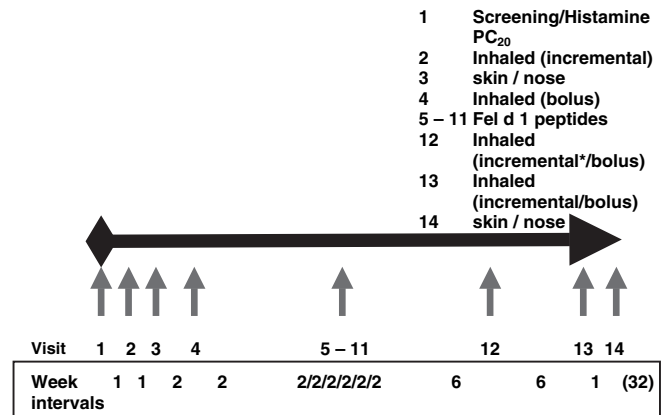


Figure 1. Study flow chart. Volunteers were initially screened by skin prick test, radioallergosorbent test (RAST) and history. Those fulfilling the inclusion criteria, were entered into either the open, or placebo-controlled arm of the study depending upon whether they experienced a single early asthmatic reaction or a dual reaction to inhaled incremental allergen challenge on visit 2. Open and DBPC study subjects underwent the same procedures and measurements, with the exception that in the open study allergen PD₂₀ was compared by incremental challenge and in the DBPC the response to bolus challenge was compared with area under the curve late-phase reaction measurements. *Inhaled allergen challenges in the open study were incremental (‘pre-treatment’ visit 3 vs ‘post-treatment’ visit 12 and visit 13). Inhaled allergen challenges in the DBPC study were bolus (‘pre-treatment’ visit 4 vs ‘post-treatment’ visit 12 and visit 13).

use were performed at Nova Laboratories Ltd, Leicester, UK. The sequences were: (i) EICPAVKRDVDLFLTGT, (ii) FLTGTPEYVEQVAQY, (iii) EQVAQYKALPVVLENA, (iv) KALPVVLENARILKNCV, (v) RILKNCVDKMTTEEDKE, (vi) KMTEEDKENALSLLDK, (vii) KENALSVDKIYTSPL, (viii) LTKVNATEPERTAMKK, (ix) TAMKKIQDCYVENGLI, (x) SRVLDGLVMTTISSSK, (xi) ISSKDCMGEAVQNTV, (xii) AVQNTVEDLKLNTLGR. In a previous study it was determined that they produced <2% histamine release from basophil-enriched PBMC, *in vitro*, over the concentration range 0.02–100 µg/ml (1). In contrast, whole cat extract (0.02–100 µg/ml) induced up to 40% release of the total histamine, in a dose response fashion, as previously reported (1).

Clinical measurements

Forced expiratory flow rate (FEV₁) was recorded at 5, 15, and 30 min and hourly for 6 h after each dose of peptides. A late phase asthmatic reaction (LPR) to intradermal Fel d1 peptides is defined as a reduction in FEV₁ of 20% (or greater) below baseline measured between 1 and 6 h from peptide administration. The peptide preparation was dispensed by an independent individual with no patient contact. Peptides or placebo were administered to subjects by a blinded investigator (CA). Clinical outcomes [skin tests, spirometry and histamine PC₂₀ (open study only)] were measured by a second blinded investigator (Research Nurse) using previously described methods (1, 2).

Nasal allergen challenge was performed with volunteers breath-holding in maximum inspiration. One actuation of 0.1 ml of the cat allergen solution (Hal Labs, Haarlem, the Netherlands) was sprayed

into each nostril from a metered-dose, atomized pump spray. Volunteers were asked to gently mouth breathe for 1 min after each allergen spray. This was to prevent inadvertent inhalation of the allergenic extract. The nasal inspiratory flow rate (NIFR) was measured using the in-check inspiratory flow meter (Clement Clarke International Ltd, Harlow, Essex, UK) at baseline, 5, 15, 30, 60 min and hourly after the challenge for a further 5 h. The number of sneezes and the extent of nasal blockage was recorded for the first 15 and 60 min and the weight of nasal secretions was measured by the change in weight of paper tissues used for the first 15 and 60 min.

Statistical analysis

Statistical analysis was performed with the aid of a commercial software package (Minitab, State College, PA) and $P = 0.05$ or less, was considered as significant. All interventions were analysed on paired data and the Wilcoxon signed rank test.

Results

Baseline characteristics and completion of study

The baseline characteristics of the subjects are shown in Table 1. The active and placebo groups were well

Table 1. Baseline Characteristics

Characteristics	Active (double-blind)	Placebo (double-blind)	Active (open study)
Number	8	8	12
Sex			
Men	3	2	4
Women	5	6	8
Age	37.5 (30–49)	39.1 (28–50)	30.5 (23–52)
Baseline FEV ₁ (% predicted)	101.3 (87.4–113.4)	99.8 (84–109.5)	105.6 (92.3–109.6)
Total IgE (IU/ml)	343.5 (54–1454)	271.1 (62–725)	418.5 (37–2223)
Cat RAST (IU/ml)	13.4 (1.04–34)	11.5 (0.64–101)	16.4 (0.35–48.6)
Eosinophils ($\times 10^9/l$)	0.35 (0.1–1.0)	0.24 (0.1–0.4)	0.31 (0.2–0.6)

Values are number or mean (range). RAST = radio-allergo-sorbent test.

matched for gender, age, baseline FEV₁, cat RAST, total IgE and blood eosinophil counts. Seven individuals in the placebo group and six in the active treatment group, were skin test positive to three or more allergens. Twenty-eight subjects completed the entire study; the remaining two (1 placebo and 1 open study) dropped-out due to inability to provide the time commitment.

Adverse events

None of the subjects developed an early (within 1 h) asthmatic reaction following i.d. administration of Fel d 1 peptides. There were no systemic anaphylactic reactions to the peptides. Cutaneous weal and flare reactions were observed with both active and placebo injections, but were more frequent and of greater magnitude in subjects receiving peptides. In some cases a dose-response effect was observed.

Late asthmatic reactions

As expected five of the subjects receiving active treatment in the double-blind study and three in the open study, had isolated LARs. One subject in the placebo group had an LAR. Late asthmatic reactions were well tolerated (reported as being 'similar to being exposed to a cat') and did not require treatment with epinephrine, nebulized bronchodilators or corticosteroids. Two subjects required treatment with 200 µg salbutamol delivered through an Accuhaler device. All reactions resolved within 8 h of peptide administration.

Effects of treatment

Double-blind study. There was a significant within group difference in the cat allergen-induced late (but not the early) asthmatic reaction at FU2 in those receiving active ($P = 0.03$) but not those receiving placebo (Fig. 2). There

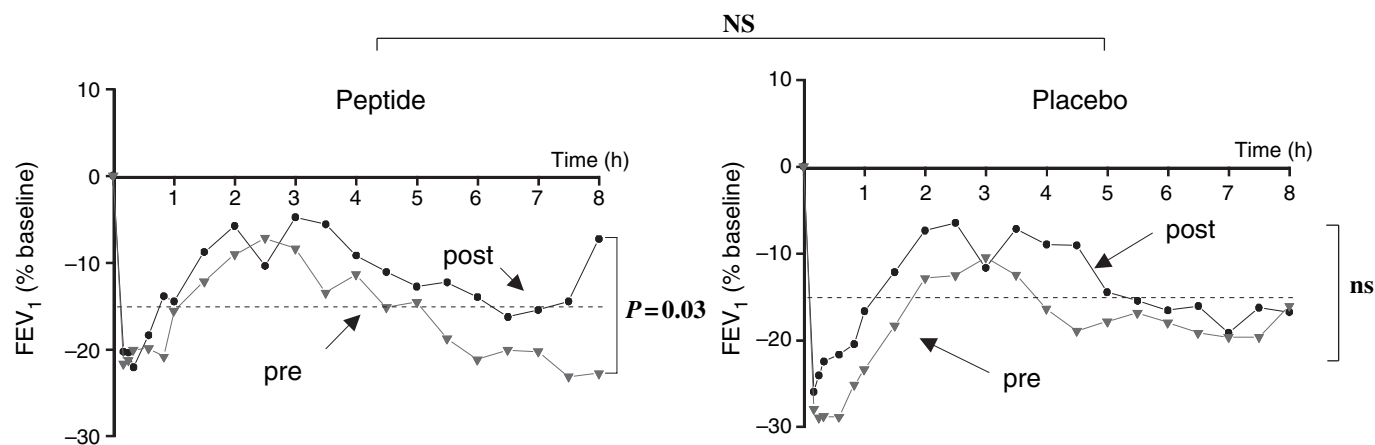


Figure 2. Effect of Fel d 1 peptide therapy on the late asthmatic reaction elicited by whole cat dander. Eight cat allergic subjects were treated with a total of 294 µg of Fel d 1 peptides in incremental doses and eight with placebo in a double-blind fashion. Whole cat allergen-induced changes in forced expiratory volume (FEV₁) were measured at baseline (pre) and at 12 weeks (post).

Table 2. Changes in Quality of Life in cat asthmatics before and after T-cell peptide therapy

Field	Within group (active Rx only)	Subject numbers (active/placebo)	Between group
Asthma			
Symptoms	0.823	6/5	0.429
Activity limitation	0.076	6/5	0.014
Emotional function	0.590	6/5	0.391
Environmental stimuli	0.062	6/5	0.125
Rhinitis			
Activities	0.374	7/7	0.804
Sleep	0.125	7/7	0.024
Non-nose/eye symp	0.035	7/7	0.031
Practical problems	0.016	7/7	0.440
Nasal problems	0.015	7/7	0.015
Eye symptoms	0.502	7/7	0.897
Emotional function	0.290	7/7	0.426

Quality of life was assessed using the 'Juniper' Asthma QOL and Rhinitis QOL (4,5). Subjects received either active or placebo treatment. Data were available for 17 patients in all. Six were excluded from the analysis of the asthma QOL (numbers 2, 5, 7, 8, 11 and 15) and three from the analysis of the rhinitis QOL (numbers 5, 11 and 15) due to incomplete datasets. The significance of the change from pre- to post-treatment was tested with a paired *t*-test or Wilcoxon signed rank test as appropriate. The probability and the test are shown in the table. The difference in the change between the two groups was tested either by *t*-test, or Wilcoxon rank sum test as appropriate.

were no significant between-group differences at FU2. No significant within-, or between-group differences in the early or late asthmatic response were observed at FU1. No changes were observed, at either follow up, in the nasal outcomes. There were significant improvements in several QOL outcomes (recorded at FU2 only) in subjects receiving active treatment (Table 2). These were asthma [activity limitation ($P = 0.014$); rhinitis [sleep ($P = 0.024$), non-nose/non-eye symptoms ($P = 0.031$), nasal problems ($P = 0.015$)].

Open study. Single early responders (open study), but not dual asthmatic responders receiving active treatment, had significant decreases in the number of sneezes ($P = 0.05$), weight of nasal secretions ($P = 0.04$) and nasal blockage scores ($P = 0.01$) 15 min after allergen challenge (Fig. 3). There were no differences in the magnitude of the early asthmatic reaction or the histamine PC₂₀ (median value 6 mg/ml pre- and 5.8 mg/ml post-peptide therapy). Quality of life outcomes were not measured in this group.

Discussion

This was a pilot study designed to determine whether Fel d 1-derived T-cell peptides had effects on upper and lower airway clinical outcomes in cat-allergic subjects. We found a small but significant inhibition of the LPR to inhaled allergen challenge in dual responders at the

second follow up in patients receiving the active preparation. The data indicate that the clinical effect of this dosing regimen was quite modest. However, the study involved small numbers. The results are consistent with the work of Pène et al. (7) who showed that Fel d 1-derived peptides IPC-1 and IPC-2 increased the allergen PD₂₀. It is possible that a longer follow-up period, i.e. 9–12, months may have revealed a more impressive effect on the late-phase response, but this was logistically impossible because the patient population we were studying were itinerant. However, it is relevant that the QOL asthma symptom score, at least as far as activity limitation is concerned, was significantly better after treatment with the active preparation.

Although we administered three times the dose of a previous study, this high concentration may not necessarily improve efficacy. Recent animal experiments (8) indicate that peptide-induced T-cell hyporesponsiveness may be effectively achieved at low dose, with reduced efficacy at higher doses. Furthermore, local reactions induced by intradermal peptide may have induced a pro-inflammatory rather than a tolerogenic response as indicated in other systems. In earlier studies, lower peptide doses were employed and were associated with promising clinical surrogate outcomes. For example, a single dose of 5 µg of each of the 12 peptides in a mixture resulted in a 50% reduction in the magnitude of the cutaneous late-phase reaction to allergen challenge (1). In another study (9), subjects received peptides starting at lower doses (0.1, 1.0, 5.0, 10, 25 µg at 2 week intervals). Interestingly, none of these subjects experienced LAR following peptide dosing. Treatment was associated with significantly improved PC₂₀ and virtual abrogation of the cutaneous LPR to allergen challenge. Thus, it may be important to deliver peptides in low doses and with a greater dose interval, so as not to initiate pro-inflammatory signals (such as mast cell degranulation at the point of injection), in order to achieve optimal effect.

Mechanistic data from the double-blind arm of this study have been reported elsewhere. There was a significant reduction in peripheral blood mononuclear cells (PBMC) proliferation and IL-13 production from blood T-cell cultures (10). This reduction in allergen-specific T-cell responses was not associated with alteration of the suppressive ability of peripheral blood CD4+CD25+ T-cells following peptide therapy. These results indicate that 'natural' regulatory T-cells may not have an important role in peptide-induced hyporesponsiveness. However, we have recently defined a suppressor activity within the CD4+ PBMC population after peptide therapy (11), suggesting the induction of an 'adaptive' population of regulator/suppressor cells. The latter class of cells have been shown to reside in both the CD4 and CD8 compartment and may be CD25-negative.

In this study, as previously, there was a number of peptide-induced late asthmatic reactions all of which were

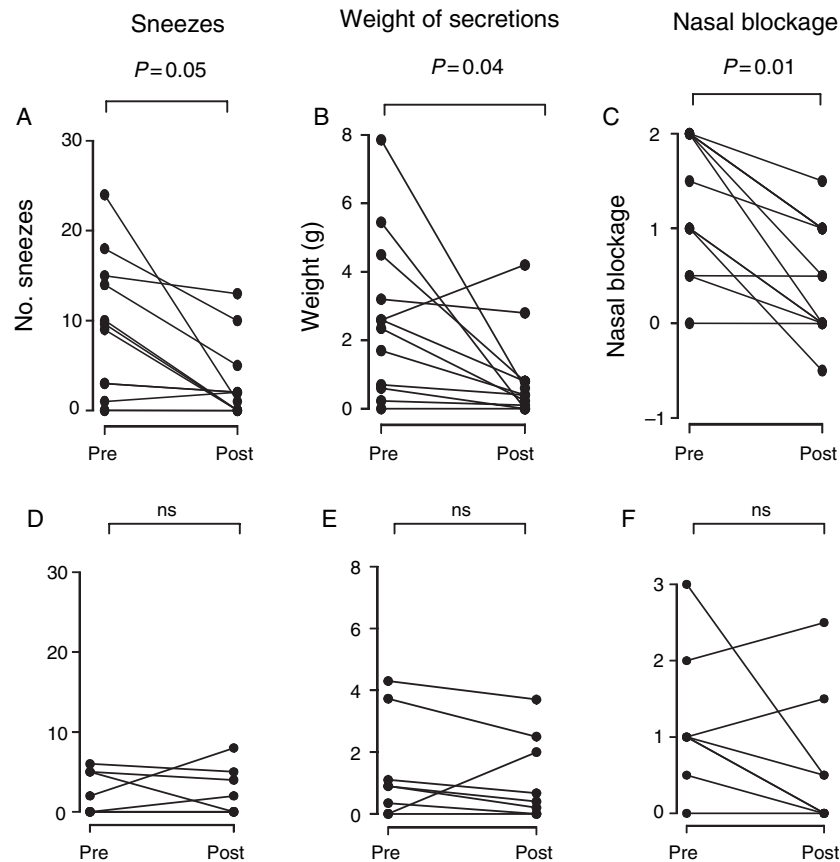


Figure 3. The effect of Fel d 1 peptides on nasal symptoms induced by whole cat dander. Cat allergic subjects were treated with a total of 274 µg of Fel d 1 peptides in incremental doses in an open fashion. Whole cat allergen-induced nasal outcomes (15 min values) were performed at baseline (pre) and at 12 weeks (post). A, B and C are single early responders (the open study) and D, E and F the subjects in the double-blind study (dual responders) who received active treatment.

of a similar pattern and magnitude to those previously reported (1, 2, 12). Data suggest that peptide-induced LARs may be minimized by commencing the induction course with small doses of peptide i.e. 0.1 µg as described above and also by extension of the dosing interval.

Decreases in the number of sneezes, weight of nasal secretions and degree of nasal blockage 15 min after challenge were observed in the open study, but not in the subjects receiving active treatment in the double-blind study. Interestingly, the baseline sneezing and weight of secretion tended to be lower in the subjects receiving active treatment in the double-blind study (i.e. subjects who had dual reactions) than in the open study (subjects who had single early asthmatic reactions) (Fig. 3). Although these differences were not significant, it suggested that cat-allergic subjects with a single early response had a higher degree of nasal reactivity than those with a dual early- and late-phase response. It should also be noted that the IgE concentrations were non-significantly elevated in the subjects in the open study as opposed to the 16 subjects in the double-blind study. Nevertheless, despite the negative nasal challenge data in

the double-blind study, there were several significant improvements as assessed by the QOL evaluation. Thus, with rhinitis; interference with sleep, non-nose/non-eye symptoms and nasal problems improved after therapy.

In summary, the results from this relatively small study indicates that T-cell peptide therapy may be of benefit in upper and lower airway allergic disease, but clearly larger numbers are required for firm conclusions. The results are encouraging although the significant nasal outcomes were from the open arm of the study (and this limitation is accepted). It is now important to perform a dose-ranging study with larger numbers in order to determine what concentrations of peptides provide optimal efficacy.

Acknowledgments

The authors are grateful to Justine Arbery (Research Nurse) who was responsible for patient recruitment, clinical observations, data management and documentation, and Jackie Turner for statistical analyses. The work was supported by the Medical Research Council (UK), Asthma UK and the British Medical Association (BMA).

References

1. Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001;**167**:1734–1739.
2. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;**360**:47–53.
3. Müller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;**101**:747–754.
4. Juniper EF, Thompson AK, Ferrie PJ, Roberts JN. Validation of the standardized version of the Rhinoconjunctivitis Quality of Life Questionnaire. *J Allergy Clin Immunol* 1999;**104**:364–369.
5. Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest* 1999;**115**:1265–1270.
6. Khan LN, Kon OM, Macfarlane AJ, Meng Q, Ying S, Barnes NC et al. Attenuation of the allergen-induced late asthmatic reaction by cyclosporin A is associated with inhibition of bronchial eosinophils, IL-5, GM-CSF and eotaxin. *Am J Respir Crit Care Med* 2000;**162**:1377–1382.
7. Pene J, Desroches A, Paradis L, Lebel B, Farce M, Nicodemus CF et al. Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T-cells of patients allergic to cats. *J Allergy Clin Immunol* 1998;**102**:571–578.
8. Apostolou I, Von Boehmer H. In vivo instruction of suppressor commitment in naive T-cells. *J Exp Med* 2004;**199**:1401–1408.
9. Alexander C, Oldfield WL, Shirley K, Larche M, Kay AB. A dosing protocol of allergen-derived T cell peptide epitopes for the treatment of allergic disease. *J Allergy Clin Immunol* 2001;**107**:716 abs.
10. Smith TRF, Alexander C, Kay AB, Larche M, Robinson DS. Cat allergen peptide immunotherapy reduces CD4+ T cell responses to cat allergen but does not alter suppression by CD4+ CD25+ T cells: double-blind placebo-controlled study. *Allergy* 2004;**59**:1097–1101.
11. Verhoef A, Alexander C, Kay AB, Larche M. Allergen-based peptide immunotherapy is associated with functional modifications in allergen-specific T cell subpopulations. *J Allergy Clin Immunol* 2004;**113**:S255.
12. Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;**189**:1885–1894.