

Mechanisms of disease

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

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Summary

Background Some patients with asthma who are allergic to cats and are injected intradermally with short, overlapping, T-cell peptides derived from Fel d 1 develop late asthmatic reactions to the peptides, which are associated with a reduction in late-phase skin reactions induced by whole allergens and bronchial hyporesponsiveness to the peptides on the second injection. We aimed to ascertain the effect of multiple injections on the magnitude of the early and late phase skin reactions to intact allergens.

Methods After a 9-week run-in period, we randomly assigned patients with asthma and allergies to cats to receive either Fel d 1 peptides (90 µg in increasing divided doses) or placebo. The primary outcome was late-phase cutaneous reactions to whole cat dander. Outcomes were measured at baseline, 4–8 weeks, and 3–9 months. Analysis was by intention to treat.

Findings 16 patients were randomly assigned to the peptides, and eight to placebo. All patients completed the course of injections. Four of the 16 patients on Fel d 1 peptides had initial late asthmatic reactions, but could be desensitised to the higher dose of peptide. Patients in the peptide group but not the placebo group had a significant reduction in the size of their late reaction to whole cat dander between baseline and both follow-ups, but the difference between groups was not significant (first follow-up, difference -422.8 mm² [95% CI -1115.0 to 269.4], $p=0.43$; second follow-up -1180.8 mm² [-2216.8 to -144.8], $p=0.058$). The size of the late reaction to Fel d 1 significantly differed between treatment groups at both follow-ups. At second follow-up, the size of the early reaction to Fel d 1, but not to whole cat dander was significantly reduced in those on peptides compared with those on placebo. The concentration of interferon γ and of interleukin 4 and 13, and the amount of proliferation, significantly decreased between baseline and second follow-up, and the concentration of interleukin 10 was significantly higher in patients on peptides, however, none of these values differed significantly between groups. Patients on peptides had a significantly greater decrease in the concentration of interferon γ and interleukin 13, and in the amount of proliferation between baseline and first follow-up than did those on placebo.

Interpretation Several, short, overlapping Fel d 1 T-cell peptides have potential in treatment of cat allergy.

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Introduction

Unpredictable anaphylactic reactions mediated by IgE restrict the usefulness of conventional immunotherapy specific to whole allergens for treatment of atopic allergic disease. Tolerance of FEL D 1 by peripheral T cells can be induced in both naive and primed mice by subcutaneous injection of peptides from this major cat allergen.¹ Since short peptides derived from the allergen inhibit T-cell function but have restricted ability to cause anaphylaxis owing to loss of three dimensional B cell epitopes, these peptides could be effective as therapeutic vaccines. Accordingly, Norman and colleagues² and others^{3–5} attempted to treat patients allergic to cats by subcutaneous injection of two peptides (IPC1 and IPC2) that spanned a large proportion of chain 1 of Fel d 1. In some studies,^{2–4} IPC1 and IPC2 were effective at high doses, but administration of these peptides was associated with allergic symptoms that arose between 10 min and 6 h after injections. Because of the length of this peptide (27 aminoacids), the immediate reactions induced by IPC1 and IPC2 may have been the result of cross linking of IgE. Thus, we synthesised three peptides derived from chain 1 of Fel d 1. The peptides were small (16–17 residues) so that they could be presented to T cells without antigen processing and so that they would be unlikely to cross link allergen-specific IgE. At first, we were interested in the late (6 h) reactions described by Norman and colleagues,² which we postulated were mediated by T cells. Nine of 40 patients with asthma and allergies to cats who were injected with these peptides developed a late asthmatic reaction, which was dependent on T cells and restricted to specific HLA haplotypes, without first developing an early response dependent on IgE or mast cells.⁶

We then prepared 12 short overlapping peptides derived from Fel d 1, which spanned most of chain 1 and chain 2 and presumably bind to the many MHC restriction elements present in an outbred population.⁷ The 12 peptides provoked late asthmatic reactions in a dose-dependent fashion, with 50% of patients developing a reaction at the highest dose. However a second injection was associated with an attenuation of this peptide-specific hyper-responsiveness, with up to 40 weeks needed for return to baseline values. The cutaneous late-phase reaction to whole cat dander was also inhibited, as were proliferation of peripheral blood mononuclear cells induced by peptide and whole allergens and production of Th2 (interleukin 4 and 13) and Th1 (interferon γ) cytokines in cultures. Therefore a single injection of allergen-derived peptides induced tolerance to subsequent peptide injection in the target organ (the lung), reduced late-phase cutaneous responsiveness to whole allergen, and changed the reactivity of T cells in vitro.

In this placebo-controlled, double-blind study, we aimed to ascertain whether individuals who are allergic to cats can tolerate increasing, divided doses of multiple, short, peptides derived from Fel d 1 and whether this tolerance

GLOSSARY

ACTIVATION-INDUCED CELL DEATH

Apoptosis induced in lymphocytes following activation through the antigen receptor.

EARLY AND LATE PHASE ALLERGIC REACTIONS

Allergic reactions provoked in vivo by allergen challenge (usually of the nose, skin, eyes, or by inhalation). The reactions are often biphasic with an early (mast cell/histamine) phase arising within minutes and a late (inflammatory cell) phase arising 3–9 h later.

FEL D 1

Cat (*Felis domesticus*) allergen 1.

inhibits the EARLY AND LATE-PHASE ALLERGIC REACTIONS induced by whole cat dander. We also aimed to determine whether late allergic reactions induced by peptides were associated with the subsequent change in these established clinical surrogate markers of allergy.^{8–10} Other outcomes included the allergen inhalation PD₂₀, production of Th1/Th2 and regulatory cytokines by blood mononuclear cells, and tolerance of cats.

Methods

Patients

Patients were enrolled by an investigator (WLGO) and the study nurse, and allocated to receive either active or placebo treatment by a random allocation sequence generated by the study statistician at the start of the study. This sequence was held by an independent investigator who dispensed the study drugs.

Patients in the study group received 5 µg, 10 µg, 25 µg, and 50 µg (total dose 90 µg) peptide at 3–4 day intervals over 2 weeks and were observed for 6 h after each injection. Individuals who developed a late-phase asthmatic reaction had the same dose given on the next or subsequent occasion and did not have their dose increased until the FEV₁ fell by less than 20% from baseline in response to the peptide. Subsequent doses were adjusted so that every patient received a total dose of 90 µg peptide. To maintain masking, five injections of the same volume of either active peptide or placebo were given. The patients, investigators administering the treatments, and investigators doing the clinical and laboratory analyses remained unaware of the randomisation status until the study was completed.

The ethics committee of the Royal Brompton and Harefield Hospitals NHS Trust approved the study. Patients were recruited by advertisement between October, 1998, and September, 1999. The study took place between October, 1998, and July, 2000, at the Royal Brompton Hospital, London, UK. We obtained written witnessed informed consent from all patients. 49 patients were screened by telephone, and 24 fulfilled the entry criteria and were subsequently enrolled. The patients' age ranged from

Sequences used to synthesise overlapping peptides

EICPAVKRQVDLFLTG
FLTGTPDEYVEQVAQY
EQVAQYKALPVLLEN
KALPVLLENARILKNCV
RILKNCVDAKMTTEEDKE
KMTTEEDKENALSLLDK
KENALSVDKIYTSPL
LTKVNATEPERTAMKK
TAMKKIQDCYVENGLI
SRVLDGLVMTTISSSK
ISSSKDCMGEAVQNTV
AVQNTVEDLKLNTLGR

	Active (n=16)	Placebo (n=8)
Characteristics		
Sex		
Men	6	4
Women	10	4
Age	40.0 (35.0–46.3)	36.0 (30.0–42.8)
Baseline FEV ₁ (L/min)	93.43 (87.49–107.27)	98.29 (96–107.98)
Methacholine PC ₂₀ (g/L)	0.82 (0.21–1.93)	0.78 (0.45–1.32)
Total IgE (IU/mL)	206 (75–384)	117 (30–758)
Cat RAST* (IU/mL)	13.2 (7.3–38.3)	15.2 (8.7–46.6)
Eosinophil (×10 ⁹ /L)	0.2 (0.18–0.30)	0.2 (0.18–0.40)
Number taking inhaled corticosteroid	8	5
Beclomethasone dipropionate equivalence (µg/day)	1000 (325–1000)	400 (300–900)

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

Baseline characteristics

25 to 50 years (median 36 years). We included patients if they had had a history of symptoms on exposure to cats in the past 12 months, evidence of more than 20% reversibility of the forced expiratory volume in 1 s (FEV₁) after inhaled β₂-agonists, and a greater than 20% fall in FEV₁ (PC₂₀) in response to 4 g/L or less of methacholine. Patients had a positive skin prick test to Fel d 1 and a cutaneous late-phase response to intradermal challenge with whole cat dander and Fel d 1 resulting in induration of at least 3 cm in diameter. They also had an FEV₁ of greater than 70% of predicted normal and had not had previous allergen injection immunotherapy. Patients were non-smokers and had no history of current illness or clinically important abnormalities in routine haematology, biochemistry, or urinalysis. Eight patients in the active group and five on placebo were receiving inhaled corticosteroids (table). Other patients took inhaled bronchodilators as needed. No patient had received oral corticosteroids within the previous 6 months. 15 of the 16 patients in the active group and all in

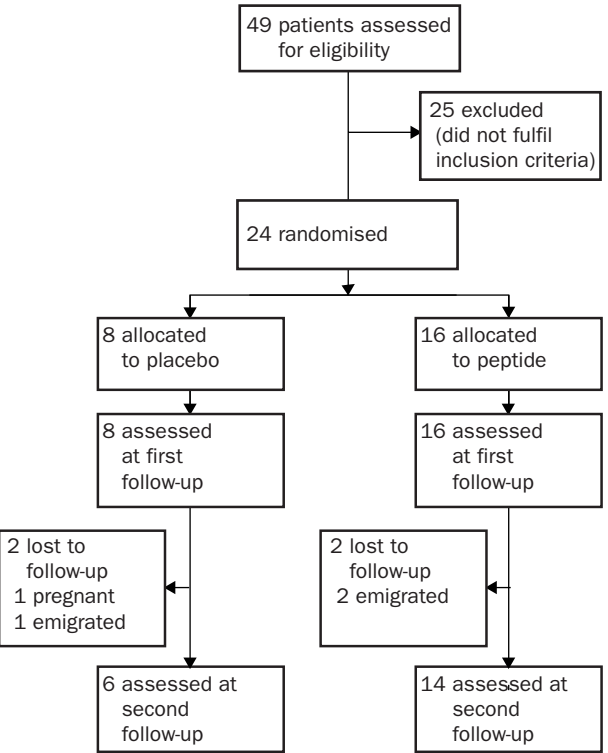


Figure 1: Trial profile

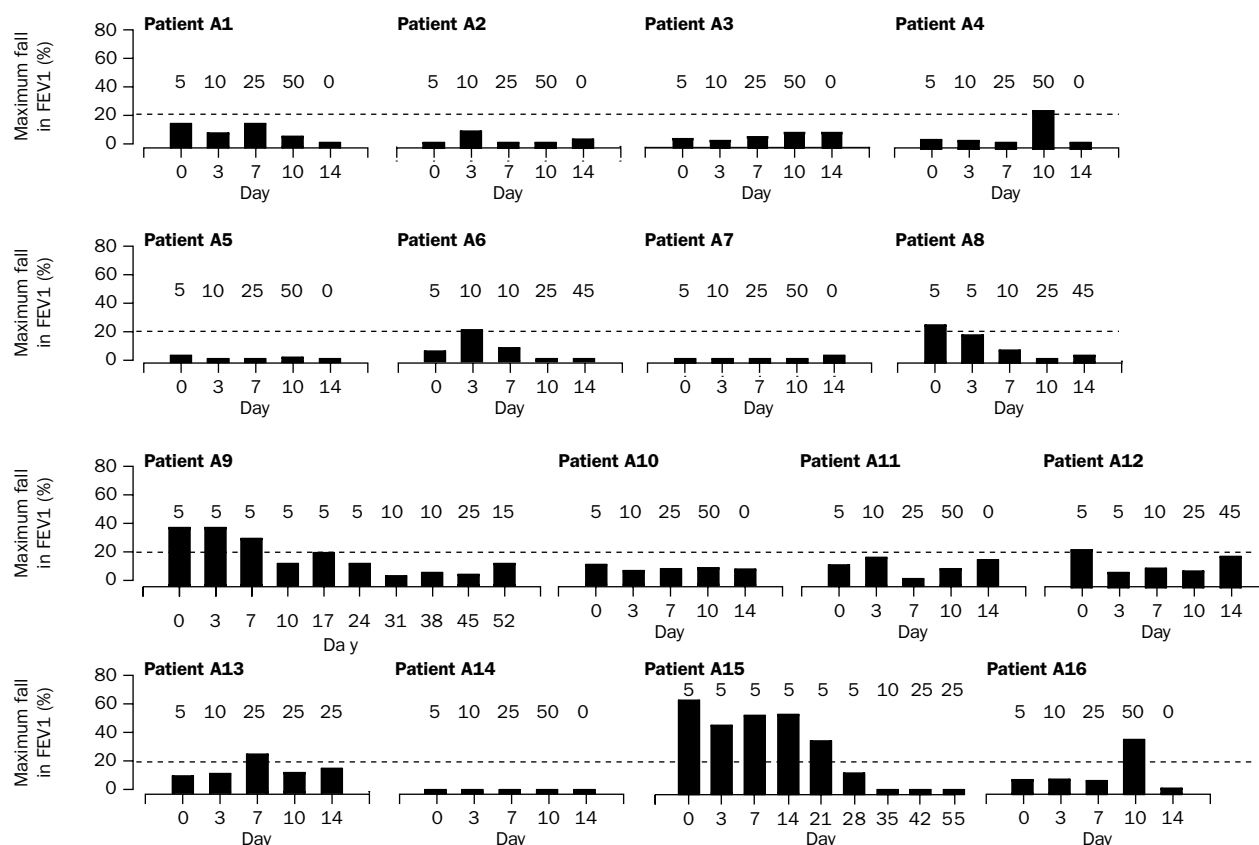


Figure 2: Effect of repeated intradermal injections of Fel d 1-peptides on the magnitude of the peptide-induced late asthmatic reaction

Numbers over each bar indicate the dose of peptide (in µg) administered on that day.

the placebo group had intermittent exposure to cats; three on active treatment and two on placebo owned cats. Cat ownership was not associated with differences in clinical outcomes or frequency of late allergic reactions induced by the peptides. All other patients had intermittent contact.

Study design

The study comprised four phases: a 9-week enrolment and run-in period for baseline measurements, a minimum of 2 weeks of intradermal administration of Fel d 1 peptides or placebo, a 4–8 week follow-up period with repeat baseline measurements, and a 3–9 month follow-up period with repeat baseline measurements. We assessed patients who had a positive skin test to grass or trees outside the pollen season.

The primary outcome was the ability of Fel d 1 peptides to inhibit the late-phase cutaneous reaction to whole cat dander (on the volar surface of the arm). On the basis of data from a previous study,⁷ eight patients on peptides and eight on placebo would give 80% power to detect a 24% reduction in the cutaneous late-phase reaction. However, results of the previous study⁷ suggested that 50% of those receiving peptide would develop late allergic reactions to the initial doses. Thus, we recruited 16 people with asthma to receive peptides to assess whether late allergic reactions induced by the peptide were an essential prerequisite for subsequent induction of tolerance to whole cat dander.

Other outcomes included the early-phase cutaneous reaction to whole cat dander extract, and purified Fel d 1, inhaled whole cat allergen PD₂₀, and the methacholine PC₂₀; the blood mononuclear cell proliferative response; production in cultures of interleukin 4 and 13, production

of interferon γ and interleukin 10 by peripheral blood mononuclear cells; and tolerance of cats on the basis of a symptom score. Tolerance of cats was assessed by a modification of a previously validated visual analogue scale.³ Patients were asked at the end of the study whether their ability to tolerate cats had changed.

Peptide synthesis and validation

We synthesised twelve overlapping peptides spanning most of Fel d 1 by F-moc chemistry at the Advanced Biotechnology Centre, Faculty of Medicine, Imperial College, London, UK. The panel shows the 12 sequences used.

The sequences produced less than 2% histamine release from basophil-enriched peripheral blood mononuclear cells in vitro over 0.02–100.00 mg/L peptide. By contrast, whole cat extract (0.02–100.00 mg/L) induced up to 40% release of the total histamine, in a dose-response fashion.⁷

Procedures

The primary assays for proliferation of peripheral blood mononuclear cells were completed as previously described.⁷ Peripheral blood mononuclear cells were cultured at 10^6 cells/mL in the presence of whole cat dander at a concentration of 100 mg/L for 6 days before assessment of ³H-thymidine incorporation. Stimulation indices (used to allow comparisons before and after stimulation) were calculated by comparison of the wells containing antigen with those containing medium alone.

For cytokine assays, we cultured peripheral blood mononuclear cells 5×10^6 cells/mL in the presence of whole cat dander (100 mg/L), for 6 days before collection of culture supernatants. Supernatants were frozen at

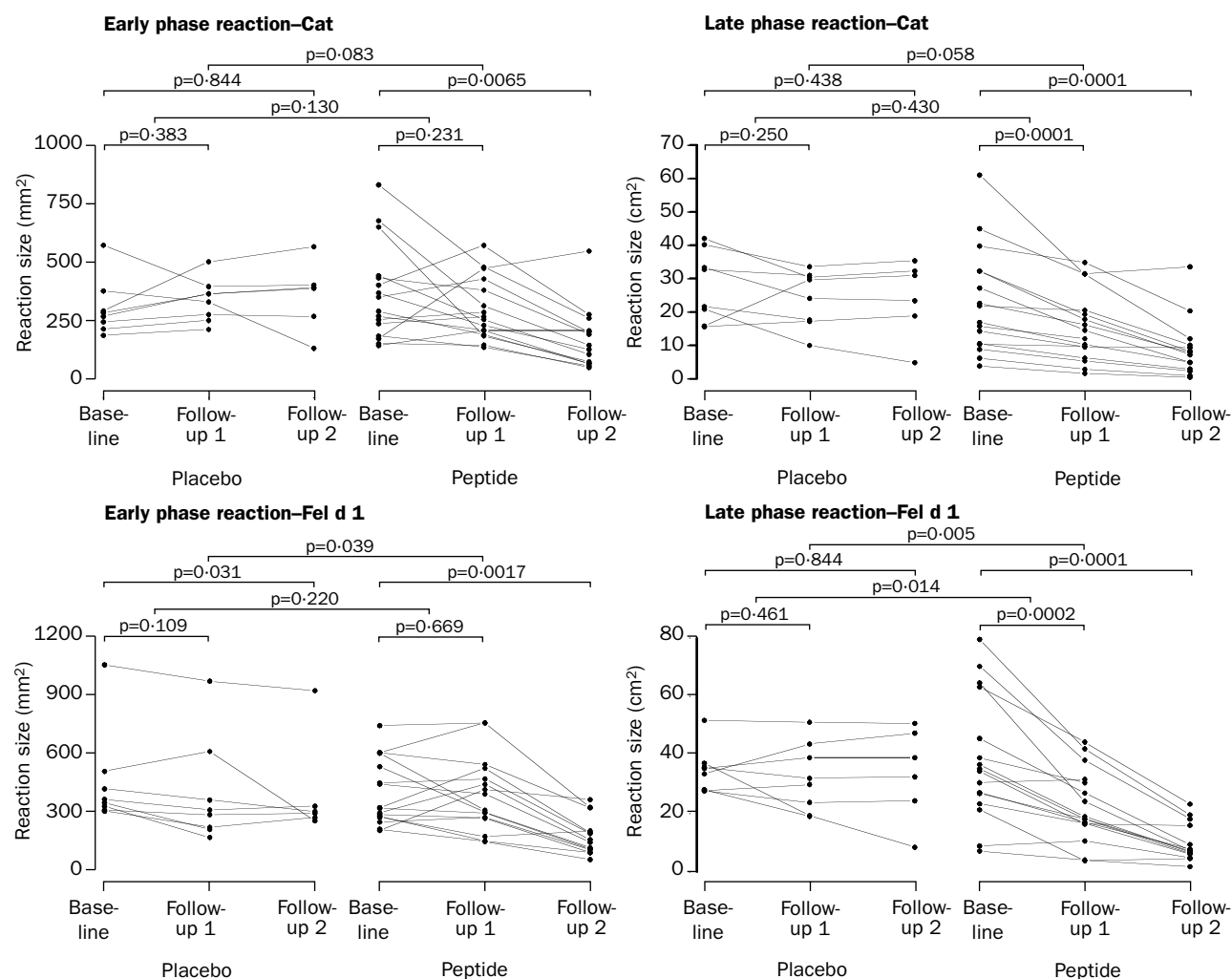


Figure 3: Effect of treatment on early-phase and late-phase cutaneous reactions to whole cat dander and purified Fel d 1

–20°C before analysis. We measured interleukin 4, 13, and 10, and interferon gamma by ELISA (Pelikine, CLB, Amsterdam, Netherlands).

Statistical analyses

Differences in the magnitude of the early and late phase skin reactions to whole cat allergen or Fel d 1 were compared between the active and placebo group with Wilcoxon's rank sum test. The groups were compared by testing for any difference in the change. We tested proliferation and production of cytokines for normality with a modified Shapiro-Wilks test. Significance of changes within groups were tested with a Wilcoxon's rank sum test. Differences between groups were assessed with a Kruskal-Wallis non-parametric ANOVA. Multiple comparisons were not done, so values were judged as significant if they were 0.05 or less.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or in the writing of the report.

Results

The table shows patients' baseline characteristics. The active and placebo groups were well matched for sex, age, FEV₁, PC₂₀ methacholine, cat radio-allergo-sorbent test, and blood eosinophil counts. About half the patients in each group were taking inhaled corticosteroids. Six of eight in the

placebo group, and 14 of 16 in the active treatment group had a positive skin test to three or more allergens. 20 patients completed the study; the remaining four (two active, two placebo) completed the first but not the second follow-up because of either pregnancy or emigration (figure 1).

None of the patients developed an early (within 1 h) asthmatic reaction after intradermal administration of Fel d 1 peptides. No patient had a systemic anaphylactic reaction to the peptides. Cutaneous weal and flare reactions were recorded with active and placebo injections. Eight of 16 patients in the active group had isolated late asthmatic reactions: two of eight patients in the placebo group also had decreases in FEV₁ of more than 20% starting 90–210 min after administration of the peptide, which were possibly due to spontaneous diurnal variation. Late asthmatic reactions were tolerated well ("similar to being exposed to a cat") and did not need treatment with epinephrine, nebulised bronchodilators, or corticosteroids. Two patients needed treatment with 200 µg salbutamol delivered through an Accuhaler device. All reactions resolved within 8 h of peptide administration.

After the first injection (5 µg) of peptide, two patients receiving active treatment (A9 and A15) had reductions in FEV₁ of 37% and 62%, respectively (figure 2). These patients needed ten and nine injections, respectively, and they gradually tolerated successive doses up to a total dose of 90 µg (but the interval between later injections was

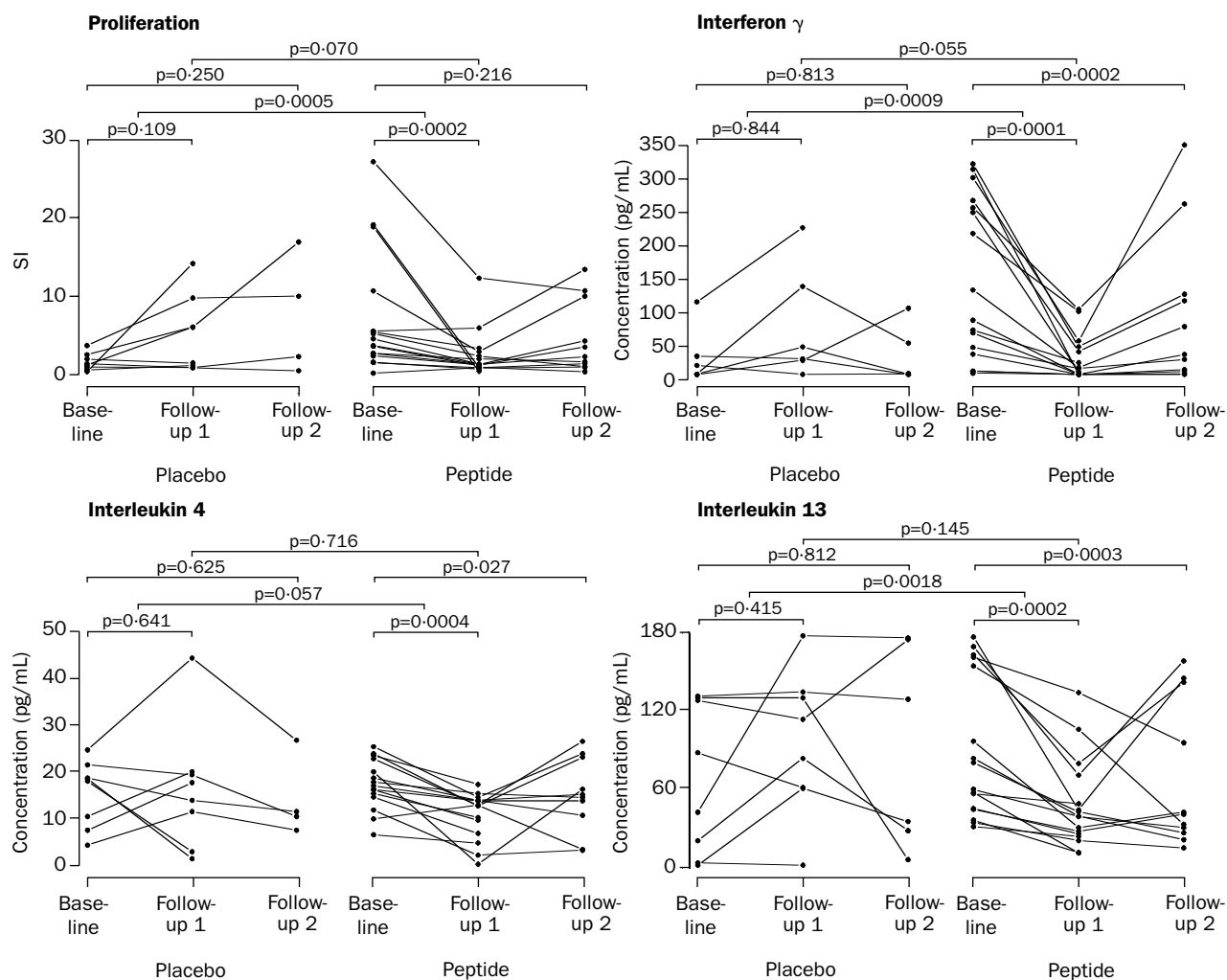


Figure 4: Effect of treatment on proliferative response and production of interleukin 4 and 13 and interferon gamma by peripheral blood mononuclear cells stimulated by whole cat dander in culture

increased from 3 or 4 days to 7 days). Two other patients (A8 and A12) had late asthmatic reactions with decreases of 24% and 21%, respectively, after the first injection (5 μ g) of peptide (figure 2). They did not have further decreases in FEV₁ of more than 20% to the subsequent 5 μ g, 10 μ g, 25 μ g, or 45 μ g doses. Thus patients could be desensitised to late asthmatic reactions induced by the peptide. Although patients A9 and A15 were protocol violations, we did not exclude them from subsequent analyses since we believed that they would not appreciably affect the objective clinical and laboratory outcomes.

Four patients had late asthmatic reactions on the second or subsequent doses of peptide. These were A4 (23% reduction at 50 μ g), A6 (20% reduction at 10 μ g), A13 (25% reduction at 25 μ g), and A16 (35% reduction at 50 μ g). Two of eight patients in the placebo group had decreases in the FEV₁ of 25% and 30%, respectively.

12 patients in the active group received 5 μ g, 10 μ g, 25 μ g, and 50 μ g peptide and a placebo injection and two (A8 and A12) received 5 μ g, 5 μ g, 10 μ g, 25 μ g, and 45 μ g of the peptides. Thus all but two patients had five visits during the treatment phase; the other two patients received ten and nine injections, respectively (figure 2).

In the placebo group, the size of the early or late reactions to whole cat dander or Fel d 1 did not differ between baseline and first follow-up or between baseline and second follow-up (figure 3). In those receiving

peptide, the early reactions to cat dander and Fel d 1 were significantly smaller at the second follow-up than at baseline (figure 3), and the late reactions to whole cat dander and Fel d 1 were significantly smaller at both follow-up times than at baseline (figure 3). The change in the reaction to whole cat dander between baseline and second follow-up did not differ between patients on peptide and those on placebo (difference -1180.8 mm², 95% CI -2216.8 to -144.8 ; figure 3). The change in the reaction to Fel d 1 between baseline and second follow-up was much greater in patients receiving peptide than in those on placebo (-2585.9 mm², -4334.1 to -837.6 ; figure 3).

Patients' reactions to the PD₂₀ allergen or PC₂₀ methacholine did not differ between treatment groups. The median PD₂₀ concentrations were 1274 Squillion Units (SQU)/mL (range 378–2994) at baseline, 1952 SQU/mL (327–7302) at first follow-up, and 2412 SQU/mL (914–3079) at second follow-up for those receiving active treatment; and 779 SQU/mL (246–3649) at baseline, 2266 (925–4892) SQU/mL at first follow-up, and 1794 SQU/mL (802–2361) at second follow-up for those on placebo. The median PC₂₀ methacholine concentrations were 0.82 g/L (0.21–1.92) at baseline and 1.26 g/L (0.47–1.98) at first follow-up for those on peptides; and 0.78 g/L (0.51–1.24) at baseline and 1.45 g/L (0.41–2.72) at first follow-up for those on placebo.

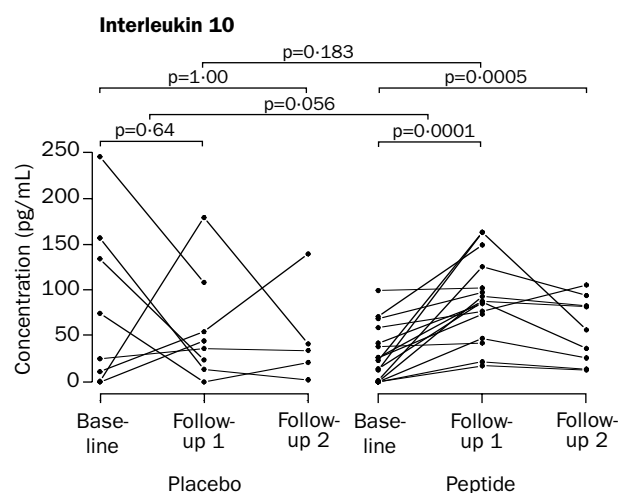


Figure 5: **Effect of treatment on production of interleukin 10 by peripheral blood mononuclear cells stimulated by whole cat dander in culture**

For whole cat dander, proliferation of peripheral blood mononuclear cells and release of interferon γ , interleukin 4, 13 (figure 4), and 10 (figure 5) from peripheral blood mononuclear cells did not differ between baseline and either follow-up assessment in those on placebo. In the active group, proliferation of peripheral blood mononuclear cells was inhibited between baseline and first follow-up (figure 4). The amount of inhibition differed between the active and placebo groups (figure 4). In the active group, inhibition of interleukin 4 was greater at first follow-up than at baseline (figure 4), inhibition of interleukin 13 and interferon γ was greater at both follow-up assessments than at baseline (figure 4), and production of interleukin 10 was greater at both follow-up assessments than at baseline (figure 5). Production of interferon γ and interleukin 13 was also greater in the active group than in the placebo group between baseline and first follow-up (figure 4). In-vitro data for two patients in the active group and two in the placebo group were not available at second follow-up. Early and late phase cutaneous reactions to whole cat dander and Fel d 1 were not associated with change in cytokine production from peripheral blood mononuclear cells. However, the study was not powered to test this relation.

Using a five-point visual analogue scale, patients receiving Fel d 1 peptides had a significant improvement in their ability to tolerate exposure to cats at the second follow-up ($p=0.001$). The amount of improvement did not differ between the two groups (figure 6).

Late asthmatic reactions induced by peptides were not associated with inhibition of the skin reactions at either follow-up. Thus at both follow-ups, the change in early and late reactions to whole cat dander and Fel d 1 did not differ between patients who developed a late asthmatic reaction and those who did not (whole cat dander $p=0.18$, Fel d 1 $p=0.72$).

Discussion

Our results show that treatment with a desensitising vaccine (based on many short, overlapping, HLA-binding, T-cell peptides derived from Fel d 1) inhibited early and late phase allergic reactions to whole allergens and modulated proliferation and production of interleukin 4, 10, and 13, and of interferon γ by peripheral blood mononuclear cells. By contrast with the results of our previous study,⁷ in which we used a single dose, our findings show that multiple

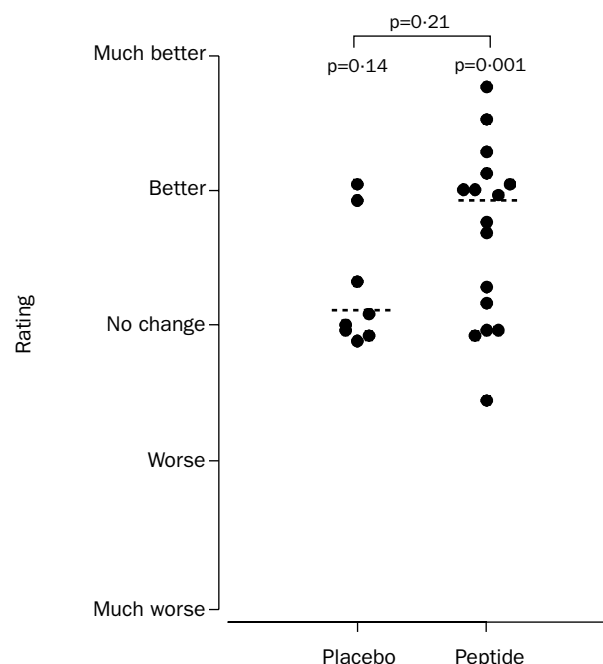


Figure 6: **Effect of treatment on subjective ability to tolerate exposure to cats on the basis of a five-point visual analogue scale**

Assessments were done at second follow-up.

dosing with peptides inhibits early and late phase skin reactions and that treatment is associated with increases in interleukin 10.

Although skin test reactivity was inhibited to the second follow-up assessment, cytokine measurements showed a trend to a return to baseline concentrations at second follow-up, suggesting that these changes were transient. Thus, changes in skin reactivity may not be related to changes in concentrations of inflammatory cytokines. However, concentrations of interleukin 10 (an anti-inflammatory cytokine), followed changes in skin reactivity more closely than did the inflammatory cytokines interleukin 4 and 13 and interferon γ , suggesting that this cytokine may have a more dominant negative effect than the others. Furthermore, the direct relation between production of cytokines by peripheral blood cells and changes at sites of allergen challenge is unclear. As with traditional whole allergen immunotherapy, booster injections or a more long-term course of treatment are probably needed to maintain the induced immunological changes.

At the start of the study we could not exclude the possibility that a late asthmatic reaction induced by peptides was an essential prerequisite for subsequent tolerance (thus the need to have twice as many patients in the active group than in the placebo group). However, our data showed that surrogate markers of allergy can be changed without an initial late asthmatic reaction. Two patients on placebo had a fall in FEV₁ of more than 20%. In previous studies,^{6,7} changes of this magnitude had not been recorded on the control (saline) day. Exclusion of the two protocol violations, who needed more than five injections to complete the course because of many late asthmatic reactions, did not change the outcome of the final statistical analyses. We have shown that gradual increases in dose (starting at 0.1 μ g) with a 10–14 day interval between doses leads to inhibition of the late-phase reaction induced by whole allergens without the unwanted late asthmatic reactions induced by the peptide.¹¹

Early and late-phase cutaneous reactions can be used as surrogates of immediate-type hypersensitivity and chronic allergic inflammation, respectively.⁸ Inhibition of the late phase reaction preceded attenuation of the early-phase reaction by several weeks (figure 3), a finding in keeping with that recorded for whole allergen immunotherapy.^{8,9} The late-phase reaction is believed, at least in part, to be dependent on T cells, possibly through the elaboration of cytokines acting through eosinophil and basophil cells.¹⁰ Inhibition of the early phase might be because of an effect on the number of mast cells or activation of IgE, or both. Although T cells might affect the early reaction by producing the interleukin 4 and 13 necessary for synthesis of IgE, cat-specific IgE did not change (data not shown)—an expected finding since IgE concentrations can take several years to decrease after allergen immunotherapy.¹² However, T cells provide cytokines such as interleukin 3, 4, and 9, which act in local differentiation of mast cell precursors, and interleukin 10 inhibits release of histamine from mast cells stimulated by anti-IgE and IgE.¹³ Therefore, peptide therapy might change mast cell numbers (as shown after successful whole allergen immunotherapy¹⁴) or change release of histamine through elaboration of interleukin 10.

Possible mechanisms underlying T-cell-specific tolerance or hyporesponsiveness induced by peptides include lack of response from absence of costimulation,¹⁵ ACTIVATION-INDUCED CELL DEATH,¹⁶ change from a Th2 to a Th1 cytokine profile,⁹ induction of regulatory T cells,^{17–19} or combinations of these mechanisms. Patients receiving peptides had a significant reduction in proliferation and production of interleukin 4 and 13, and interferon γ induced by whole allergens (figure 4). Therefore, interferon γ responses (recorded after successful immunotherapy with whole allergen extracts⁹) had not increased after administration of T-cell peptides. Downregulation of Th1 and Th2 cytokines after administration of peptide has, however, been previously described in mice¹ and human beings²⁰ with allergic disease.

Our finding of increased concentrations of interleukin 10 lends support to the role of regulatory T cells in tolerance induced by peptides. Akdis and colleagues,²¹ using whole bee venom, have also shown that successful immunotherapy in individuals sensitive to bees was associated with elaboration of interleukin 10.

We were able to show induction of specific peptide bronchial hyporesponsiveness in patients who developed a late asthmatic reaction to the initial (5 μ g) injection, since they subsequently tolerated higher doses up to a total of 90 μ g. Although we could not show an effect on the whole allergen PD₂₀ or methacholine PC₂₀, the study was not powered for these outcomes. Furthermore these patients had severe asthma as a result of exposure to cats (half were taking inhaled corticosteroids), received low doses (90 μ g) of peptides (previous studies with IPC1/IPC2 used doses of up to 3000 μ g² and 4500 μ g),⁴ might have had too short a follow-up time for an observable effect, and had multiple sensitivities (most were skin-test positive to three or more allergens). High doses of vaccine might improve inhalational outcomes in people with less severe asthmatic reactions to cats. Our results suggest that multiple, short, overlapping Fel d 1 peptides have potential in treatment of cat allergy.

Contributors

All investigators contributed to study design and the writing of the report.

Conflict of interest statement

A B Kay and M Larché are cofounders of an Imperial College spinout company (Circassia) whose aim was to develop MHC-based T-cell vaccines for treatment of allergic disease. Circassia is now a wholly owned subsidiary of Powderject Pharmaceuticals. A B Kay and M Larché hold stock in, and have consultancy agreements with, Powderject.

Acknowledgments

We thank Karen Shirley (research nurse) who recruited patients, made clinical observations, and managed and documented the data; Jackie Turner for statistical analyses; Martin Chapman (University of Virginia, Charlottesville, USA) for the purified Fel d 1 for in vivo challenges, and ALK (UK) for whole cat dander extract for in vitro assays.

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References

- 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 1. *Proc Natl Acad Sci USA* 1993; **90**: 7608–12.
- Norman PS, Ohman JL, Long AA, et al. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996; **154**: 1623–28.
- Maguire P, Nocodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin Immunol* 1999; **93**: 222–31.
- Pene J, Desroches A, Paradis L, 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**: 571–78.
- Simons FE, Imada M, Li Y, Watson WT, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 1996; **8**: 1937–45.
- Haselden BM, Kay AB, Larché M. IgE-independent MHC-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999; **189**: 1885–94.
- Oldfield WLG, Kay AB, Larché 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**: 1734–39.
- Durham SR, Walker SM, Varga EM, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**: 468–75.
- Varney VA, Hamid QA, Gaga M, 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.
- Kay AB. Allergy and allergic diseases. First of two parts. *N Engl J Med* 2001; **344**: 30–37.
- Alexander C, Oldfield WLG, Shirley KE, Larché 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.
- Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973; **52**: 472–82.
- Royer B, Varadaradjalou S, Saas P, Guillosson JJ, Kantelip JP, Arock M. Inhibition of IgE-induced activation of human mast cells by interleukin-10. *Clin Exp Allergy* 2001; **31**: 694–704.
- Durham SR, Varney VA, Gaga M, et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1999; **29**: 1490–96.
- Mueller DL, Jenkins MK, Chiodetti L, Schwartz RH. An intracellular calcium increase and protein kinase C activation fail to initiate T cell proliferation in the absence of a costimulatory molecule. *J Immunol* 1990; **144**: 3701–09.
- Kabelitz D, Pohl T, Pechhold K. Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol Today* 1993; **14**: 338–39.
- Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL. A critical role for transforming growth factor- β but not IL-4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. *J Exp Med* 1996; **183**: 2669–74.
- Groux H, O'Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; **389**: 737–42.
- Bridoux F, Badou A, Saoudi A, et al. Transforming growth factor beta (TGF- β)-dependent inhibition of T helper cell 2 (Th2)-induced autoimmunity by self-major histocompatibility complex (MHC) class II-specific, regulatory CD4(+) T cell lines. *J Exp Med* 1997; **185**: 1769–75.
- Müller U, Akdis CA, Fricker M, 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**: 747–54.
- Akdis CA, Blesken T, Akdis M, Wüthrich B, Blaser K. Role of interleukin-10 in specific immunotherapy. *J Clin Invest* 1998; **102**: 98–106.