

Late asthmatic reactions provoked by intradermal injection of T-cell peptide epitopes are not associated with bronchial mucosal infiltration of eosinophils or T_H2-type cells or with elevated concentrations of histamine or eicosanoids in bronchoalveolar fluid

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Background: Isolated late asthmatic reactions can be provoked by intradermal challenge of allergen-derived T-cell peptide epitopes.

Objective: The purpose of this study was to determine whether the isolated LAR is associated with the local accumulation of inflammatory cells, the expression of T_H2 cytokines, and the production of pharmacologic mediators.

Methods: A randomized, placebo-controlled, crossover study design was used. The investigation involved bronchial and skin biopsies and bronchoalveolar lavage (BAL) fluids from 8 cat-allergic subjects who developed significant late asthmatic reactions 6 hours after intradermal injection of Fel d 1 chain 1–derived peptides (FCIPs).

Results: Immunostaining of bronchial biopsy specimens showed no changes in the numbers of eosinophils, neutrophils, basophils, mast cells, CD3⁺, CD4⁺ or CD8⁺ T cells, CD25⁺ cells or macrophages, or cells mRNA⁺ for IL-4, IL-5, or IL-13 when the FCIP day was compared with the diluent control day.

There were also no significant differences in eosinophil numbers, either in BAL fluids or in peripheral blood after FCIP challenge. Furthermore, there were no significant alterations in the concentrations of histamine, histamine-releasing factors, or eicosanoids (LTC₄/D₄/E₄, PGD₂, PGE₂, TXB₂, PGF_{2α}) in BAL fluids. FCIPs induced a significant ($P < .05$) elevation in CD8⁺ cells in the skin and an unexpected decrease in IL-5 in BAL fluids ($P = .043$).

Conclusion: Part of the asthma process might involve T

cell-dependent airway narrowing with no requirement for IgE, mast cells, or infiltrating inflammatory cells. (*J Allergy Clin Immunol* 2001;108:394–401.)

Key words: T lymphocyte, Fel d 1, asthma, bronchoscopy, peptide epitope

We recently showed that intradermal injection of T-cell peptide epitopes derived from Fel d 1, the major cat allergen,¹ provoked isolated late asthmatic reactions (LARs) in 9 of 40 cat-allergic subjects.¹ The reactions appeared to be IgE-independent, inasmuch as no immediate reaction was observed at the site of injection and in vitro the peptides did not produce histamine release from peripheral blood basophils. Evidence for T-cell dependency of these LARs included the demonstration of MHC class II restriction of peptide recognition in responding individuals. Thus our present hypothesis is that T-cell activation, as an initiating event, can lead directly to airway narrowing in atopic asthmatic subjects.

It was previously demonstrated that in asthma, either at baseline^{2–5} or after allergen challenge,^{4,6,7} there was local inflammation of the airways, as shown by increases in eosinophils, T_H2-type cells, and mast cells. Provoked asthma induced by inhalation of whole allergen is characterized by an early-phase asthmatic reaction and a late-phase asthmatic reaction. The former is believed to be largely mast cell-dependent, whereas the LAR is associated with infiltration of inflammatory cells into the bronchial mucosa. Previous studies have shown that depending on the protocol, whole allergen produces increases in the numbers of eosinophils,^{6,7} basophils,⁴ neutrophils^{6,7} and CD4⁺ T_H2-type lymphocytes⁸ in bronchoalveolar lavage (BAL) fluid and/or bronchial biopsy (BB) specimens up to 24 hours after inhalational allergen challenge. Evidence that CD4⁺ cells play a critical role in eosinophil recruitment was provided by the finding of mRNA⁺ T cells for the T_H2-type (IL-4 and IL-5) and eosinophil-active (IL-3, IL-5, and GM-CSF) cytokines in BAL fluid⁸ and BB specimens.⁷ Expression of these T_H2 cells was elevated both at baseline² and after allergen-

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Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
BB: Bronchial biopsy
FC1P: Fel d 1 chain 1–derived peptide
HAS: Human albumin supplement
LAR: Late asthmatic reaction

induced LAR,^{7,8} and IL-4 and IL-5 mRNA colocalized largely to CD4⁺ T cells.⁵ Activation and accumulation of eosinophils in the airways leads to release of granule-derived basic proteins, leukotrienes, and prostaglandins, all of which are believed to play a role in the maintenance of airway narrowing.⁹

For these reasons, we hypothesized that the T-cell peptide epitope-induced LAR would be similar to whole allergen challenge in that it would be associated with increases in T_H2-type cells in and around the bronchial mucosa, which would in turn lead to the infiltration of eosinophils, basophils, and other inflammatory cells. We also predicted that products of eosinophils, basophils, and T cells, such as granule-derived basic proteins, prostaglandins, cysteinyl leukotrienes, histamine, and histamine-releasing factors, would be detected in BAL fluids after peptide-induced LARs.

MATERIALS AND METHODS

Study design

All subjects were documented cat-allergic asthmatic individuals, as defined previously.¹ Thirty-four people entered the study. At each initial assessment, the study was explained in detail and the patient completed an asthma and allergy questionnaire that recorded sensitivity to allergens, asthma symptoms, and general health and medication information. Each of the 34 patients was given a physical examination, and allergic status was assessed by skin prick tests to a panel of common aeroallergens (Aquagen SQ; ALK, Horsholm, Denmark), as well as measurements of total serum IgE concentrations and allergen-specific IgE (Phadebas PRIST and CAP systems, respectively; Pharmacia, Uppsala, Sweden). On entering the study (ie, on the screening day), each subject reported a history of wheezing and shortness of breath on exposure to cats and had positive skin prick test results, RAST, and immediate and late skin responses to cat dander. Full blood count and differential counts were performed through use of a Technicon H1 cell counter (Bayer AG, Leverkusen, Germany). Baseline lung function was determined at initial assessment by peak flow and spirometry. The FEV₁ and forced vital capacity were measured through use of a dry wedge bellows spirometer (Vitalograph, Buckingham, United Kingdom). Bronchial responsiveness to methacholine was measured through use of a Wright nebulizer, according to the method of Cockcroft et al.¹⁰ Subjects were excluded if they had received Fel d 1 chain 1–derived peptides (FC1Ps) in the previous year, had a baseline FEV₁ of less than 80% predicted, had taken oral corticosteroids in the previous 3 months, had a history of any serious concomitant illness, smoked, or had a history compatible with respiratory infection in the 4 weeks preceding any of the study days. β_2 -agonists were withheld on the study day, and inhaled steroids were discontinued for a minimum of 2 days before each study day. All patients gave written informed consent, and the study was approved by the Royal Brompton and Harefield NHS Trust Ethics Committee.

At the first study visit (visit 1), all patients were challenged with 30 BU ALK cat extract by intradermal injection; each of them had a resulting cutaneous late-phase reaction from which a biopsy was taken 6 hours later. On each study day, spirometry was performed at 5 minutes, 15 minutes, and hourly thereafter for 6 hours after intradermal challenge. The study was postponed if the baseline FEV₁ fell below 80% predicted on any study day. (The maximal change observed of the baseline study day was 6% in comparison with the original screening day.). As expected, none of the subjects had an early asthmatic reaction or a LAR in response to intradermal whole cat extract on visit 1. On study visit 2, which was at least 4 weeks after visit 1, each patient was randomly assigned to receive either 80 μ g of the 3 FC1Ps or control ALK diluent (the patient, but not the operator, being blinded as to whether peptides or control was being administered). Twenty subjects were assigned to FC1P and 14 subjects to control. Four of the 20 subjects who received FC1P developed an isolated LAR (defined as a fall in FEV₁ of 20% or more) beginning more than 3 hours after the intradermal challenge. At 6 hours, fiberoptic bronchoscopy with BB, BAL, and skin biopsy were performed on the 4 responders. No further measurements were performed on the 16 subjects who did not respond to FC1P on visit 2. Each of the 14 subjects randomized to receive intradermal injection of control diluent on visit 2 underwent fiberoptic bronchoscopy with BB, BAL, and skin biopsy at 6 hours. At study visit 3, the 4 peptide responders from visit 2 were challenged with control diluent intradermally, and at 6 hours, fiberoptic bronchoscopy with BB, BAL, and skin biopsy was repeated. The 14 subjects receiving control on visit 2 were challenged intradermally with FC1P on visit 3, and in the 4 responders, BB, BAL, and skin biopsy were performed at 6 hours. The 10 nonresponders were not studied further. In total, therefore, there were 8 subjects who developed, by 6 hours, an isolated LAR after intradermal injection of FC1P and from whom paired samples were obtained. Their clinical characteristics are shown in Table 1. Three of the 8 reactors also reported nasal congestion at the time that they developed FC1P-induced respiratory symptoms. The FEV₁ data from 6 of the 8 patients were used in a previous study.¹ Two of the patients received FC1P for a second time; they were both known responders and had received an identical dose of the 3 peptides 18 months earlier. Three of the 8 subjects were cat-owners. The study protocol is summarized in the flow chart shown in Fig 1.

Intradermal testing and skin biopsies

All intradermal skin tests were performed between 8:00 and 8:30 AM. The reaction sizes were measured at 15 minutes, 1 hour, 3 hours, and 6 hours; biopsy specimens were taken at 6 hours and processed as described previously.¹¹

Fiberoptic bronchoscopy

Fiberoptic bronchoscopy with BAL and BB was performed at 6 hours after intradermal administration of either control (ALK diluent) or FC1P peptides (80 μ g), as described in detail elsewhere.⁹ BAL fluid was processed as described previously.²

Immunohistochemistry

Cryostat sections (6–10 μ m) were freshly cut from biopsy specimens, mounted on 0.1% poly-L lysine-coated slides, and air-dried overnight at 37°C. mAb staining was detected through use of the alkaline phosphatase-antialkaline phosphatase method, as described previously.⁷ Normal human serum (20%) was used to prevent non-specific binding of the second- and third-layer antibodies. A mouse IgG1 myeloma protein was used as a negative control. The mAbs used were CD3, CD4, and CD25 (Becton Dickinson, Cowley, Oxford, United Kingdom); MBP, CD8, and CD68 (Dakopatts, High Wycombe, United Kingdom); antineutrophil elastase and human

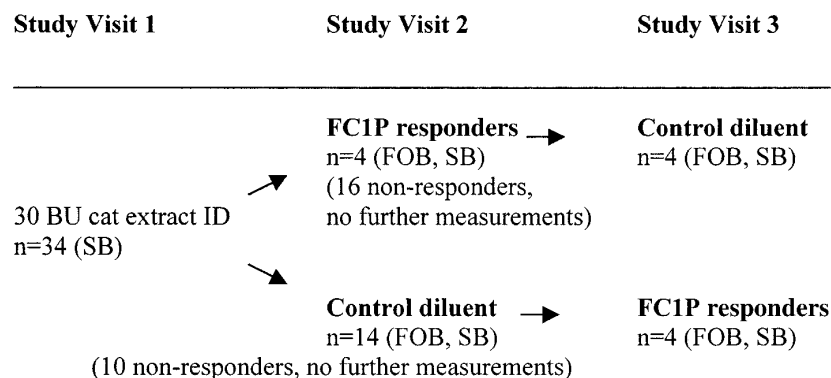


FIG 1. Study protocol flow chart. SB, Skin biopsies; FOB, fiberoptic bronchoscopy.

TABLE I. Subject characteristics according to clinical status

	No. of subjects	Age* (y)	Male/female	Total serum IgE* (IU/mL)	Whole cat extract serum IgE* (IU/mL)	Serum IgE to Fel d 1* (IU/mL)	Skin prick test diameter ALK cat* (mm)	Median PC ₂₀ methacholine* (mg/mL)
Cat-allergic asthmatic	20	29 (19-46)	10/10	356.5 (152-810)	3.09 (0.62-100)	3.4 (<0.2-86)	7.1 (5-7)	2.0 (0.3-3.9)
Non-cat-allergic asthmatic	10	33 (23-49)	5/5	313.0 (35-1039)	<0.35	<0.2	0	2.44 (0.096,3.7)
Cat-allergic rhinitic	10	37 (37-58)	4/6	98.5 (50-201)	4.25 (0.78-29.7)	4.28 (<0.2-18)	6.2 (4-12)	>16
Normal control	10	32 (23-41)	4/6	29.0 (4-152)	<0.35	<0.2	0	>16

*Median (range).

mast cell tryptase (Chemicon International, Inc, Temecula, Calif); BB1 (a gift from Dr A. F. Walls, University of Southampton, United Kingdom); and anti-IL-5 (from the American Type Culture Collection, Rockville, Md; JESI-39D10). Polyclonal rabbit antimouse immunoglobulin and alkaline phosphatase-antialkaline phosphatase method reagents were purchased from Dakopatts.

In situ hybridization

In situ hybridization was performed through use of radiolabeled riboprobes, as described in detail elsewhere.^{5,7}

Peptide synthesis

The 3 FC1P peptides for injection were synthesized by Fmoc chemistry, as described previously.¹ Briefly, peptides were purified to greater than 95% by high-pressure liquid chromatography and subjected to sequence and mass analysis and independent analysis for sterility and the absence of endotoxin. Peptide sequences used in this study were as follows: FC1P1, LFLTGTPDEYVEQVAQY; FC1P2, EQVAQYKALPVLVLENA; FC1P3, KALPVLVLENARILKNCV.

ELISAs for cytokines

IL-5 was measured in the laboratory of Dr D. Huston (Baylor Medical Center, Houston, Tex) according to a previously described technique.¹² The sensitivity of the assay was 3 pg/mL⁻¹.

Histamine measurement

Histamine content in BAL fluids and after basophil stimulation was measured by means of a commercially available competitive ELISA kit (Immunotech, Marseilles, France), according to the manufacturer's instructions.

Determination of histamine releasing activity

Dispersed mast cells from foreskins of newborns and basophil-enriched peripheral blood polymorphonuclear cells were isolated as described previously.¹³ A total of 75 μ L of each cell suspension was incubated for 40 minutes at 37°C with an equal volume of BAL fluid, HBSS-human albumin supplement (HBSS-HAS) buffer (spontaneous release), or HBSS-HAS buffer containing goat antihuman IgE at a final concentration of 50 mg/ μ L⁻¹ (positive functional control). Release of total basophil and mast cell histamine content was obtained by boiling 75 μ L of cell suspension and HBSS-HAS buffer. Supernatants of the pelleted cell preparations were stored at -20°C until histamine content was measured.

Measurements of eicosanoids

Total LTC₄, LTD₄, and LTE₄ were measured in BAL fluid by enzyme immunoassay involving the use of cysteinyl-leukotriene polyclonal serum (Cayman Chemical, Ann Arbor, Mich) after prior purification on C18 columns (Altech, Los Altos, Calif). The recovery limit was determined through use of 4000 cpm of tritiated LTC₄ standard (Dupont NEN Research Products, Boston, Mass).

Prostanoids were measured by modified stable isotope dilution assay involving the use of gas chromatography-negative ion chemical ionization-mass spectrometry, as described previously.¹⁴ Briefly, deuterium-labeled internal standards of PGD₂, PGE₂, PGF_{2 α} , and TXB₂ were added to 3 mL of BAL fluid; the samples were then acidified to pH 3 with 1 mol/L HCl and extracted through use of C18 columns (Waters Associates, Milford, Mass). Prostanoids were eluted with ethyl acetate/heptane (50:50 [v/v]). The elute was evaporated under nitrogen, and prostanoids were converted to pentafluorobenzyl

esters by treatment with a mixture of 12.5% pentafluorobenzyl bromide and 10% N,N-diisopropylethylamine in acetonitrile (Aldrich Chemical Company, Milwaukee, Wis). The reagents were dried under nitrogen, and the residue was subjected to thin-layer chromatography (Whatman Incorporation, Clifton, NJ) through use of the solvent ethyl acetate/methanol (98:2 [v/v]). Compounds migrating in the regions of the appropriate pentafluorobenzyl standards were scraped and extracted from the silica gel with ethyl acetate. The methoxime derivatives of PGD₂, PGE₂, and TXB₂ were made by treatment with 0.5% methoxamine hydrochloride in pyridine (Aldrich). The compounds were then converted to trimethylsilyl ether derivatives by incubating with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco, Bellefonte, Pa) in pyridine. The reagents were dried under nitrogen and samples redissolved in dodecane. PGD₂, PGE₂, PGF_{2α}, and TXB₂ were quantified by measuring the ratio of the intensity of ion m/z 524/530/531/532, m/z 524/528, m/z 569/573, and m/z 614/617, respectively. Analysis was performed through use of a Nermag R10-R10C mass spectrometer (Fairfield, NJ) operating in the negative ion mode and coupled to a Varian Vista 6000 gas chromatograph (Sunnyvale, Calif) through use of a 15-m DB 1701 fused silica capillary column (J and W Scientific, Folsom, Calif).

Statistical analysis

Data were analyzed through use of a Minitab statistical package (release 7; Minitab, Inc, State College, Pa). All between-subject and within-subject comparisons were analyzed through use of the Wilcoxon signed rank test for paired data. A *P* value of less than .05 was taken as significant. Correlations were performed on ranked data through use of Minitab.

RESULTS

Cutaneous and asthmatic reactions to intradermal Fel d 1 chain 1 peptides

Six of the 8 patients studied had no visible local cutaneous reaction to the FC1P. Each of 2 subjects developed a wheal that was slightly larger than that observed with diluent alone at 15 minutes (though smaller than that observed with whole allergen). One subject had a visible late cutaneous reaction to intradermal injection of FC1P at 6 hours. None of the 26 FC1P nonresponders had late cutaneous reactions to the peptides. All 34 subjects developed immediate and late-phase cutaneous reactions after intradermal injection of whole cat dander.

Each of the 8 FC1P responders had a marked and progressive decrease in FEV₁ that commenced at 2 to 4 hours after challenge (Fig 2, Table II). This was not observed in the 8 subjects with either the control diluent or intradermal whole cat dander given on visit 1 (ie, there were 2 control days). Area-under-curve analysis revealed a significant difference between the diluent and FC1P challenge days and the intradermal whole cat dander and FC1P challenge days (*P* < .001). Before bronchoscopy, airway obstruction was reversed by nebulized albuterol; responses ranged from 74% to 110% of baseline (data not shown).

Immunohistochemical staining of bronchial and cutaneous biopsy specimens

There were no increases in any of the phenotypic or activation markers—CD3⁺, CD4⁺, CD8⁺, and CD25⁺ cells, MBP⁺ and Congo red⁺ eosinophils, elastase⁺ neutrophils, BB1⁺ basophils, CD68⁺ macrophages, and

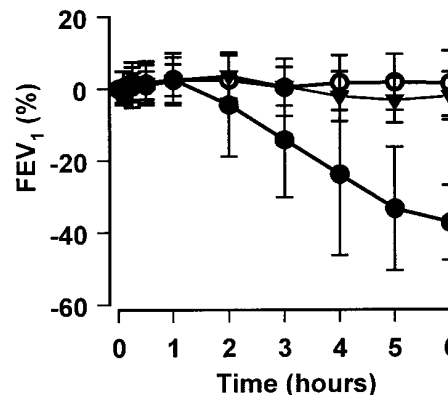


FIG 2. Change in FEV₁ after intradermal challenge with Fel d 1 chain 1 peptides (FC1P; solid circles), diluent control (open circles), and cutaneous testing with intradermal cat dander (solid triangles). Area-under-curve analysis showed a significant difference between the FC1P and diluent challenge days (*P* < .001) but no significant difference between placebo and intradermal cat dander.

tryptase⁺ mast cells—or in 2G6 (antiecotaxin; data not shown) in the BB specimens after FC1P challenge in comparison with diluent challenge (Fig 3). There was also no evidence of increased eosinophil degranulation, inasmuch as the degree of extracellular MBP⁺ staining was similar between FC1P and control biopsy specimens. There were also no changes in peripheral blood eosinophils after challenge with FC1P in comparison with the diluent control day (data not shown). However, immunohistochemical staining of intradermal challenge sites showed a significant increase (*P* < .05; Wilcoxon signed rank test) in CD8⁺ cells after Fel d 1 peptide challenge in comparison with diluent challenge sites. In contrast, there were no increases in CD8⁺ cells with whole cat dander (Table III). As expected, there was a significant increase in the number of Congo red⁺ cells with intradermal whole cat dander (*P* = .05) and a tendency toward an increase in MBP⁺ eosinophils (*P* = .078). No change in the number of eosinophils at the site of intradermal Fel d 1 peptides was observed. No other phenotypic markers (CD3, CD4, CD25, neutrophil elastase, BB1 basophils, CD68, and mast cell tryptase) or ecotaxin (2G6)⁺ cells increased in number significantly at 6 hours in the skin after challenge with either FC1P or whole cat dander (Table III).

There was no significant correlation between any of the cell markers and the magnitude of the LAR.

In situ hybridization of BB specimens for expression of IL-4, IL-5, IL-13, and IFN-γ mRNA

No difference was observed at baseline or after peptide challenge in BB specimens in the number of cells mRNA⁺ for IL-4, IL-5, IL-13, or IFN-γ (Fig 4).

Analysis of mediators and cells in BAL fluid

May Grunwald-Giemsa staining of cytopins from the BAL cell pellet showed no difference between the FC1P and control days in the numbers of macrophages, neu-

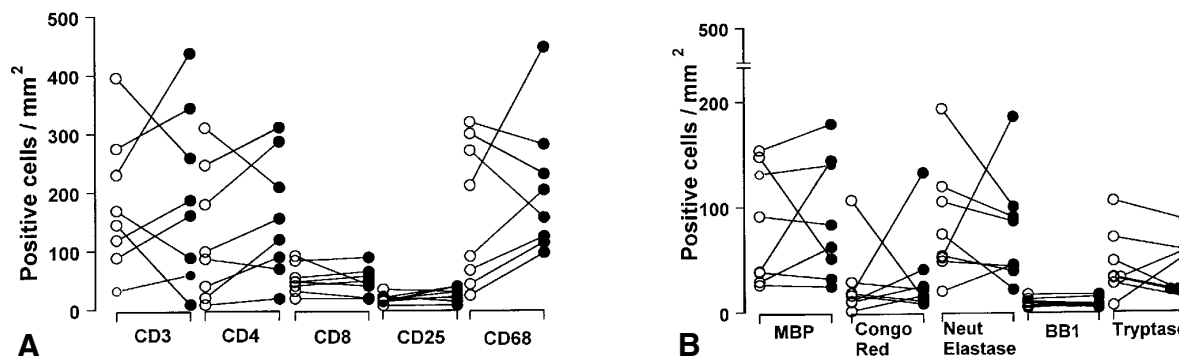


FIG 3. Immunohistologic findings of BB specimens obtained 6 hours after diluent or FC1P challenge. **A**, Biopsy specimens were studied for macrophages (T-cell markers CD3, CD4, CD8, CD25, and CD68). **B**, Biopsy specimens were also studied for eosinophils (MBP), neutrophils (Neut elastase), basophils (BB1), and mast cells (tryptase). Eosinophils were also stained with Congo red. Solid circles, FC1P; open circles, diluent control.

TABLE II. Clinical characteristics of patients injected with intradermal FCIP

Patient no.	Baseline FEV ₁ at screening (% predicted)	PC ₂₀ methacholine (mg/mL)	RAST cat (IU/mL)	Reaction to intracutaneous whole cat dander (mean diameter in cm)		FEV ₁ (% change baseline)
				15 minutes (weal size)	6 hours (induration)	
1	98	2.5	>100	2.05	9.65	-35
2	107	2	34	1.30	4.65	-25
3	87	<2	12	1.75	4.40	-37
4	87	<2	10	1.65	7.00	-59
5	101	1.6	>100	1.95	5.80	-37.5
6	80	2	35	1.95	6.20	-61
7	91	0.52	87	1.85	8.50	-34
8	95	3.2	60	1.45	5.80	-31

The magnitude of the early and late cutaneous reactions to ALK cat dander (30 BU) and FCIP (40 µg injected in 2 sites) in comparison with diluent alone are shown, as is the maximal fall in FEV₁ after peptide challenge.

TABLE III. Immunohistology of cutaneous biopsies obtained 6 hours after injection of FC1P, whole cat dander, or diluent control

mAb	Control (median)	FC1P (median)	Median difference (95% CI)	P value (FC1P vs control)	Cat dander (median)	Median difference (95% CI)	P value (cat dander vs control)
CD3	32	31	8 (-4,20)	.44	25	1 (-7,11)	.73
CD4	29	25	-4 (-10,12)	.83	22	3 (-18,12)	1.00
CD8	5	17	6 (-1,25)	<.05	4	-2 (-2,25)	.44
CD25	8	13	0 (-3,11)	.73	9	-2 (-6,7)	1.00
MBP (eosinophils)	1	1	0 (0,3.4)	.27	7	6 (0,40)	.07
Congo red (eosinophils)	0	1	0 (-2,1)	.93	5	3 (0,15)	.05
Elastase (neutrophils)	55	44	-11 (-70,43)	.44	23	-32 (-64,9)	.06
Tryptase (mast cells)	27	24	-1 (-6,4)	.53	30	3 (-2,9)	.36
BB1 (basophils)	2	7	4 (-2,17)	.15	2	0 (-1,17)	.29
CD68 (macrophages)	52	74	15 (-14,34)	.18	63	4 (-7,39)	.44
CD1a (dendritic cells)	22	21	-2 (-9,13)	.94	9	-12 (-28,3)	.11
2G6 (eotaxin)	30	25	-6.5 (-15,5)	.96	31	6.5 (-4,19)	.56

The median number of cells positive for T-cell markers (CD3, CD4, CD8), CD25, eosinophils (MBP, Congo red), neutrophils (neutrophil elastase), eotaxin (2G6), mast cells (tryptase), basophils (BB1), macrophages (CD68), and dendritic cells (CD1a) are shown. Each result is expressed as the total number of positive cells per square millimeter.

trophils, eosinophils, lymphocytes, and epithelial cells. Similar findings were observed for Congo red⁺ eosinophils (Fig 5).

There were also no significant differences in the concentration of histamine or ECP in BAL supernatants

between the diluent and peptide challenge days (Fig 5). BAL fluids from the peptide and diluent days were also incubated with blood basophils to detect histamine releasing factor(s). The respective percent-release values for peptide versus diluent BAL fluid for subjects 1

through 8 were as follows: 0.0 vs 5.0; 0.0 vs 0.0; 0.0 vs 1.0; 1.0 vs 2.0; 4.2 vs 3.9; 47.0 vs 1.7; 1.5 vs 2.9; and 4.5 vs 3.6. Mast cell release was also assessed for subjects 1 and 4. BAL-induced histamine was less than the spontaneous release for each subject. There were also no differences in eicosanoids (prostaglandins, thromboxane, and leukotrienes C₄, D₄, and E₄) between FC1P and diluent challenge (Fig 6).

There was an unexpected and significantly low concentration of IL-5 in BAL after peptide challenge in comparison with the control day ($P = .043$). However, there were no differences in the numbers of IL-5 protein positive cells in BB specimens.

Discussion

We recently described IgE-independent isolated LARs elicited by intradermal administration of allergen-derived T-cell peptide epitopes. The evidence that linear, allergen-derived, peptide-induced LARs are T cell-dependent includes the following. First, as described previously,¹ the FC1P peptides induced T-cell proliferation but not histamine release from peripheral blood basophils. Second, responsiveness of subjects with asthma, in terms of the development of LAR, appears to be MHC class II-restricted and requires binding of peptides to the appropriate HLA-DR.¹ Thus the fact that only 8 of our 34 subjects reacted might be largely attributable to MHC class II recognition of the peptides by the responders but not by the nonresponders.¹ More recently, we have shown that when 12 peptides spanning chain 1 and 2 of Fel d 1 were administered, the percent of responders was approximately 50%.¹⁵ Third, injection of FC1P was associated with hyporesponsiveness to the second injection. This peptide-induced "tolerance" returns to baseline by approximately 1 year¹ (2 of the 8 subjects had had FC1P on a previous occasion 18 months earlier). Peptide-induced T-cell tolerance is a well-established immunologic mechanism in a variety of biological and clinical situations.¹⁶ Finally, we have shown that whereas FC1P induces proliferation of peripheral blood T cells from cat-allergic asthmatics, cat-allergic rhinitics, and normal controls, only cat-allergic asthmatics respond with a high IL-5/IFN- γ ratio.¹⁷

There were no significant differences between the FC1P responders and nonresponders in terms of baseline FEV₁, PC20 methacholine, or cutaneous early and late reactions to whole cat dander. However, the RAST of the nonresponders was significantly lower in the present study, though this had not been observed previously.¹ We do not believe that the LARs were due to spontaneous deterioration of asthma. There were 2 control days (ie, whole cat extract and peptide diluent), and patients were stable at baseline. The peptide-induced LARs were appreciable, percent changes in FEV₁ ranging from -25% to -61%.

We have considered the possibility that FC1P-induced LAR is not disease-specific and that non-cat-allergic asthmatics might respond in a similar fashion to these

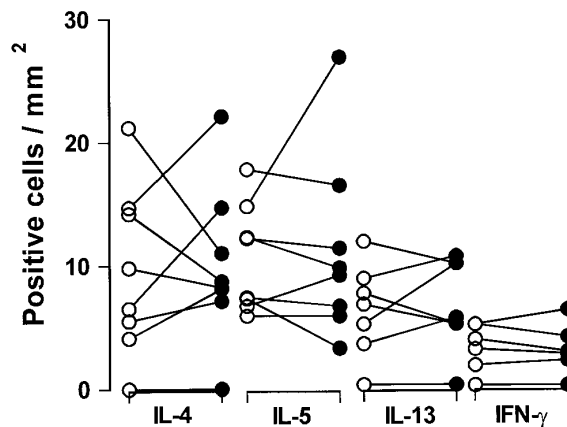


FIG 4. In situ hybridization of BB specimens for cells mRNA+ for IL-4, IL-5, IL-13, and IFN- γ . No differences were observed between FC1P peptide challenge (solid circles) and diluent (open circles).

peptides. Such studies would be difficult and would include administration of FC1P to a wide range of subjects with asthmatics, at varying doses and over a long period of time. If non-cat-allergic individuals with asthma did react to FC1P, this could be the result of T-cell sensitization in the absence of an IgE response, as we have previously demonstrated.¹ Nevertheless, we believe that FC1P-induced LARs are immunologically specific and represent a novel method for studying nonimmediate asthmatic reactions.

In the present study, we show that unlike the LAR to inhaled whole allergen, T-cell peptide epitope-induced LARs were not associated with local bronchial mucosal T_H2 activation, with eosinophil or basophil infiltration, or with detectable release of mediators, including histamine, leukotrienes, cytokines, and histamine releasing factors. These findings suggest that it might be possible to dissociate T cell-induced asthmatic reactions from IgE-dependent and eosinophil-dependent mechanisms in human beings, as has been described in animal models.¹⁸⁻²⁰ It also raises the possibility that at least in part of the asthma process, there are pathways to airway narrowing that are T cell-dependent and do not necessarily involve mast cells, basophils, or eosinophils. Therefore, although the findings we report in this study are largely negative, we believe that they are important because they demonstrate a provoked asthma model in human beings that apparently does not involve inflammatory cells (including eosinophils), T_H2 cytokines, or pharmacologic mediators such as histamine and leukotrienes. However, we cannot exclude the consideration that though there was no change in the total number of cells, there might have been a higher proportion of activated cells on the peptide day.

There was a significant decrease in the concentration of IL-5 in BAL when the peptide challenge day was compared with the control day. The reason for this finding is unclear, especially inasmuch as there was no difference between the 2 study days in the numbers of IL-5 mRNA or protein-positive cells in BB specimens.

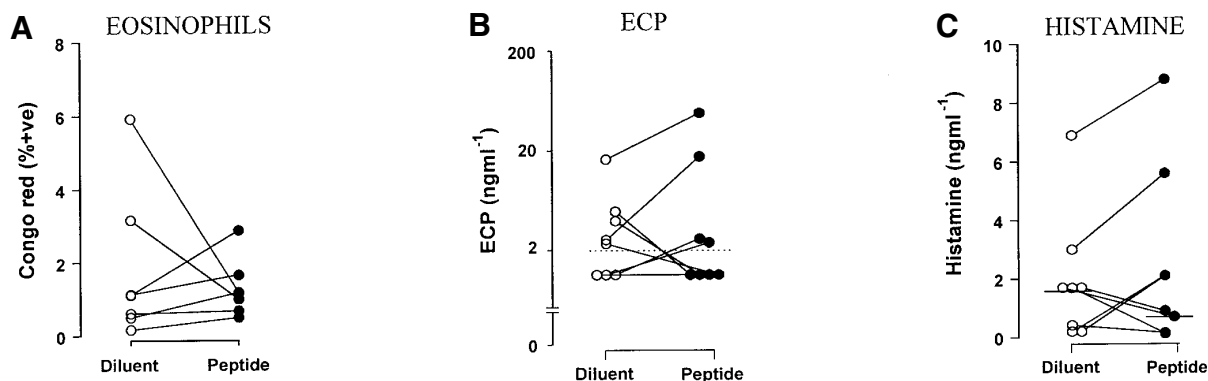


FIG 5. Measurements in BAL fluid. Congo red⁺ eosinophils in cytopins (**A**), ECP concentrations (**B**), and histamine concentrations (**C**) 6 hours after diluent (*open circles*) or FC1P challenge (*closed circles*). No significant differences in concentrations were observed between study days. Changes in the concentrations of ECP correlated with changes in histamine concentrations ($r = 0.82$, $P < .02$). Dotted line defines assay detection limits.

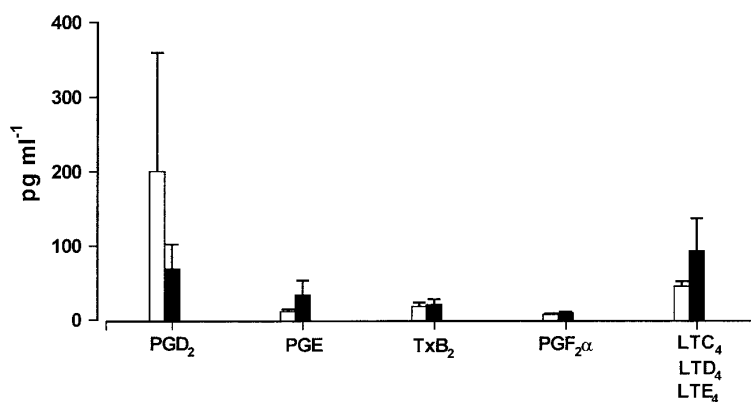


FIG 6. Concentrations of prostaglandin D_2 , prostaglandin E, thromboxane B_2 , prostaglandin $F_{2\alpha}$, and leukotrienes C₄/D₄/E₄ in BAL supernatant after diluent (*open bars*) and FC 1P (*solid bars*) challenge. No significant differences were observed between the 2 study days.

A further explanation of our findings is that endobronchial mucosal biopsies and measurements in BAL might not reflect pathology that is deeper in the bronchial wall, given that the route of challenge was intradermal rather than by inhalation. Related to this is the fate of intradermally injected peptides. Thus, after intradermal challenge, FC1P peptides might be absorbed into the bloodstream via uptake in the capillary bed of the skin. They might reach the airways directly and cause local activation of mucosa-associated T cells, leading to bronchoconstriction. The possibility of direct interactions between activated T cells and airway smooth muscle cannot be excluded, but this might not be observed in superficial mucosal BB specimens. Alternatively, T cells might be activated at the site of injection or in regional lymph nodes and induce bronchoconstriction remotely by release of soluble mediators. Again, in this situation, cellular infiltration would not be observed in BB specimens.

The onset of bronchoconstriction in a LAR without airway eosinophil infiltration is in keeping with other findings both in human beings and in experimental animals. In a recent preliminary study, a humanized anti-IL-5

mAb had no effect on the magnitude of the allergen-induced LAR or the degree of airway hyperresponsiveness (AHR)²¹; although the antibody reduced blood and sputum eosinophils, its effect on eosinophil activation is unknown. Furthermore, in a murine model, Hogan et al¹⁸ demonstrated that AHR was dependent on CD4⁺ T cells but not on IL-4 or IL-5. In addition, blockade of IL-13 abolished AHR and mucous hypersecretion independently of eosinophils and without effecting allergen-specific IgE in a mouse model of asthma,^{19,20} suggesting that IL-13 can cause AHR by IgE-independent and eosinophil-independent mechanisms. However, in our present study, there was no evidence of increased numbers of IL-13 mRNA⁺ cells in BB specimens; furthermore, IL-13 protein was undetectable in BAL fluid. Eosinophilic bronchitis, presenting with chronic cough and sputum eosinophilia, can occur without evidence of airway obstruction.²² Taken together, these observations support the view that airways eosinophilia is not a prerequisite to the airway narrowing associated with the LAR or AHR.

We have considered the possibility that peptide-induced LARs are, at least in part, due to neuropeptide

release from nerve cells in response to neurotrophins such as nerve growth factor.²³ However, neuropeptides release histamine from lung mast cells,²⁴ and histamine was not detected in BAL.

Further evidence that peptide-induced allergic reactions differ from those described for whole allergen came from findings in the skin. Thus, in contrast to the increase induced by whole allergen challenge, there was no change in eosinophil numbers at the site of injection of FC1P in comparison with diluent control injection. We did, however, observe an increase in CD8⁺ cells (but not, surprisingly, CD4⁺) in the skin after peptide challenge. Previously, we demonstrated that repeated administration of T-cell peptide epitopes was associated with inhibition of the LAR, suggesting T-cell hyporesponsiveness.^{1,25} It is therefore interesting, to speculate that the CD8⁺ cells found in the skin after peptide injection might be involved in the early events associated with subsequent downregulation of the LAR. In support of this, CD8⁺ cells have been shown to be increased after successful immunotherapy.²⁶

In our previous study,¹ none of the subjects who developed LARs to FC1P had cutaneous reactions to the peptides, and the peptides did not induce histamine release when incubated with peripheral blood basophils. In this study, 2 subjects had small, immediate skin reactions in comparison with diluent. These reactions might have been the result of an osmotic effect from the high molar concentration of peptide injected at 1 site. It is unlikely that these reactions were IgE-mediated, inasmuch as there was no evidence of mast cell degranulation in cutaneous biopsy specimens, BB specimens, or BAL fluid.

The mechanism and cellular characteristics of the occasional late-phase skin reaction observed with FC1P, as observed both here and in other, unpublished investigations, are the subject of ongoing studies. A detailed time-course study of the molecular immunopathology of peptide-induced late-phase skin reactions is currently being performed.

In conclusion, these data raise the possibility that there are mechanisms by which T cells directly induce asthmatic reactions independently of inflammatory cells and lipid mediators.

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