

Proliferation and release of IL-5 and IFN- γ by peripheral blood mononuclear cells from cat-allergic asthmatics and rhinitics, non-cat-allergic asthmatics, and normal controls to peptides derived from Fel d 1 chain 1

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Background: In general, T cells from normal, nonatopic individuals respond to aeroallergens with synthesis and release of IFN- γ . In contrast, release of T_H2-type cytokines by activated lymphocytes is a feature of allergic rhinitis and atopic asthma. **Objective:** The purpose of this study was to determine differences in T-cell recognition of epitopes within allergenic sequences, in terms of proliferation and cytokine production, in subjects with atopic asthma compared with subjects with allergic rhinitis and normal controls.

Methods: Proliferative responses and IL-5/IFN- γ release patterns from PBMCs from cat-allergic asthmatic, cat-allergic rhinitic, and non-cat-allergic asthmatic subjects and nonatopic normal controls were determined in primary cultures. Cells were challenged with 7 overlapping peptides spanning chain 1 of the major cat allergen, Fel d 1.

Results: The 4 groups did not differ with respect to the ability to mount proliferative responses to Fel d 1 peptides. In all groups, the IFN- γ responses were predominantly to the amino terminus peptides. Cat-allergic and non-cat-allergic asthmatic subjects (and not cat-allergic rhinitic subjects and normal controls) made IL-5 responses to most of the Fel d 1 peptides, the result being a mixed (T_H0) cytokine response at the N-terminus and a restricted (T_H2) response at the C-terminus.

Conclusion: Proliferative and IL-5/IFN- γ responses of T cells from asthmatic and atopic rhinitic subjects and normal controls to allergen peptides can be dissociated. Furthermore, differing cytokine responses to peptides derived from a single

antigen suggest that certain domains of the molecule might preferentially induce IL-5 rather than IFN- γ and as a result could be more important in disease pathogenesis. (*J Allergy Clin Immunol* 2001;108:349-56.)

Key words: Asthma, allergy, peptide, T lymphocyte, cytokine

Exposure to common aeroallergens leads to activation of CD4⁺ T lymphocytes and release of cytokines.¹ The effector function of activated T cells is in part dependent on the profile of cytokines released. On this basis, CD4 T cells have been divided into 2 broad subsets, T helper 1 (T_H1) and T helper 2 (T_H2).² Thus, T cells from nonatopic adults in vitro produce predominantly IFN- γ , typical of T_H1 cells.³ Production of IFN- γ has been associated with autoimmune inflammatory conditions.⁴ In contrast, IL-4, IL-5, and IL-13 have been associated with T_H2-type responses characteristic of allergic disease.^{5,6} Whether the activated CD4 cell develops a T_H1 or a T_H2 cytokine profile in vivo is dependent on the genetic background of the host⁷ as well as on a variety of local factors, including the affinity of the TcR-peptide-MHC trimeric complex,⁸⁻¹⁰ dose of antigen,^{11,12} and cytokine milieu.¹³

To map "immunodominant" epitopes within a single allergen, the ability of allergen-derived peptides to stimulate T cells has been assessed either by short-term cultures of PBMCs^{14,15} or by growing T-cell lines and/or T-cell clones.^{16,17} Polyclonal T-cell responses are thought to reflect the diversity of T-cell responses to allergens more clearly than do T-cell clones, inasmuch as a bias toward certain T-cell clones arises during the in vitro cloning procedure.

To determine whether recognition of a particular epitope is associated with atopy, comparisons have been made of in vitro proliferative and cytokine responses of PBMC and/or T-cell lines from atopic and normal individuals, cultured in the presence of allergen-derived peptides. Patterns of proliferation are generally the same between groups.¹⁸⁻²⁰ However, some studies have demonstrated a tendency toward greater proliferation in

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Abbreviations used

PC: Provocative concentration
 PPD: *Mycobacterium tuberculosis* purified protein derivative
 TFA: Trifluoroacetic acid

atopic individuals than in normal individuals.^{15,17,21} Cytokine release by T-cell lines from atopic individuals in response to allergen-derived peptides demonstrated a high IL-4:IFN- γ production ratio in response to defined epitopes.²¹ In addition, within a single allergen molecule, individual T-cell epitopes might induce differing patterns of cytokine production,²² depending on atopic status.

It has been shown in vitro that T cells from atopic asthmatic individuals release increased amounts of IL-5 and IL-13 in response to whole allergens in comparison with T cells from normal controls and asymptomatic atopic controls; in addition, there was a trend toward increased IL-5 release in comparison with what was seen in rhinitic subjects.^{6,23} In particular, enhanced expression of IL-5 is a characteristic feature of asthma and correlates with disease severity and airway hyperresponsiveness.²⁴⁻²⁶ However, comparisons of intramolecular patterns of T-cell proliferation and cytokine production by asthmatic, rhinitic, and normal individuals, for the sake of determining whether certain regions can reflect asthmatic rather than atopic status, have not been made.

In the present study, therefore, we compared in vitro responses to 7 overlapping peptides from Fel d 1 between individuals in 4 clinically distinct groups (cat-allergic asthmatics, non-cat-allergic asthmatics, cat-allergic rhinitics, and nonatopic controls). HLA class II typing was performed on all subjects to exclude any obvious bias resulting from selections of individuals expressing particular MHC haplotypes. The specific hypothesis being tested was that asthmatics would demonstrate differences in proliferation and cytokine production in comparison with rhinitics and normal controls and that T-cell recognition of these regions within the Fel d 1 molecule might be associated with atopic asthma in response to Fel d 1.

METHODS

Volunteer recruitment

Fifty volunteers were recruited by advertisement and from the Royal Brompton Hospital Allergy Clinic. The study was approved by the Ethics Committee of the Royal Brompton and Harefield NHS Trust. Volunteers were recruited into 4 groups: cat-allergic asthmatic (20 subjects), non-cat-allergic asthmatic (10 subjects), cat-allergic rhinitic (10 subjects), and normal control (10 subjects). All subjects were nonsmokers, and none had a history of any other significant concomitant illness, previous immunotherapy, or recent (less than 3 months) oral corticosteroid use. All study volunteers had received BCG vaccination against tuberculosis. The clinical details, skin test reactivities, serum IgE to cat and Fel d 1 results, and methacholine challenge test (PC₂₀) results are shown in Table I. Cat atopic status was confirmed by a history of asthma or rhinitis on exposure to cat and by positive skin prick test and RAST results for cat. Each cat-allergic asthmatic had a history of wheezing on exposure to cat and demonstrated airways hyperresponsiveness with a

PC₂₀ of less than 4 mg/mL methacholine or greater than 20% reversibility with inhaled β_2 agonists. Bronchial responsiveness to methacholine was measured through use of a Wright nebulizer by the method of Cockcroft et al.²⁷ Cat-rhinitic subjects had no evidence of airway obstruction or airway hyperresponsiveness. Non-cat-asthmatic patients fulfilled the same criteria of bronchial hyperresponsiveness and airway reversibility but had a negative history of symptoms on exposure to cat and negative skin prick testing and RAST results for cat dander. Normal controls did not react to 8 common aeroallergens on skin prick testing and had no evidence of airway obstruction or airway hyperresponsiveness.

Fel d 1-specific IgE was determined for patients in each group, as previously described.²⁸ There was no significant difference in the serum IgE concentrations to cat dander, Fel d 1, or skin prick testing for cat dander between cat-allergic asthmatics and cat-allergic rhinitics. Median values of specific IgE for Fel d 1 are shown in Table I.

HLA analysis

Class II MHC haplotyping was performed as previously described.^{29,30}

Peptide synthesis

Seven peptides spanning chain 1 of Fel d 1 were synthesized by Fmoc chemistry at the Advanced Biotechnology Centre, Imperial College School of Medicine, Charing Cross Hospital, London. Peptides were supplied as lyophilized TFA salts and subjected to sequence and mass analysis. The amino acid sequence of the overlapping peptides are as follows: peptide 1 (1-17), EICPAVKRDVDFLTGT; peptide 2 (12-28), LFLTGTPDEYVEQVAQY; peptide 3 (23-38), EQVAQYKALPVLVLENA; peptide 4 (29-45), KALPVLVLENAIRILKNCV; peptide 5 (39-55), RILKNCVDKMTTEEDKE; peptide 6 (48-63), KMTTEEDKENALSLDDK; peptide 7 (54-69), KENALSLDDKIYSPL.

Proliferation assays

PBMCs were freshly isolated from 100 mL of blood by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). The cells were washed twice in heparinized HEPES buffered medium (Sigma, Poole, United Kingdom) and resuspended in Roswell Park Memorial Institute medium supplemented with 5% pooled human AB serum, (Sigma) 2 mmol/L L-glutamine, 100 μ g/mL streptomycin, and 100 U/mL penicillin (Sigma). Cultures were established in 96-well flat-bottomed plates at 2×10^5 cells per well for proliferation assays in a total volume of 200 μ L of medium. A minimum of 16 replicates at each of 3 concentrations (1, 33, and 100 μ g/mL) were prepared. Sixteen replicates of medium acted as the negative control, and purified protein derivative (10 μ g/mL) of *Mycobacterium tuberculosis* (PPD; Evans, Leatherhead, UK) acted as the positive control. Cells were incubated at 37°C in a humidified incubator gassed with 5% CO₂ in air for 6 days. Cultures were pulsed with 37 kBq tritiated (³H) thymidine for 8 hours, harvested onto glass fiber filters, and counted in a microplate β -counter (Top Count; Packard, Pangbourne, United Kingdom).

Measurement of cytokines

Before pulsing with tritiated thymidine, 50 μ L of supernatant from the 100 μ g/mL were collected from 16 replicate culture wells and, after mixing, stored in aliquots at -20°C before the determination of cytokine levels for each patient by ELISA. IFN- γ concentrations were maximal at 100 μ g/mL, and IL-5 was detectable only at this concentration. In a dose-finding study, cytokine levels were found to be maximal in cultures at 100 μ g/mL of peptide, and all assays were therefore performed on these supernatants. IFN- γ was measured through use of Pelikine compact kits (CLB, Amsterdam, The Netherlands)

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).

according to the manufacturer's instructions. The sensitivity of this ELISA was <2 pg/mL. IL-5 was assayed by ELISA, as previously described³¹; the sensitivity of this assay was typically 2.5 pg/mL.

Statistical analysis

Proliferative responses. The proliferative responses of 16 replicate wells were compared at each concentration of antigen (peptides and PPD) with background counts (medium alone) for individual subjects by Mann-Whitney analysis. Significant differences ($P < .05$) were noted as positive responses. Stimulation index was calculated as a ratio between proliferative responses in antigen- or allergen-stimulated culture and in medium alone. Proliferative responses between groups were compared by χ^2 analysis with Yates correction.

Cytokine analysis. Many of the values for IL-5 and IFN- γ were below the level of detection; therefore, a value of 1.5 was substituted for these values so that they could be included in the analysis. The ratio of IL-5 to IFN- γ was calculated for each patient for each peptide. The sum over all 7 peptides was calculated for IL-5 and IFN- γ . The ratio of the sums was calculated for each patient. Variation-over-group within each peptide was analyzed through use of a Kruskal-Wallis 1-way nonparametric ANOVA; when this was significant, it was further investigated through use of pairwise Wilcoxon rank-sum tests. Variation-over-peptide within each group was analyzed through use of a nonparametric Friedman 2-way ANOVA; when this was significant, it was further investigated through use of the least significant difference method. Variation over each group for the sum over peptides was analyzed through use of a Kruskal-Wallis nonparametric ANOVA; where this was significant, it was further investigated through use of pairwise Wilcoxon rank-sum tests.

RESULTS

Proliferative responses do not differ between groups

The data are expressed as both the percents of each group giving a proliferative response (Fig 1) and the mean stimulation indexes for asthmatic, rhinitic, and control subjects (Fig 2). Positive proliferative responses were determined by Mann-Whitney analysis of 16 replicate cultures with peptide and compared with medium alone (Fig 2). There were no differences among the 4 groups in the percent of individuals with positive proliferative responses (Fig 1) or in the stimulation index (Fig 2) with

respect to any of the 7 peptides. Peptide 5 (aa 39-55) appeared to induce proliferation in a greater proportion of rhinitics than of asthmatics (Fig 1); however, this did not achieve significance by χ^2 analysis (with Yates correction). Significant responses to all regions of chain 1 of Fel d 1 were observed. None of the peptides induced proliferation in PBMCs from all subjects, indicating that the stimulation was unlikely to be due to mitogenlike activity. Of the 50 subjects, 49 reacted to one or more of the peptides, and all reacted to PPD. A greater frequency and magnitude of proliferative responses tended to be associated with the amino terminus of the molecule—in particular, in peptides 1 (aa 1-17) and 3 (aa 23-38; Fig 1).

Responses at the carboxy terminus of Fel d 1 induce a high IL-5:IFN- γ ratio in cat-allergic asthmatic subjects

In all 4 groups, analysis of cytokine production with respect to each individual peptide revealed a relatively increased IFN- γ response of cells challenged with peptides from the amino terminus of the molecule (Fig 3). In addition, there was an increased frequency of IFN- γ responses to these peptides (data not shown). In contrast, IL-5, which in general was detected only in the 2 asthmatic groups, was elicited by all 7 peptides (except 5 and 7 in the non-cat asthmatics). In cat-allergic asthmatics, responses to peptides 4 through 7 at the C terminus (aa 29-69) consisted primarily of IL-5, with levels of IFN- γ that were lower than those observed at the N terminus, thus generating an increased IL-5:IFN- γ ratio in this region of the molecule. The reverse (ie, a low IL-5:IFN- γ ratio) was observed in the cat-allergic rhinitics (IL-5:IFN- γ ratios in comparison with cat-allergic asthmatics: peptide 4 [aa 29-45], $P = .0004$; peptide 5 [aa 39-55], $P = .0055$; peptide 6 [aa 48-63], $P = .0019$; peptide 7 [aa 54-69], $P = .0004$), the non-cat asthmatics (IL-5:IFN- γ ratios in comparison with cat-allergic asthmatics: peptide 4 [aa 29-45], $P = .039$; peptide 5 [aa 39-55], $P = .033$), and the normal controls (IL-5:IFN- γ ratios in comparison with cat-allergic asthmatics: peptide 7 [aa 54-69], $P = .013$). Normal controls produced no IL-5 and negligible IFN- γ in response to peptide 5 (aa 39-55) and peptide 6 (aa 48-63).

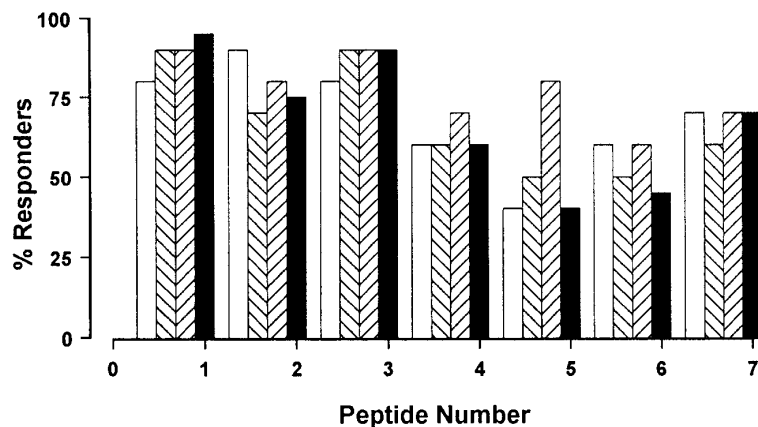


FIG 1. Percent of subjects in each group giving a proliferative response at 1 or more concentrations of the 7 peptides. Groups: cat-allergic asthmatics (*black bars*), non-cat-allergic asthmatics (*down-hatched bars*), cat-allergic rhinitics (*up-hatched bars*), and normal controls (*open bars*).

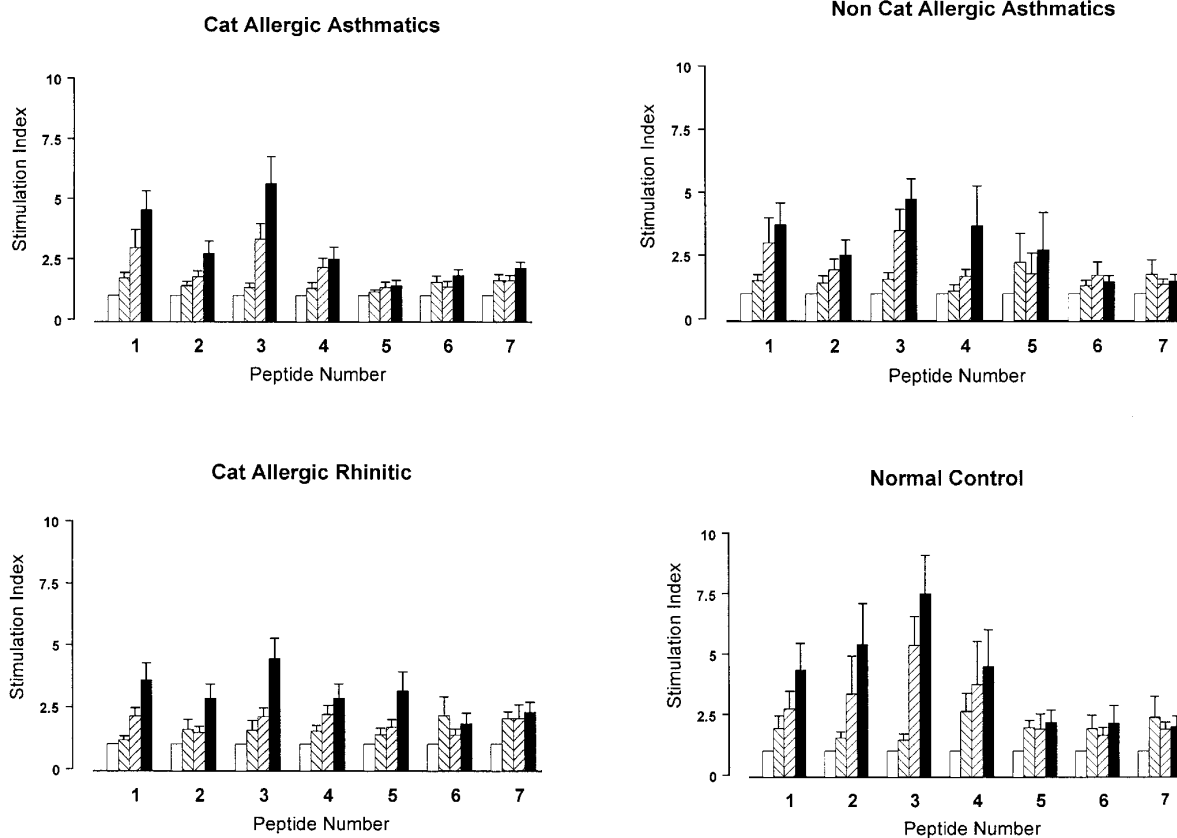


FIG 2. Mean stimulation index for each clinical group at 3 peptide concentrations: medium alone (*open bars*), 1 µg/mL (*down-hatched bars*), 33 µg/mL (*up-hatched bars*), and 100 µg/mL (*black bars*). No differences in magnitude were observed among groups. Median (range) counts per minute: cat-allergic asthmatics, medium 1,327 (416-4,354) and 100 µg/mL peptide 3,818 (379-24,337); non-cat-allergic asthmatics, medium 1,412 (334-4,405) and 100 µg/mL peptide 3,002 (463-19,299); cat-allergic rhinitics, medium 1,588 (542-4,456) and 100 µg/mL peptide 3,943 (619-21,143); normal controls, medium 1,241 (258-3,866) and 100 µg/mL peptide 3,243 (615-17,629). Error bars represent SEMs.

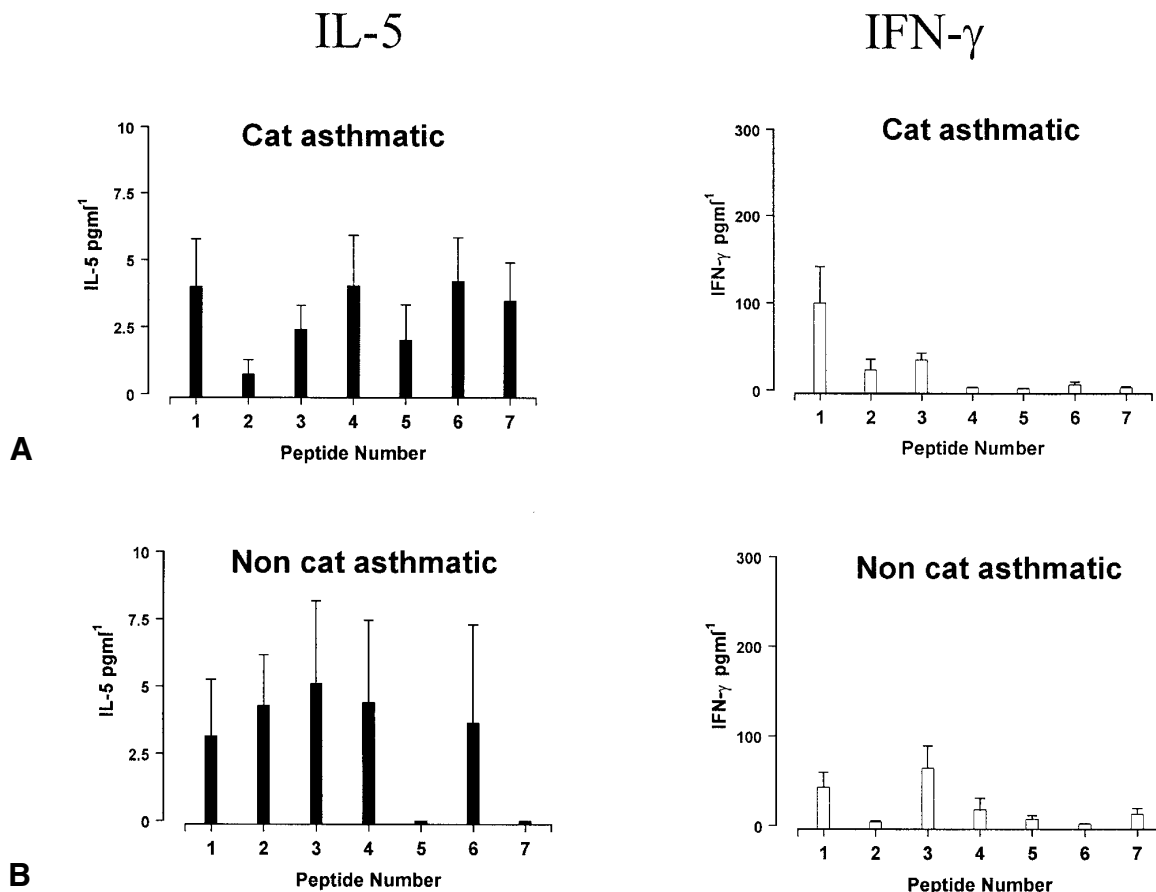


FIG 3. IL-5 and IFN- γ production from asthmatics, rhinitics, and controls. **A,** Cat-allergic asthmatics: means and SEs for IL-5 release (black bars) and IFN- γ (open bars) release induced by Fel d 1 peptides from all PBMC primary cultures. Responses to peptides 4 to 7 at the C-terminus (aa 29-69) consisted primarily of IL-5 with very little IFN- γ , generating a high IL-5:IFN- γ ratio. **B,** Non-cat-allergic asthmatics: means and SEs for IL-5 (black bars) and IFN- γ (open bars) release induced by Fel d 1 peptides from all PBMC primary cultures. IL-5:IFN- γ ratios in comparison with cat-allergic asthmatics: for aa 29-45 (peptide 4), $P = .039$; for aa 39-55 (peptide 5), $P = .033$. **C,** Cat-allergic rhinitics: means and SEs for IL-5 (black bars) and IFN- γ (open bars) release induced by Fel d 1 peptides from all PBMC primary cultures. IL-5:IFN- γ ratios in comparison with cat-allergic asthmatics: for aa 29-45 (peptide 4), $P = .0004$; for aa 39-55 (peptide 5), $P = .0055$; for aa 48-63 (peptide 6), $P = .0019$; for aa 54-69 (peptide 7), $P = .0004$. **D,** Normal controls: means and SEs for IL-5 (black bars) and IFN- γ (open bars) release induced by Fel d 1 peptides from all PBMC primary cultures. IL-5:IFN- γ ratio in comparison with cat-asthmatics: for aa 54-69 (peptide 7), $P = .013$. No IL-5 was produced and negligible IFN- γ was produced in response to peptides aa 39-55 (peptide 5) and aa 48-63 (peptide 6).

Overall IL-5:IFN- γ ratio in the 7 peptides was increased in cat-allergic asthmatics

IL-5 and IFN- γ were measured in pooled supernatants of 16 replicates from cultures with each of the 7 peptides (100 μ g/mL; previously determined optimum dose), medium, and PPD. No significant difference was observed in total IL-5 or IFN- γ production among cultures from the different groups (data not shown). However, the overall IL-5:IFN- γ ratio for the 7 peptides was significantly increased in cat-allergic asthmatics in comparison with cat-allergic rhinitics ($P = .02$) and normal controls ($P = .031$) and tended to be higher than in the non-cat-allergic asthmatics (Fig 4). IL-5 and IFN- γ were also detected in PPD cultures. No difference was observed in IFN- γ production between groups in response

to PPD, though IL-5 was elevated in PBMCs from asthmatic individuals in comparison with rhinitic individuals.

DISCUSSION

In the present study, PBMCs from cat-allergic asthmatics and control groups were challenged in vitro with synthetic peptides spanning chain 1 of the major cat allergen, Fel d 1. No differences were observed in proliferation profiles between atopic and nonatopic subjects. However, differences were visible in patterns of IL-5 and IFN- γ release between the asthmatic and nonasthmatic groups, with an elevated IL-5 :IFN- γ ratio in the cat-allergic asthmatic subjects in comparison with the cat-allergic rhinitic and normal subjects. In addition, in the asthmatic subjects, cytokine production varied in response to challenge

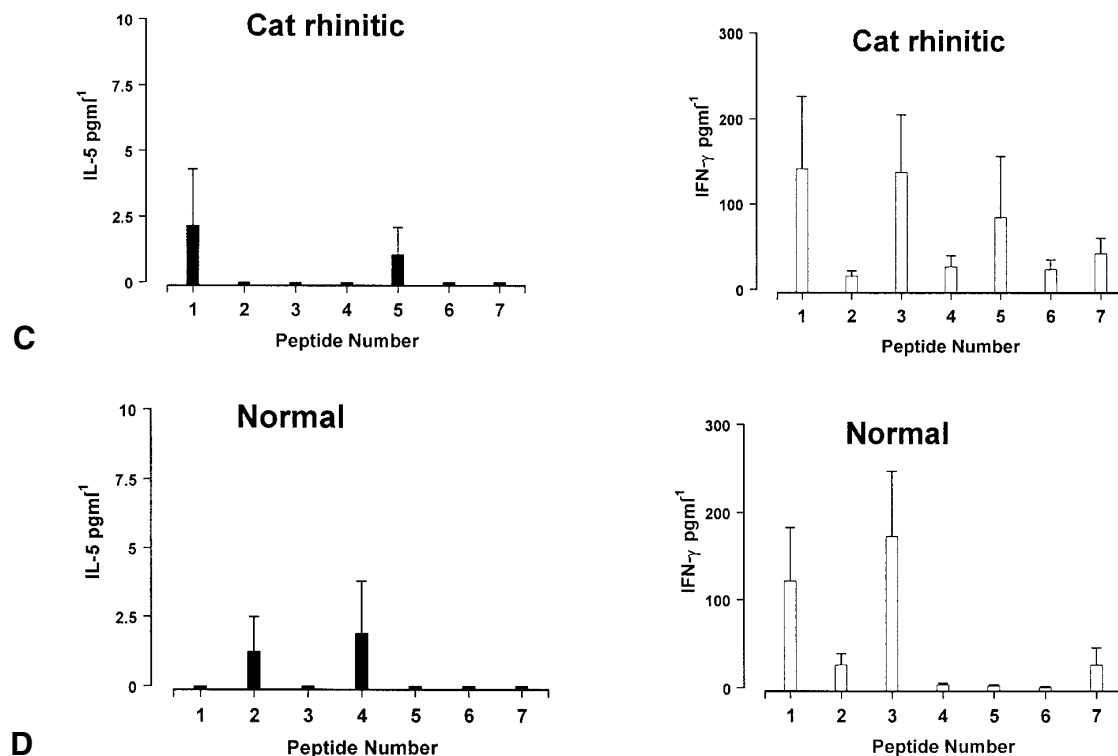


FIG 3. Continued.

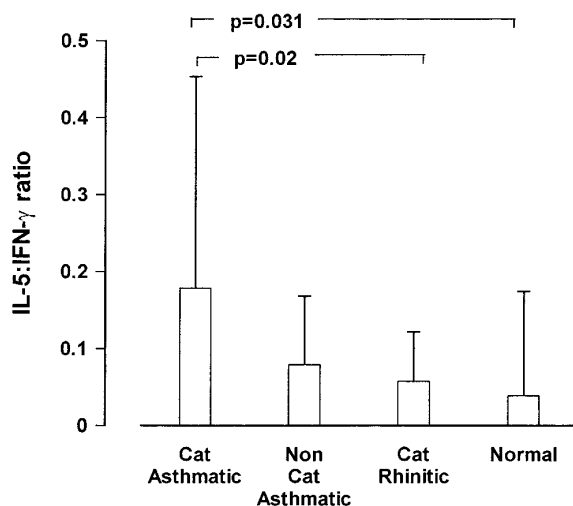


FIG 4. Cytokine ratios. IL-5:IFN- γ ratio in PBMC cultures from cat-allergic asthmatics, non-cat-allergic asthmatics, cat-allergic rhinitics, and normal controls released in response to peptides 1-7 spanning chain 1 of Fel d 1. Cytokines were measured by ELISA, as described.

with peptides from different regions of the Fel d 1 protein. These observations suggest that within individual proteins, recognition of certain regions might be associated with a more T_H2 -like phenotype whereas others elicit T_H0 responses. These findings could be of importance in the design of peptide-based desensitization strategies.

Mark et al¹⁸ studied proliferative responses to peptides from Fel d 1 in T-cell lines from atopic and nonatopic individuals. In agreement with our own data, the pattern of epitope recognition observed between groups was not different. However, responses were generally of greater magnitude in the atopic group. Investigating primary PBMC responses to Der p 2 peptides, O'Hehir et al¹⁵ observed similar patterns of proliferation in sensitized and nonsensitized subjects. The magnitude of the responses was variable but not substantially different between groups. Similarly, patterns of Bet v 1 epitope recognition between atopic and nonatopic individuals were identical in a study using T-cell clones and lines in vitro.¹⁹ Thus there is general agreement in the literature that sensitized and nonsensitized individuals have similar patterns of allergen-derived peptide recognition, though some studies report brisker responses from sensitized subjects.

IFN- γ and particularly IL-5 could occasionally be detected in the absence of significant proliferative responses, suggesting that proliferation can be dissociated from cytokine release. Peptides derived from the amino terminus of the molecule appeared to induce the largest proliferative responses, a finding in agreement with data obtained from short-term T-cell lines.^{7,11} These results suggest that when the immunogenicity of epitopes is being determined, with a view toward targeting areas for desensitization, proliferative responses alone might not be good predictors of which regions are linked to pathogenicity.

All subjects were typed for DRB and DQ. No HLA associations were observed between clinical groups or

with individual peptide responses (data not shown). However, our study was clearly underpowered to detect significant associations of MHC class II haplotype with reactivity to Fel d 1 peptides. Then again, this might reflect the ability of peptides to bind to more than one MHC molecule, in keeping with the observations of Texier et al³² and ourselves.³³

The observation of a high IL-5:IFN- γ ratio in asthmatics is in agreement with previous studies investigating expression of IL-5 in bronchial biopsies from asthmatic subjects and synthesis *in vitro* by T cells in response to whole allergen.^{24,26} IL-5 plays an essential role in the terminal differentiation,³⁴ mobilization,³⁵ priming, and survival of eosinophils.^{36,37} Interestingly, the non-cat-allergic asthmatic subjects, though having negative skin tests and serum Fel d 1-specific IgE, were clearly sensitized, as shown by clearly detectable cat allergen-induced proliferation and production of IL-5 and IFN- γ . Thus a T_H2 response can exist with a weak or undetectable IgE response.

In a previous investigation,³³ we administered 3 peptides (corresponding to peptides 2, 3, and 4 in the present study) to cat-allergic asthmatics and were able to induce isolated late asthmatic reactions in some individuals. Using HLA-DR-transfected fibroblast lines, we were able to demonstrate MHC restriction of responses to peptides 3 and 4 together with induction of IL-5 *in vitro*. In the present study, these 2 peptides have been shown to induce differing cytokine response profiles in sensitized asthmatics at the population level. Peptide 3 induced both IL-5 and IFN- γ , whereas peptide 4 induced IL-5 (in some cases at very high levels) in the relative absence of IFN- γ . On the basis of these findings, the direct contribution of cytokine production patterns to asthma pathogenesis remains unclear. However, the findings described here are reflective of a group rather than of an individual. Thus production of IL-5 to a particular peptide, unopposed by IFN- γ , might contribute to the pathogenesis and maintenance of asthma.

On the basis of the findings described in this study, epitopes toward the carboxy terminus of the molecule might be particularly relevant in this respect. *In vivo* challenge studies with peptides (for example, through induction of late asthmatic reactions) might clarify this issue. In the absence of clear associations between cytokine production patterns to particular peptide-MHC combinations, the mechanisms underlying skewing of responses in some individuals toward IL-5 production remain enigmatic.

Differential cytokine responses according to atopic status have been demonstrated to 2 peptides of the allergen Par o 1 (p92 and p96).²² Peptide p96 induced IFN- γ , IL-4, and IL-5 production from T-cell clones of volunteers regardless of atopic status. However, high levels of IL-4 and IL-5 were produced in response to p92 by clones from producers of high levels of IgE only. In our study, IL-5 and IFN- γ production in response to individual peptides is linked to asthmatic status rather than serum IgE concentration. In the present study, concentrations of IL-4 were below the limits of detection.

In conclusion, we have observed no differences in patterns of T-cell proliferation in response to challenge with Fel d 1 peptides. Differences were observed in cultures from allergic asthmatics with respect to cytokine production, with an increased IL-5:IFN- γ ratio in comparison with all other groups. In addition, particular regions within a single molecule were found to favor the production of IL-5 in the relative absence of IFN- γ . Although the low levels of cytokines detected under these conditions should be interpreted with caution, such differential production of disease-related cytokines implies that some T-cell epitopes might be more relevant to pathogenesis than others—a possibility that could have implications for peptide or whole allergen-based immunotherapy. These subtle differences might be masked in studies in which atopic individuals are grouped together without clinical characterization. Should allergens be targeted for manipulation as a future treatment strategy, careful cytokine mapping, rather than assessment of proliferative responses, might be more helpful in effective therapy design.

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