

Allergen-Derived T Cell Peptide-Induced Late Asthmatic Reactions Precede the Induction of Antigen-Specific Hyporesponsiveness in Atopic Allergic Asthmatic Subjects¹

William L. G. Oldfield, A. Barry Kay,² and Mark Larché

Allergen-derived peptides can induce T cell tolerance in naive and Ag-primed mice. This is preceded by transient T cell activation. In humans, intradermal administration of short allergen-derived T cell peptide epitopes provokes IgE-independent isolated late asthmatic reactions (LARs) in sensitized subjects. In this study, we determine whether, as in mouse models, such peptides produce hyporesponsiveness to rechallenge with peptides, or whole allergen, either clinically or in terms of in vitro T cell responses. We found that a second injection of cat allergen (Fel d 1)-derived T cell peptides was associated with a marked reduction, or absence, of the LAR, and that up to 40 wk was required for return to baseline values. The cutaneous late-phase reaction to whole cat dander was also inhibited, even in subjects who did not experience an initial LAR. These observations were associated with a significant decrease in peptide- and whole allergen-induced proliferation of PBMCs and the production of IL-4, IL-13, and IFN- γ in cultures. Thus, allergen-derived peptides induce tolerance to subsequent peptide injection in the target organ (the lung), reduce late-phase cutaneous responsiveness to whole allergen, and alter in vitro T cell reactivity. *The Journal of Immunology*, 2001, 167: 1734–1739.

It is well established that T lymphocytes, particularly CD4⁺ Th2 cells, play a central role in the pathogenesis of atopic allergic disease, including asthma, and that modulation of T cell function can ameliorate symptoms (1).

Several studies in mice have shown that the development of T cell tolerance in vivo is preceded by transient T cell activation. Webb et al. (2) reported that the clonal elimination of V β 6⁺ cells responding to a superantigen was preceded by marked expansion of these cells, whereas Vidard et al. (3) found that before the establishment of specific T cell tolerance to OVA, T cells displayed transient responsiveness and synthesized IL-2 upon antigenic stimulation in vitro. Similar findings were reported by Hoyne et al. (4) using an immunodominant peptide derived from house dust mites. In this model, a strong, transient T cell CD4⁺ response in vitro preceded the development of tolerance in vivo.

Peripheral T cell tolerance can also be induced in both naive and primed mice by s.c. injection of peptides from the major cat allergen, Fel d 1 (5). We previously demonstrated that short Fel d 1-derived peptides directly initiated T cell-dependent, late asthmatic reactions (LARs),³ without the requirement for an early IgE/mast cell-dependent response, in cat-allergic asthmatics (6). LARs occurred only in those individuals expressing appropriate HLA restriction elements for one or more of the injected peptides. In the present study, we have tested the hypothesis that in humans, as in mouse models of tolerance, allergen-derived peptides induce hy-

poresponsiveness to rechallenge with peptides either clinically or in terms of in vitro T cell responses.

It has also been proposed that allergen-specific immunotherapy, as with other forms of immune modulation, may involve mechanisms by which nonresponsiveness induced to one epitope of a molecule confers nonresponsiveness either to other epitopes within the whole molecule (intramolecular epitope suppression) (7), adjacent molecules (bystander tolerance), or is passed to the next generation of regulatory T cells (infectious tolerance) (8). For this reason, we speculated that in vivo administration of Fel d 1 peptides would inhibit the late-phase allergic reaction, and in vitro T cell reactivity, induced by whole cat allergen.

Materials and Methods

Subjects and study design

Cat-allergic asthmatics were recruited, diagnosed, and assessed as described in detail elsewhere (6). The study received prior approval from the Ethics Committee of the Royal Brompton and Harefield Hospitals National Health Service Trust, (London, U.K.), and written witnessed informed consent was obtained. Twenty-four subjects were recruited in three groups of eight subjects into an open dose-ranging study. Group 1 received 1 μ g peptide, group 2 received 2.5 μ g peptide, and group 3 received 5 μ g peptide. On study day 1 (control day), subjects had venous blood withdrawn for proliferation and cytokine assays and then received an intradermal (ID) injection of whole cat dander extract (30 BU; ALK-Abello, Horsholm, Denmark) into the volar surface of the left forearm. The cutaneous reaction was recorded after 15 and 360 min, as previously described (9). Forced expiratory volume in 1 s (FEV₁) was measured at 5, 15, 30, and 60 min, and hourly thereafter for an additional 5 h. At least 2 wk later, subjects received Fel d 1-derived peptides via the ID route. Clinical monitoring of skin and lung reactions was the same as study day 1. Two weeks after peptide challenge, the eight subjects in group 3 (5 μ g peptide) had a second ID challenge with whole cat dander, and blood was withdrawn for proliferation and cytokine assays. Clinical monitoring was performed as on day 1. To assess the development of peptide-specific bronchial hyporesponsiveness, the seven subjects (one from group 1, two from group 2, and four from group 3) who developed a LAR on initial peptide challenge during the pilot study were rechallenged with the same dose of Fel d 1-derived peptides between 2 and 14 wk later. A positive late-phase asthmatic reaction was defined as before, i.e., a fall of >20% in FEV₁ from baseline (6). The magnitude, as well as the frequency, of the LARs was dose dependent. Thus, the LARs observed with 5 μ g produced a proportionately greater decrease in the FEV₁, and a more sustained reaction, than the 1 and 2.5 μ g

Department of Allergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, London, United Kingdom

Received for publication April 10, 2001. Accepted for publication June 5, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This study was supported by the National Asthma Campaign (U.K.).

² Address correspondence and reprint requests to Dr. A. Barry Kay, Department of Allergy and Clinical Immunology, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse Street, London, SW3 6LY U.K. E-mail address: a.b.kay@ic.ac.uk

³ Abbreviations used in this paper: LAR, late asthmatic reaction; FEV₁, forced expiratory volume in 1 s; ID, intradermal; PPD, purified protein derivative.

doses. The decrease in the FEV₁ in the 5 μ g group was significantly greater than either the 2.5 μ g group ($p = 0.031$), or the 1 μ g group ($p = 0.015$), or the control day ($p = 0.008$). Clinical monitoring was performed as described (6). A further seven subjects who developed a LAR following the first administration of 5 μ g of the 12 peptides received a second injection of the same dose between 3 days and 68 wk later as part of a separate study.

Peptide synthesis and validation

Sixteen overlapping peptides from chains 1 and 2 of Fel d 1 were synthesized by F-moc chemistry at the Advanced Biotechnology Center, Imperial College School of Medicine (London, U.K.). The sequences were: chain 1, 1) EICPAVKRDVDFLTGT, 2) LFLTGTPEYVEQVAQY, 3) EQVAQYKALPVVLENA, 4) KALPVVLENAIRILKNCV, 5) RILKNCVDAKMT EEDKE, 6) KMT EEDKENALSLLDK, 7) KENALSVDKIYTSPL; chain 2, 8) VKMAETCFIFDVFFA, 9) CFIFDVFFAVANGNEL, 10) GNELLL KLSLTQVNTAT, 11) LTKVNTATEPERTAMKK, 12) TAMKKIQDCYVEN GLI, 13) CYVENGLISRVDGLV, 14) SRVDGLVMTTISSSK, 15) ISSS KDCMGEAVQNTV, 16) AVQNTVEDLKLNTLGR. Peptides 8, 9, 10, and 14 were relatively insoluble due to a high proportion of hydrophobic residues, and were consequently excluded from the injected, in vivo, preparation. The remaining 12 peptides were mixed and used for in vivo studies. They produced less than 2% histamine release from basophil-enriched PBMC, in vitro, over the concentration range 0.02–100 μ g/ml. In contrast, whole cat extract (0.02–200 μ g/ml) induced up to 40% release of the total histamine, in a dose-response fashion, as previously reported (6).

Primary PBMC proliferation assays

These were performed as previously described (6). Stimulation indices (used to allow pre-post comparisons) were calculated by comparing the Ag-containing wells with those containing medium alone.

Cytokine assays

PBMCs were cultured at 5×10^5 cells/well in the presence of individual peptides (100 μ g/ml), whole cat dander (100 μ g/ml), or purified protein derivative (PPD; 10 μ g/ml), for 6 days before the collection of culture supernatants. Supernatants were frozen at -20°C before analysis. IL-4, IL-13, and IFN- γ were analyzed in duplicate by ELISA (Pelikine, CLB, Amsterdam, The Netherlands). The sensitivity of the assays was 0.4 pg ml⁻¹ (IL-4), 1.5 pg ml⁻¹ (IL-13), and 2 pg ml⁻¹ (IFN- γ).

Statistical analyses

LARs. FEV₁ data were summarized over time for each subject for the control day and the 2 peptide days. Areas under each curve for the FEV₁ measured over 6 h were calculated using the trapezoidal rule. Differences in the areas under each curve between the control day and peptide day 1, peptide days 1 and 2, and peptide day 2 and control day were analyzed by paired t test.

Allergen-induced cutaneous reaction. Differences in the magnitude of the allergen-induced early- and late-phase skin reactions measured as described (9) were analyzed using a nonparametric Wilcoxon signed rank test.

Proliferation data. Data were tested for normality using a modified Shapiro-Wilks test. Because significant deviations from normality were found,

differences between pre and post were calculated and the changes tested with a nonparametric Wilcoxon signed rank test.

Cytokine production. Data were tested for normality using a modified Shapiro-Wilks test. No significant differences from normality were found. Differences between pre and post were calculated and the changes tested with a paired t test.

Results

Induction of peptide-specific bronchial hyporesponsiveness

Seven subjects who developed a LAR following ID challenge of Fel d 1 peptides were subsequently challenged on a second occasion, 2–14 wk later, and the results compared with the control day. The first challenge was associated with a significant ($p = 0.012$) decrease in FEV₁ compared with control (Fig. 1). The second ID challenge did not induce a LAR (second challenge vs control day, $p = 0.82$). The differences between the two Fel d 1 peptide challenges were statistically significant ($p = 0.003$).

The magnitude of Fel d 1 peptide-induced bronchial hyporesponsiveness was related to the interval between the first and second peptide challenges. As the time interval between the two injections increased from 3 days to 2 wk, there was a progressive decrease in the magnitude of the LAR on the second challenge (Fig. 2). There was then a “window” between 2 and 8 wk, when there was marked bronchial hyporesponsiveness to a second peptide challenge with no evidence of a LAR and patients were asymptomatic. This was then followed by a gradual return in bronchial responsiveness to peptide challenge over a 32- to 40-wk interval, at which time the magnitude of the second LAR was similar to the first LAR. The data in Fig. 2 were analyzed using S-Plus (Mathsoft, Cambridge, MA) and modeled using a smoothing spline with cross-validation to determine the penalty.

Attenuation of the cutaneous late-phase allergic reaction to whole allergen

The effect of a single injection of Fel d 1 peptides on the allergen-induced early- and late-phase cutaneous reaction to whole cat dander was evaluated in the eight subjects who received 5 μ g peptide (Fig. 3). No difference was observed in the magnitude of the early (15-min) reaction. There was a significant ($p = 0.014$) decrease in the cross-sectional area of the cutaneous late-phase (6-h) reaction, with all subjects showing reduced reactivity. The development of a peptide-induced LAR was not a requirement for subsequent attenuation of the late-phase skin reaction because four of eight subjects did not experience a LAR following the first injection of Fel d 1 peptides.

FIGURE 1. The effect on FEV₁ of readministering Fel d 1 peptides to cat-allergic asthmatics who previously developed a LAR following the first injection. Seven subjects who developed a LAR following ID challenge of Fel d 1 peptides (●) were challenged on a second occasion 2–14 wk later (○), and the pooled results were compared with the control day (▲). The points represent the mean ($\pm$ SEM).

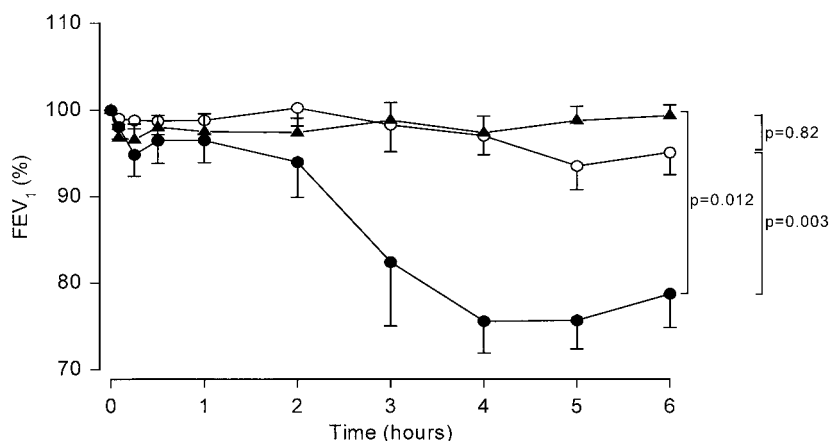
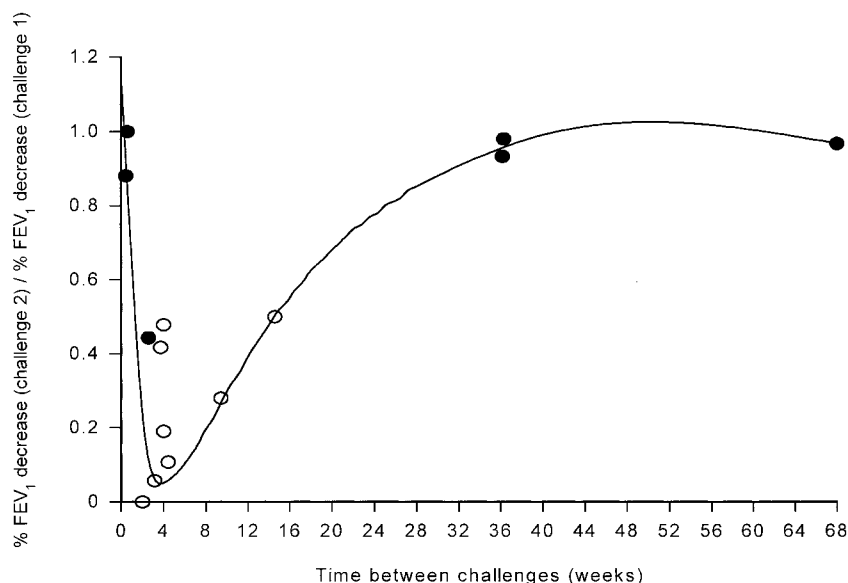


FIGURE 2. A comparison over time of the percentage decrease in the FEV₁ on the first and second challenge in cat-allergic subjects who developed a LAR following ID challenge with Fel d 1 peptides (5 μ g). In the subjects studied, the time interval between the two challenges varied from a few days to 68 wk. Asthmatics shown in the open circles had a percentage decrease in FEV₁ of <20% on the second challenge and remained asymptomatic. Those depicted by the closed circles had a LAR (i.e., a decrease in FEV₁ of >20%) on the second occasion and experienced wheezy breathlessness (as all subjects did on the first challenge).



Proliferation and cytokine production

The effect of a single administration of Fel d 1 peptides on proliferation and IL-4, IL-13, and IFN- γ production induced by the individual injected peptides is shown in Fig. 4. When pre- and postpeptide challenge samples (PBMC) were compared, responses to all peptides appeared to be reduced, although in some cases this did not achieve statistical significance. There were statistically significant decreases in proliferation (peptides 1, 11, 12, 13, 15, and 16), IL-4 (peptides 4, 6, and 12), IL-13 (peptides 1, 2, 3, 4, 5, 15, and 16), and IFN- γ concentrations (peptides 1, 2, 5, 6, and 7). In contrast, there were no significant differences in either proliferation, IL-4, IL-13, or IFN- γ (pre- vs postchallenge) to the recall Ag PPD. When the data from the individual peptides were pooled, there were significant differences (pre- vs postinjection) for proliferation (2.03 (1.68 – 4.56) vs 1.22 (1.04 – 1.52), $p = 0.007$), IL-4 (8.99 (± 1.33) vs 7.35 (± 1.25), $p = 0.002$), IL-13 (38.47 (± 5.67) vs 22.39 (± 6.30), $p = 0.015$), and IFN- γ (33.35 (± 10.31) vs 8.94 (± 4.58), $p = 0.021$). The data are expressed as median with interquartile range (proliferative response) and mean $\pm$ SE (cytokine production).

Evidence of linked suppression was obtained by studying the proliferative and cytokine responses of PBMC to whole cat dander allergen extract (Fig. 5). There were significant decreases in proliferation ($p = 0.023$) and IL-13 ($p = 0.032$), and trends for a reduction in IL-4 ($p = 0.052$) and IFN- γ ($p = 0.084$) release. Again, no significant differences were observed (pre- vs postpeptide challenge) in PPD-induced proliferation and cytokine production.

Discussion

Our present findings support the hypothesis that in humans, as in mice, transient activation of T cells, as shown by allergen-derived peptide-induced LARs, precedes the development of hyporesponsiveness to subsequent challenge. Our previous study of isolated LARs involved ID challenge with three short overlapping peptides from chain 1 of Fel d 1. In this study, we injected 12 short overlapping peptides spanning most of chain 1 and chain 2 of Fel d 1, presumably capable of binding to multiple HLA molecules. These provoked LARs in a dose-dependent fashion in about one-half of the cat-allergic subjects at the higher doses.

In an earlier study, Norman and colleagues (10) attempted to treat cat-allergic subjects by s.c. injection of two peptides (termed

IPC1 and IPC2), which spanned a large proportion of chain 1 of Fel d 1. However, IPC1 and IPC2 were 27 residues long and associated with immediate and late allergic symptoms that occurred between 10 min and 6 h after s.c. injections. In contrast, the Fel d 1 peptides in the present and previous study (6) were of relatively small size (16/17 residues) and linear configuration to enable them to be presented to T cells in the absence of Ag processing and without the ability to cross-link allergen-specific IgE.

The mechanisms involved in the inhibition of the LARs, observed on the second injection of Fel d 1 peptide (Fig. 1), may be similar to those described for classical immunological tolerance. Thus, the peptides may have induced anergy due to absence of costimulation (11), activation-induced cell death (12), a switch from a Th2 to a Th1 cytokine profile (13–15), the induction of regulatory/suppressor T cells (16–18), or combinations of these mechanisms. The very gradual return to baseline bronchial responsiveness to Fel d 1 peptide challenge over several months (Fig. 2) could support a role for any of these mechanisms.

Akdis and colleagues (19), using either whole bee venom or peptides from phospholipase A₂ (the major bee allergen), have

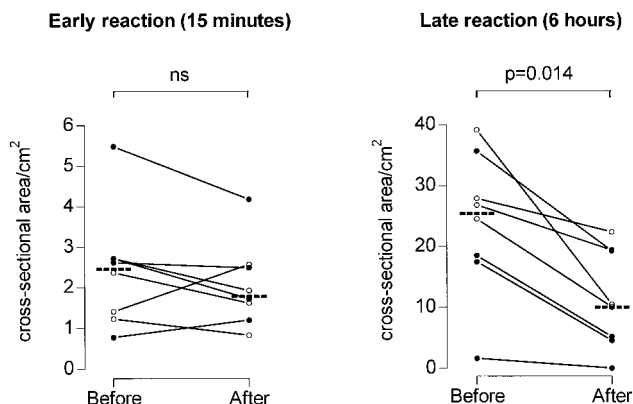


FIGURE 3. The effect of a single injection of Fel d 1 peptides (5 μ g) on the early (15-min)- and late (6-h)-phase cutaneous reaction to whole cat dander. Subjects who had a LAR on the first injection are shown in the closed circles, and those who did not are shown in the open circles. The interval between the injection of Fel d 1 peptides and the reassessment of cutaneous reactivity to whole cat dander was 2 wk.

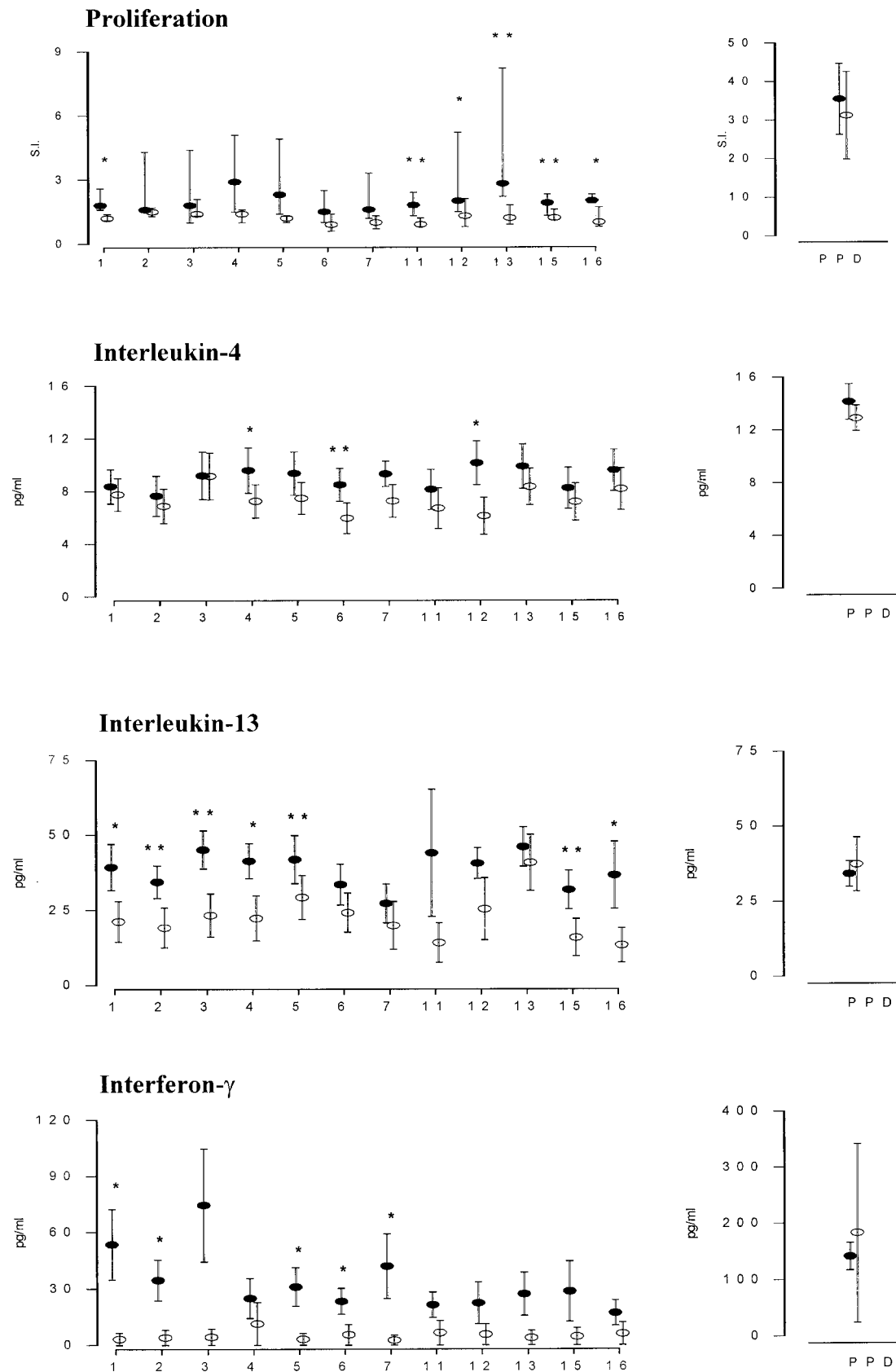
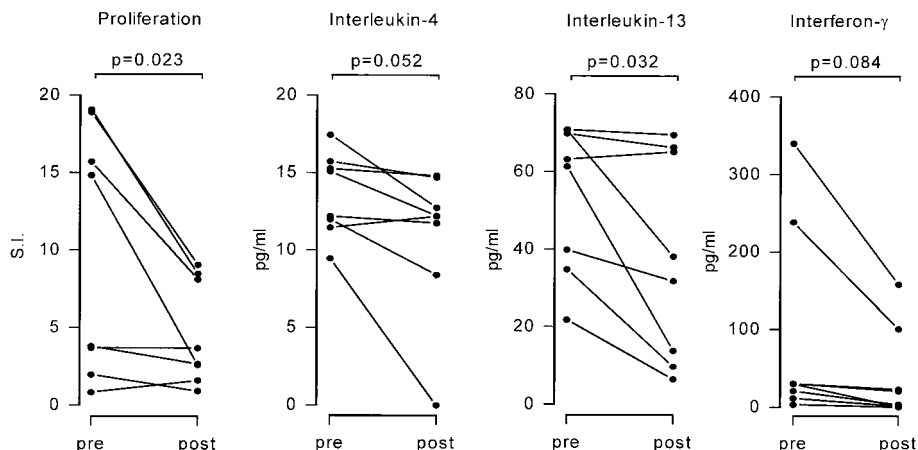


FIGURE 4. The effect of a single injection of Fel d 1 peptides (5 μ g) on proliferation and IFN- γ , IL-4, and IL-13 production by PBMCs to the 12 injected peptides. Responses to PPD are shown as a control. Preinjection values are shown by the closed circles, and postinjection by the open circles. Proliferative responses are expressed as median with interquartile range, and cytokine responses as mean $\pm$ SE. Significant changes are indicated by *, $p < 0.05$, and **, $p < 0.01$.

FIGURE 5. The effect of a single injection of Fel d 1 peptides (5 μ g) on the PBMC responses (proliferation and IFN- γ , IL-4, and IL-13 production) to whole cat dander in cat-allergic asthmatic subjects ($n = 8$).



shown that successful immunotherapy, in bee-sensitive individuals, is associated with the elaboration of IL-10 (20) (C. Akdis and K. Blaser, personal communication). It has been suggested that the IL-10 induced during venom immunotherapy gives rise to anergic Ag-specific T cells, and that these changes could be partially reversed in vitro by the addition of exogenous IL-2 or IL-15 (19). It is currently unclear whether the Ag-specific hyporesponsiveness seen in the venom model is due to classical anergy or the influence of IL-10-secreting regulatory T cells. Clonal deletion as an overall mechanism is unlikely from our findings, as we provide evidence that Fel d 1 peptides can suppress the subsequent in vivo response to whole allergen (Figs. 3 and 5).

To investigate the possibility of a peptide epitope-induced switch from a Th2 to a Th1 cytokine profile, we assessed the primary proliferative responses and cytokine production of PBMCs to both the individual peptides (Fig. 4) and whole cat dander (Fig. 5). We found that a single ID injection of 5 μ g Fel d 1 peptides was associated with a reduction in both proliferation and production of IL-4, IL-13, and IFN- γ . Thus, it appeared that an increase in IFN- γ responses (observed after successful immunotherapy with whole allergen extracts) (13–15) had not occurred after administration of peptides, at least under the conditions of our experiments. However, down-regulation of both Th1 and Th2 cytokines after peptide administration has been previously described in both murine (5) and human models of allergic disease (21).

We also assessed in vitro responses to whole allergen (Fig. 5). Again, there was a reduction in all the in vitro parameters measured, suggesting that the induction of linked/intramolecular epitope suppression had occurred. This may also contribute to the inhibition of the late-phase cutaneous response to whole allergen (Fig. 3). It has been previously shown in animal models that regulatory CD4⁺ T cells exert their suppressive effect by linked recognition of epitopes on the same or neighboring APCs (intramolecular epitope suppression) (7), adjacent molecules (bystander tolerance), or is passed to the next generation of regulatory T cells (infectious tolerance) (8). This has implications for the design of peptide-based immunotherapy, as this phenomenon means that fewer peptides may need to be administered to induce immunosuppression to the whole allergen. This hypothesis is further strengthened by our previous observation that at least one T cell epitope from Fel d 1 displays promiscuous MHC-binding characteristics (6). Additionally, we have demonstrated that tolerance to the whole allergen can be induced both in vivo (Fig. 3) and in vitro (Fig. 5), using peptides spanning only a proportion of the whole allergen. Studies are currently underway to identify critical binding residues in the four noninjected peptides, as this may allow the

replacement of one or more hydrophobic residues with alternative hydrophilic residues, thus rendering the peptide soluble. A peptide mixture spanning the whole of chain 1 and chain 2 of Fel d 1 may be more tolerogenic than the 12 peptides used in the present study, especially as these would presumably bind more HLA molecules.

The 12 Fel d 1 peptides, like FC1P, did not release histamine from blood basophils of cat-allergic subjects. These observations, taken together with the absence of peptide-induced early asthmatic reactions (even on the second challenge), suggest 1) peptide-specific IgE was not induced as a result of Fel d 1 peptide administration, and 2) Fel d 1 peptides did not cross-link preformed IgE, thereby leading to mediator release by mast cells and basophils.

It is possible that the inability of a second injection of peptides to induce a LAR was due to the induction of peptide-specific Abs (non-IgE isotype), which sequestered the peptide immediately following administration. However, this is unlikely because T cell peptides 1) inhibited the late, but not the early skin reaction, and 2) had predominant T cell effects, i.e., reduced proliferative and cytokine responses in vitro.

Asthmatics who received Fel d 1 peptides, but did not develop LARs, were still protected against subsequent challenge with whole allergen, as shown by inhibition of the late-phase cutaneous reaction (Fig. 3). Therefore, reactivity, in terms of the development of bronchospasm, was not essential for the development of hyporesponsiveness. Consequently, in a therapeutic setting, it should be possible to achieve hyposensitization or tolerance, without the unwanted LARs, by gradually increasing the dose of peptide and ensuring an optimal interval between the doses. In support of this concept, we have recently shown that a total of 41.1 μ g of a similar Fel d 1 peptide could be administered to cat asthmatics without the development of LARs, by gradual up-dosing at 14-day intervals (22). Recently, two groups attempted to treat patients with multiple sclerosis by administration of an altered peptide ligand derived from the sequence of myelin basic protein (23, 24). Both studies were halted, as many subjects developed disease exacerbations. In both situations, the dose of peptide administered on a repeated basis was 5–50 mg. Our experience with peptide-induced LARs may provide an explanation for the induction of exacerbations in multiple sclerosis patients and point the way for safer and clinically effective dose ranging for future peptide-based clinical interventions.

In conclusion, we have demonstrated that multiple, short, overlapping peptides containing T cell epitopes can induce both peptide- and whole allergen-specific hyporesponsiveness. This clinical hyporesponsiveness was associated with a marked reduction in the T cell effector response to both the peptides and the whole allergen

compatible with the induction or expansion of a population of allergen-specific regulatory cells. Further studies are in progress, involving measurements of IL-10 and TGF- β and the identification of T cell clones or lines, postpeptide therapy, which have a regulatory cell phenotype, to resolve these issues. Our data also provide further evidence that allergen-derived peptides may, in time, provide a useful alternative to specific immunotherapy in the treatment of atopic allergic disease and other conditions in which T cells contribute to pathogenesis.

Acknowledgments

Cat dander allergen extract was the generous gift of ALK-Abello.

References

- Kay, A. B. 2001. Allergy and allergic diseases: parts I and II. *N. Engl. J. Med.* 344:109.
- Webb, S., C. Morris, and J. Sprent. 1990. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* 63:1249.
- Vidard, L., L. J. Colarusso, and B. Benacerraf. 1994. Specific T-cell tolerance may be preceded by a primary response. *Proc. Natl. Acad. Sci. USA* 91:5627.
- Hoyne, G. F., B. A. Askonas, C. Hetzel, W. R. Thomas, and J. R. Lamb. 1996. Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4⁺ T cells precedes the development of tolerance in vivo. *Int. Immunol.* 8:335.
- Briner, T. J., M. C. Kuo, K. M. Keating, B. L. Rogers, and J. L. Greenstein. 1993. 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* 90:7608.
- Haselden, B. M., A. B. Kay, and M. Larché. 1999. IgE-independent MHC-restricted T cell peptide epitope-induced late asthmatic reactions. *J. Exp. Med.* 189:1885.
- Hoyne, G. F., A. G. Jarnicki, W. R. Thomas, and J. R. Lamb. 1997. Characterization of the specificity and duration of T cell tolerance to intranasally administered peptides in mice: a role for intramolecular epitope suppression. *Int. Immunol.* 9:1165.
- Cobbald, S., and H. Waldmann. 1998. Infectious tolerance. *Curr. Opin. Immunol.* 10:518.
- Frew, A. J., and A. B. Kay. 1988. The relationship between infiltrating CD4⁺ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J. Immunol.* 141:4158.
- Norman, P. S., J. L. Ohman, Jr., A. A. Long, P. S. Creticos, M. A. Geftter, Z. Shaked, R. A. Wood, P. A. Eggleston, K. B. Hafner, P. Rao, et al. 1996. Treatment of cat allergy with T-cell reactive peptides. *Am. J. Respir. Crit. Care Med.* 154:1623.
- Mueller, D. L., M. K. Jenkins, L. Chiodetti, and R. H. Schwartz. 1990. An intracellular calcium increase and protein kinase C activation fail to initiate T cell proliferation in the absence of a costimulatory molecule. *J. Immunol.* 144:3701.
- Kabelitz, D., T. Pohl, and K. Pechhold. 1993. Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. *Immunol. Today* 14:338.
- Varney, V. A., Q. A. Hamid, M. Gaga, S. Ying, M. Jacobson, A. J. Frew, A. B. Kay, and S. R. Durham. 1993. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J. Clin. Invest.* 92:644.
- Secrist, H., C. J. Chelen, Y. Wen, J. D. Marshall, and D. T. Umetsu. 1993. Allergen immunotherapy decreases interleukin 4 production in CD4⁺ T cells from allergic individuals. *J. Exp. Med.* 178:123.
- Jutel, M., W. J. Pichler, D. Skrbic, A. Urwyler, C. Dahinden, and U. R. Müller. 1995. Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN- γ secretion in specific allergen-stimulated T cell cultures. *J. Immunol.* 154:4187.
- Powrie, F., J. Carlino, M. W. Leach, S. Mauze, and R. L. Coffman. 1996. 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.* 183:2669.
- Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737.
- Bridoux, F., A. Badou, A. Saoudi, I. Bernard, E. Druet, R. Pasquier, P. Druet, and L. Pelletier. 1997. Transforming growth factor β (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.* 185:1769.
- Akdis, C. A., M. Akdis, T. Blesken, D. Wymann, S. S. Alkan, U. Müller, and K. Blaser. 1996. Epitope-specific T cell tolerance to phospholipase A₂ in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J. Clin. Invest.* 98:1676.
- Akdis, C. A., T. Blesken, M. Akdis, B. Wüthrich, and K. Blaser. 1998. Role of interleukin 10 in specific immunotherapy. *J. Clin. Invest.* 102:98.
- Müller, U., C. A. Akdis, M. Fricker, M. Akdis, T. Blesken, F. Bettens, and K. Blaser. 1998. 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.* 101:747.
- Alexander, C., W. L. G. Oldfield, K. E. Shirley, M. Larché, and A. B. Kay. 2001. A dosing protocol of allergen-derived T cell peptide epitopes for the treatment of allergic disease. *J. Allergy Clin. Immunol.* 107:S217.
- Kappos, L., G. Comi, H. Panitch, J. Oger, J. Antel, P. Conlon, L. Steinman, and The Altered Peptide Ligand in Relapsing MS Study Group. 2000. Induction of non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. *Nat. Med.* 6:1176.
- Bielekova, B., G. Goodwin, N. Richert, I. Cortese, T. Kondo, G. Afshar, B. Gran, J. Eaton, J. Antel, J. A. Frank, et al. 2000. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat. Med.* 6:1167.