

Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: Results of a phase I trial

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Background: There is a need to improve the safety and efficacy of allergen-specific immunotherapy. Long synthetic peptide-based immunotherapy was proven safe, immunogenic, and protective in preclinical trials.

Objective: To evaluate the safety and immunogenicity of an allergen-derived long synthetic overlapping peptide (LSP) immunotherapy, we designed a double-blind, placebo-controlled phase I clinical trial in patients hypersensitive to bee venom.

Methods: Patients from the active group were injected at day 0 with a mixture of 3 LSPs mapping the entire PLA2 molecule, a major bee venom allergen, in a dose-escalating protocol to a maintenance dose of 100 µg per peptide repeated at days 4, 7, 14, 42, and 70. The control group was injected with human albumin.

Results: Whereas specific T-cell proliferation in the peptide group increased up to day 14, a sharp decline was observed thereafter, ending in specific T-cell hyporesponsiveness at day 80. Serum-specific IgG4 response was enhanced, in contrast to anti-PLA2 IgE. Specific T-cell cytokine modulation was marked by increased IL-10 and IFN-γ secretion. LSP injections were well tolerated in all patients except for mild, late allergic reactions in 2 patients at day 70.

Conclusions: The results of this short-term study demonstrate that LSP-based allergen immunotherapy was safe and able to induce T_H1-type immune deviation, allergen-specific IL-10 production, and T-cell hyporesponsiveness. LSPs, which offer the advantage of covering all possible T-cell epitopes for any HLA genotype, can be considered candidates for a novel and safe approach of specific immunotherapy. (*J Allergy Clin Immunol* 2003;111:854-61.)

Key words: T_H1/T_H2, allergy, immunotherapy, bee venom, phospholipase A2, long synthetic peptides, tolerance, anergy, IL-10

Abbreviations used

BV: Bee venom
EPC: End point concentration
ID: Intradermal
LSP: Long synthetic overlapping peptide
PLA2: Phospholipase A2

The major drawback of allergen-specific immunotherapy is its inherent risk to induce anaphylaxis.^{1,2} We have therefore developed a novel strategy of immunotherapy based on long synthetic overlapping peptides (LSPs) mapping the whole sequence of phospholipase A2 (PLA2), a major bee venom (BV) allergen. As shown in our previous studies, LSPs have the advantage of covering all possible T-cell epitopes and are able to efficiently recruit specific T cells.³ This is in contrast to short synthetic overlapping peptides, which, in direct comparison, were not able to stimulate T cells as efficiently.⁴ Furthermore, the PLA2 T-cell epitope restriction pattern varied considerably from patient to patient.³ To circumvent the risk of anaphylaxis, other groups designed an immunotherapy based on a restricted panel of short T-cell epitope-covering peptides.⁵⁻⁹ Treatment of 5 patients with a mixture of 3 T-cell epitope peptides of PLA2 over a period of 2 months led to T-cell hyporesponsiveness, inhibition of T_H1 and T_H2 cytokine production, and enhanced IgG4/IgE ratio.⁵ Immunotherapy was well tolerated; however, on PLA2 challenge, mild systemic anaphylactic reactions developed in 2 patients. These approaches, based on a restricted set of peptides, required the predetermination of the patient's T-cell epitope responses, which may be highly unpredictable as a result of the patient's HLA genotype variability^{3,10,11} and represents a major burden in the conception of the vaccine. This has been partly overcome in a recent clinical trial in patients allergic to cats.¹² Both chains of *Fel d 1* were entirely mapped by short 16-mer synthetic overlapping peptides. If this phase I study led to encouraging clinical results, technically the approach may be tedious when it comes to covering the whole length of several major allergens in a combination treatment. Furthermore, this approach was limited by the level of solubility of several of the peptides.

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Despite their size (45 to 60 amino acid long), LSPs appeared to have low IgE binding activity, as suggested by our preliminary *in vitro* data,³ and thus could be considered for administration at high doses with minimal risks of exposing patients to immediate allergic reactions. In a preclinical study in the murine CBA/J model, LSP-based systemic immunotherapy induced both T-cell anergy and T_H1-immune deviation, with inhibition of specific IgE production, enhanced IgG2a response, and efficient protection of PLA2-sensitized mice on intraperitoneal challenge with native PLA2.¹³ On the basis of these supportive *in vitro* and *in vivo* data, we conducted a phase I safety and immunogenicity trial in patients hypersensitive to BV.

METHODS

Sixteen patients hypersensitive to BV were recruited from the Outpatient Clinic of the Division of Allergy and Immunology, Lausanne, Switzerland (9 men, 7 women). All patients gave informed consent to the study, and study protocols were approved by the Hospital Review Board. Criteria for enrollment were grade I to IV systemic hypersensitivity reaction to honey bee field sting (according to Müller classification¹⁴), positive anti-PLA2 and anti-whole BV-specific IgE (>0.35 kU/L as titrated by CAP system, Pharmacia, Uppsala, Sweden, and by immunoblotting), positive immediate reactions to intradermal (ID) skin test PLA2 and whole BV (presence of a wheal >5 mm with erythema at an allergen concentration ≤0.1 µg/mL), and negative ID test reactions to individual LSP and LSP mixture (≤5 mm wheal and flare reaction at peptide concentration >0.1 µg/mL).

Skin testing

ID tests with BV, PLA2, and peptides were performed as described.¹⁵ Concentrations tested ranged from 10⁻³ µg/mL to 1 µg/mL (10-fold dilution series). An ID test result was considered positive when a wheal reaction >5 mm (for BV, PLA2, and peptides) in diameter and an erythema were present at a concentration ≤0.1 µg/mL. The 0.1 µg/mL concentration was defined as the end point concentration (EPC) because higher concentrations of BV and PLA2 might induce nonspecific toxic reactions.¹⁶

Study design

The study was designed as a double-blind, randomized, 2-dose, placebo-controlled trial. At day 0, patients (n = 9) from the LSP group were injected at 30-minute intervals with, successively, 0.1 µg, 1 µg, 10 µg, 20 µg, 40 µg, 80 µg, and 100 µg of each of the 3 LSPs (cumulative dose of 251.1 µg of each LSP within 3 hours). Seven patients were then injected at days 4, 7, 14, 42, and 70 with a maintenance dose of 100 µg of each of the 3 LSPs. A maintenance dose of 300 µg of each LSP was initially injected in 2 patients up to day 42. For safety reasons (see Results section), one of the two patients was withdrawn at day 42 from further injections, the other one switched to a maintenance dose of 100 µg LSP. The immunologic results from these two patients were pooled to those of the seven other patients from the LSP group without distinction of maintenance dose. Patients from the control group (n = 7) were injected with an equivalent volume of peptide diluent only (0.3 mg/mL albumin solution, containing 4 mg/mL of phenol) (ALK/Abello, Horsholm, Denmark).

Peptide synthesis and purification

Three long synthetic peptides, LSP1-60, LSP47-99, and LSP90-134, mapping the entire 134 amino acids of PLA2 from *Apis mel-*

lifera, were synthesized on an Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City, Calif) and purified as described.¹⁷ Analytic HPLC and mass spectrometry were used to assess the purity of each peptide (>80%), which were readily soluble in PBS. On the day of injection, the peptide mixture was reconstituted in an 0.3 mg/mL albumin solution (containing 4 mg/mL of phenol) (ALK/Abello, Horsholm, Denmark) and injected subcutaneously in the deltoid area.

Reagents

Whole BV and PLA2 were purchased from Latoxan (Rosans, France). For cell culture, PLA2 was further purified by HPLC. Its cytotoxicity was inhibited by overnight reduction at 37°C with a 100 molar excess of dithiothreitol, followed by alkylation with a 1000 molar excess of *N*-ethylmaleimide. PLA2 was finally purified on a Sephadex G-25 column (Pharmacia, Uppsala, Sweden). PMA and ionomycin were purchased from Calbiochem, San Diego, Calif.

Proliferation assays

Blood was drawn immediately before each LSP injection, and PBMC were isolated from heparinized blood by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden). Before ³H-thymidine (Du Pont NEN Products Boston, Mass) incorporation, PBMC (2 × 10⁵ /well) from each donor were cultured for 6 days in octoplicates in 96-well, flat-bottom plates (Costar Corning Inc, New York, NY) in RPMI 1640 medium (Gibco, Basel, Switzerland) containing 10% AB+ serum (Swiss Red Cross, Bern, Switzerland), 2 mmol/L glutamine, 1% Na-pyruvate, 1% nonessential amino acids, 1% kanamycin (all from Gibco) with optimal concentration of LSPs (10 µg/mL) or PLA2 (10 µg/mL).⁴

Short-term T-cell lines

T-cell lines were derived from PBMC that were isolated before each injection and stimulated in 24-well plates (Nunc) (10⁶ cells/well) with a mixture of the three LSPs (10 µg/mL) for 7 days in supplemented 10% AB+ RPMI 1640 medium as described above. The short-term T-cell lines obtained were washed and restimulated for 24 hours (for IL-4, IL-5, IL-13, and TGF-β secretion) or 48 hours (for IFN-γ and IL-10) with plastic cross-linked OKT3 (1 µg/mL).¹⁸ Cell culture supernatants were collected for cytokine quantification and stored at -80°C.

Cytokine quantification

IL-4, IL-10, and IFN-γ were titrated with the use of commercially available ELISA kits (Mabtech AG, Nacka, Sweden, for IL-4, IL-10, and IFN-γ, and R&D System for IL-5, IL-13, and TGF-β), according to the manufacturer's recommendations.

Quantification of specific serum IgE and IgG4

Whole BV and anti-PLA2-specific IgE were quantified by using the Pharmacia CAP System Fluoroimmunoassay (Pharmacia Diagnostic AB, Uppsala, Sweden) as described.⁴ For quantification of specific anti-PLA2 IgG4, native PLA2 (5 µg/mL) was coated on 96-well plates (Maxisorb, Denmark) in carbonate/bicarbonate buffer, pH 9.6, for 2 hours at room temperature. Plates were blocked with milk 5%/PBS/Tween 0.05%. Serial dilutions of sera in 1% milk/Tween 0.05% were incubated for 1 hour at room temperature. Plates were washed 3 times, incubated with horseradish peroxidase-labeled anti-IgG4 mAb JDC-14 1/10,000 (Pharmingen, Hamburg, Germany), and revealed in 3,3', 5,5'-tetramethylbenzidine. Optical density was determined at 450 nm on a microtiter plate analyzer (MR5000, Dynatech Laboratories). Titers were reported to a standard serum and expressed as arbitrary standard units.

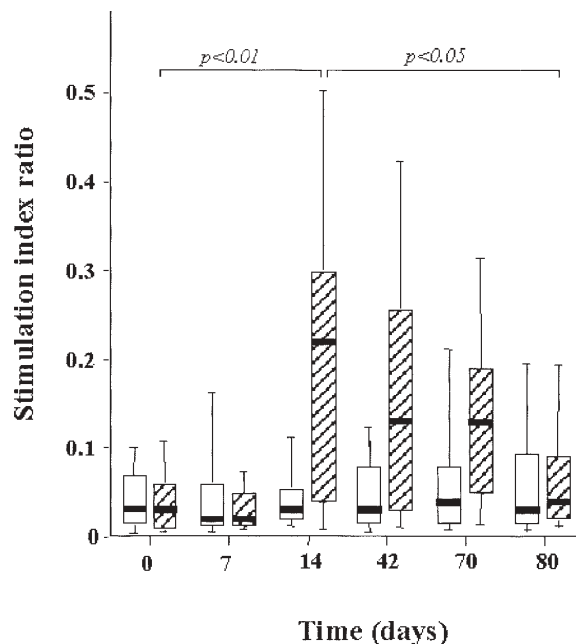


FIG 1. PLA2-derived long synthetic peptide therapy induces specific T-cell anergy in patients hypersensitive to BV. *Hatched columns*, Peptide group; *open columns*, control group (treated with albumin). Results are expressed as ratio of stimulation indexes in response to the mixture of the three LSPs reported to the stimulation indexes obtained in response to PMA (100 ng/mL)/ionomycin (1 μ mol/L) used as interassay standard. Results are presented as box-and-whisker plots with successive percentiles 5, 25, 50, 75, and 95. Medians are indicated by thick bars. Difference within groups was examined by a Friedman nonparametric ANOVA test (peptide group, $P = .035$; control group, $P = .95$), completed by a multicomparison Dunn test (peptide group: day 0 vs day 14, $P < .001$; day 14 vs day 80, $P < .05$).

Immunoblotting and dot blot analysis

Anti-BV or anti-PLA2 immunoblots were processed as described.¹⁹ For dot blot analysis, 1 μ g of whole BV, PLA2, LSPs, or human albumin was diluted 1:4 in DMSO, spotted on PVDF membranes, and dried for 30 minutes at 37°C. After blocking in nonfat milk 5%, further steps were performed as described.¹⁹ Dot densities were analyzed by scanning densitometry with an Advanced American Biotechnology scanner (Fullerton, Calif).

Statistical analysis

Differences within and between groups were evaluated by nonparametric ANOVA tests (Friedman or Kruskal-Wallis nonparametric test with multicomparison posttest, or Mann-Whitney test, respectively) or by Fisher exact test (between-group differences: responders versus nonresponders, positive responses being defined as a doubling of day 0 value), with the use of Instat 3.0 software.

RESULTS

Patient data

Patients were randomly assigned to the LSP or control (albumin) groups. In the LSP group, the mean age of patients was 39 ± 14 years (5 men, 4 women). One patient had a history of grade I hypersensitivity to BV, 7

had grade III, and 1 had grade IV, according to Müller classification. EPC for ID tests to BV was $10^{-1.7}$ μ g/mL (geometric mean). Mean serum anti-BV-specific IgE level was 21.5 ± 33.9 kU/L. In the control group, the mean age was 40 ± 10 years (4 men, 3 women). One patient previously had a grade I hypersensitivity reaction to bee venom, 3 a grade II, and 3 a grade III. EPC for ID tests to BV was $10^{-2.0}$ μ g/mL (geometric mean). Mean serum anti-BV-specific IgE level was 29.8 ± 26.1 kU/L. There was no significant difference between groups at inclusion regarding sex, age, severity of initial clinical reaction, anti-BV IgE-specific and anti-PLA2-specific IgE levels, and IgG4 antibody levels.

Long peptide immunotherapy induces T-cell anergy

As previously reported,⁴ T-cell proliferation in response to the three LSPs before the first injection at day 0 was low in either group all along the study and persisted so in the control group (Friedman, $P > .05$) (Fig 1). In contrast, there was a marked enhancement of T-cell proliferation ratio in response to the three LSPs in the peptide group, which was significant both within (Friedman, $P = .035$) and between groups (Mann-Whitney, day 14 and day 42, $P < .05$). Proliferation ratio median rose from 0.03 to 0.22 at day 14 to progressively decrease thereafter to those obtained in the control group, demonstrating an active tolerance induction. This pattern thus demonstrated that T-cell tolerance occurring after day 42 in the peptide group was preceded by a vigorous activation phase peaking at day 14.

T-cell cytokine production

IL-4 secretion by PBMCs maximally stimulated with OKT3 remained low in the peptide group (Fig 2, A). A similar pattern was observed for IL-5 and IL-13 secretion (data not shown). In contrast, we observed a striking enhancement of both IFN- γ and IL-10 secretion by LSP-specific T cells, which reached a peak at day 42 of therapy (Kruskal-Wallis, $P < .018$ and $< .012$, respectively) (Fig 2, B and C). IL-10 and IFN- γ secretion tended to decline toward day 80 (nonsignificant). TGF- β secretion stayed at background level all along the trial (data not shown). In contrast, there was no change over time in IL-4, IL-5, IL-10, IL-13, TGF- β , and IFN- γ production by PBMCs isolated from the control group (data not shown). These data were compatible with a T_H0 to T_H1 immune deviation paralleled by an enhanced production of IL-10, a cytokine that may be involved in the active T-cell tolerance induction observed (Fig 1).²⁰

Specific anti-PLA2 serum IgE and IgG4

Though the difference between the anti-PLA2 IgE levels over time in the peptide versus the control group indicated a trend toward higher IgE value in the peptide group (Fisher exact test, T14, T42, T80, $P < .03$), comparison within the groups showed that there was no significant variation of anti-PLA2 IgE levels over time (Friedman, $P > .05$) (Fig 3, A and B). In contrast, specif-

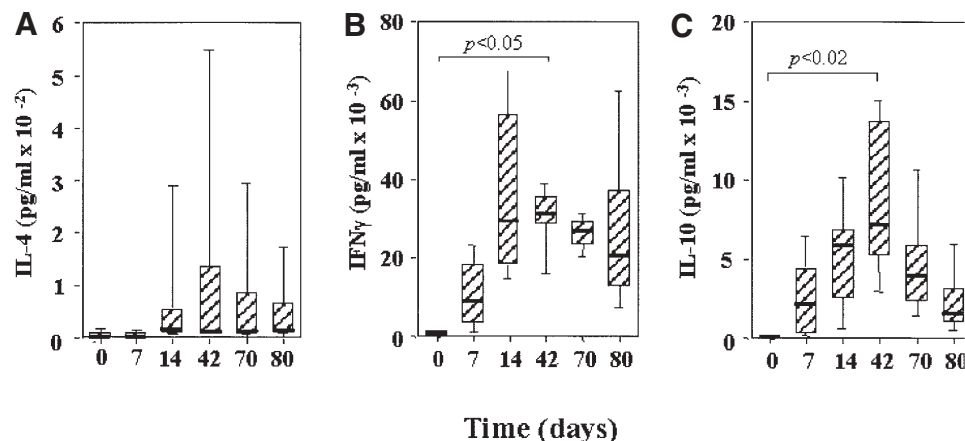


FIG 2. Long synthetic peptide therapy deviates T-cell cytokine response and strongly stimulates IL-10 secretion. Cytokines (**A**, IL-4; **B**, IFN- γ ; **C**, IL-10) from supernatants of short-term T-cell lines were measured by ELISA in cell supernatant. Results are presented as box-and-whisker plots with successive percentiles 5, 25, 50, 75, and 95. Medians are indicated by thick bars. Difference within groups for each cytokine was examined by Kruskal-Wallis nonparametric ANOVA test ($P > .05$ for IL-4, $P = .018$ for IFN- γ , $P = .012$ for IL-10), completed by a Mann-Whitney nonparametric test as mentioned in the figure (**B**, IFN- γ , day 0 vs day 42, $P < .05$; **C**, IL-10, day 0 vs day 42, $P < .02$).

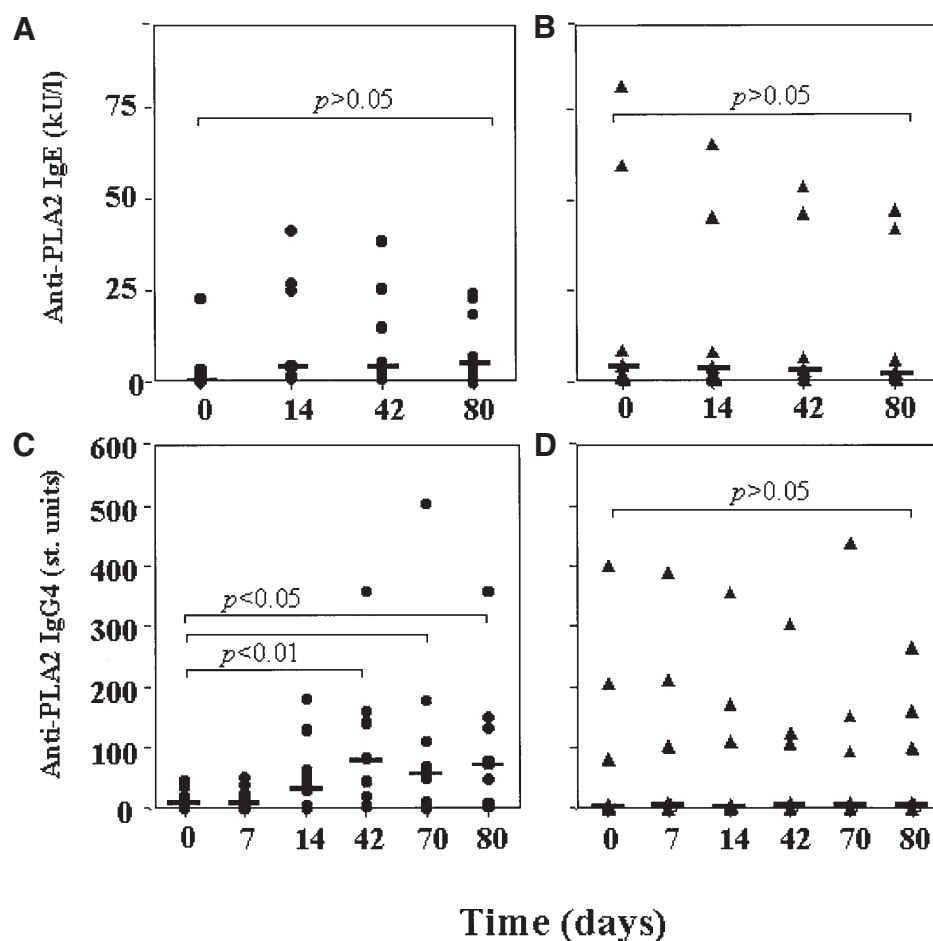


FIG 3. Anti-PLA2-specific serum IgE and IgG4. Anti-PLA2-specific serum IgE (**A** and **B**) and IgG4 (**C** and **D**) were measured in the peptide group (**A** and **C**) and the control group (**B** and **D**) at indicated time points. Median values are indicated by thick bars. Each point represents an individual value. Difference within groups was examined by Friedman nonparametric ANOVA test, completed in **C** by multicomparison posttest ($P < .01$, $P < .05$, and $P < .05$ for day 0 vs days 42, 70, and 80, respectively).

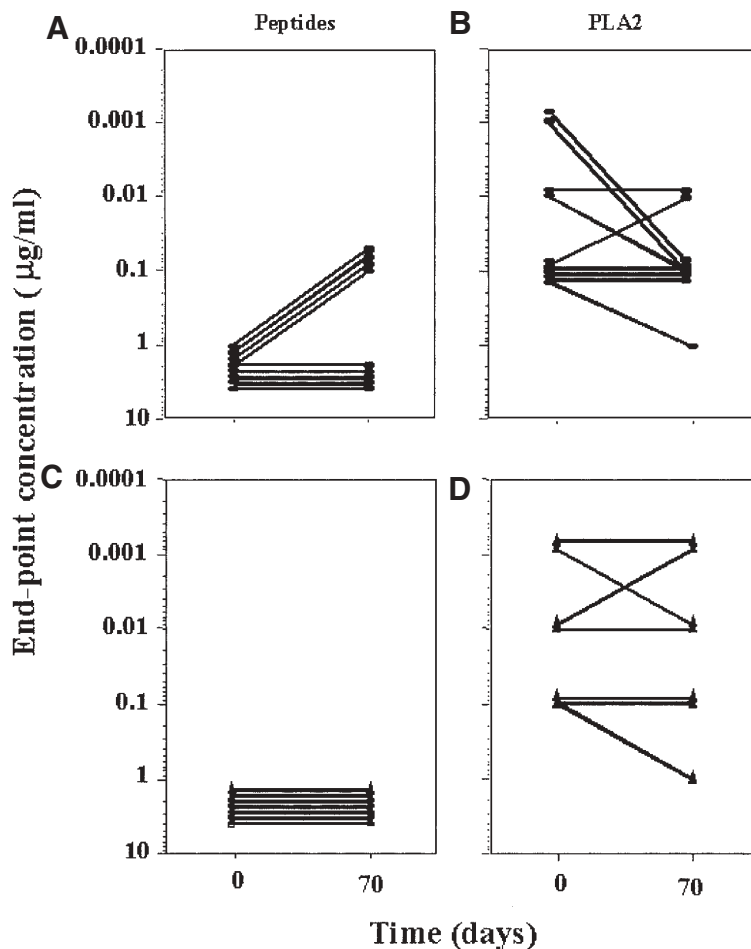


FIG 4. Intradermal skin tests with peptides and native PLA2. Results are expressed as end point concentrations (log₁₀ scale) at enrollment into the study (day 0) and after the last injection at day 70 in patients from the peptide group (A and B) and the control group (C and D), tested, respectively, with the three long synthetic peptides (as a mixture) (A and C) and with native PLA2 (B and D).

ic anti-PLA2 IgG4 antibodies steadily increased over time within the peptide group to reach significance (Friedman, $P < .001$) (Fig 3, C). Serum anti-PLA2 IgG4 levels in the control group (Fig 3, D) remained constant all over the study and significantly differed from the peptide group (Fisher exact test, T42, T70, T80, $P < .01$).

Skin immediate reactivity to intradermal tests

At the screening visit, none of the patients in the LSP or in the control group had an immediate allergic reaction to intradermal injection of any of the three LSPs separately or as a mixture ($EPC \geq 1 \mu\text{g/mL}$) (Fig 4, A and C). At the end of the trial (day 70), none of the patients from the control group had $EPC \leq 0.1 \mu\text{g/mL}$, whereas 4 of the 9 patients from the peptide group had skin reactivity to the LSP mixture at $0.1 \mu\text{g/mL}$, considered the lower limit of positivity. At day 0, all patients in the LSP and control groups had positive ID test reactions to native PLA2 (Fig 4, B and D). At the end of the trial (day 70), in the peptide group (Fig 4, B), 2 patients had increased EPC by 2

log₁₀ and 2 others by 1 log₁₀. A single patient had decreased EPC to PLA2 from 0.1 to $0.01 \mu\text{g/mL}$, whereas 4 patients did have a change in EPC to PLA2. In the control group at day 70 (Fig 4, B), 2 patients had increased EPC to PLA2 by 1 log, 1 patient had decreased EPC by 1 log, and 4 had no modification in EPC. Although globally those changes were nonsignificant between the groups, the only two patients who had markedly enhanced EPC to native PLA2 (by 2 logs) were in the LSP group.

In vitro IgE binding to long peptides

Although in dot blot assays there was a trend toward a modestly enhanced mean anti-whole BV and anti-native PLA2, IgE binding in the peptide group at day 14 and later as compared with days 0 and 7, there were no significant differences all along the study within and between the groups (Fig 5, A and B). Similarly, there were no differences in IgE binding to individual LSP within and between the groups (Fig 5, C, D, and E). Again, a nonsignificant trend toward enhanced IgE recognition of

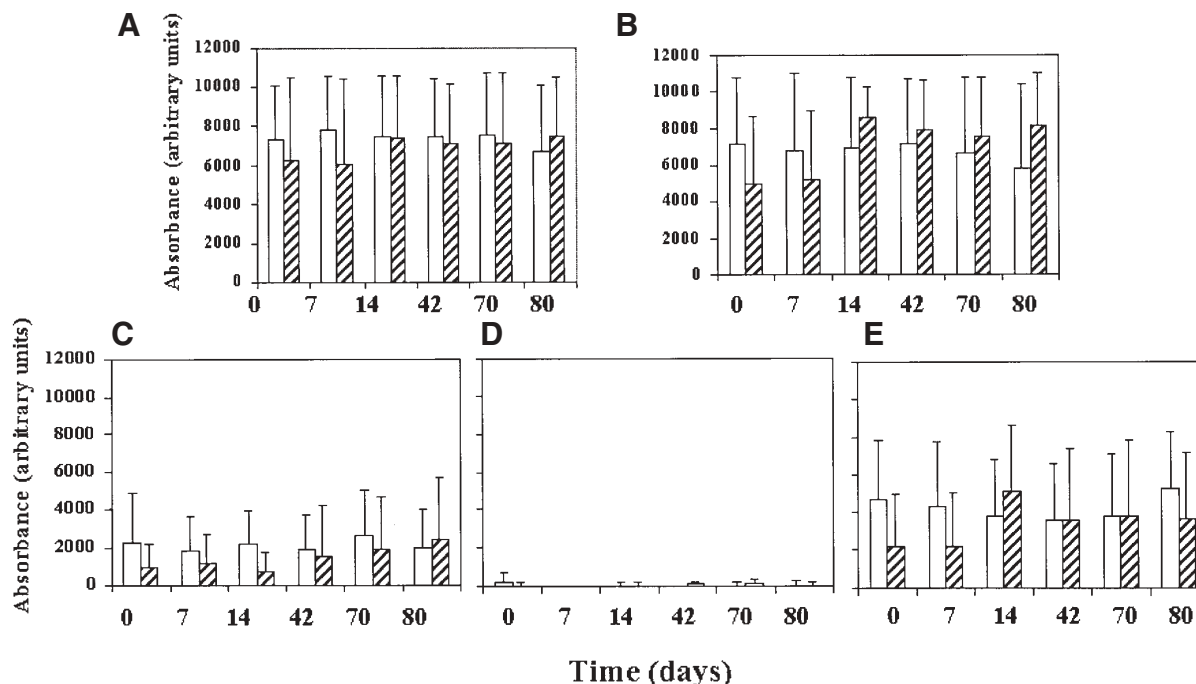


FIG 5. In vitro IgE binding to whole BV and native PLA2 (**A** and **B**) and to long synthetic peptides LSP1-60, LSP47-99, and LSP90-134 (**C**, **D**, and **E**, respectively) was analyzed by dot blot assays after each injection. Results are expressed as absorbance arbitrary units. Open columns, Control group; hatched columns, peptide group.

peptide LSP90-134 was noted in the LSP group. Both in the control and peptide groups, the C-terminal peptide LSP90-134 was binding IgE at a higher level followed by the N-terminal peptide LSP1-60. IgE binding to the internal peptide LSP47-99 was undetectable.

Safety evaluation study

At day 0, despite the injection of sharply increasing LSP doses up to a cumulative dose of 250 μ g of each peptide within 3.5 hours (100 μ g LSP group), none of the patients had local nor systemic reactions. In two patients, mild, late (>2 hours) local reactions (erythema) occurred after peptide injection at days 14, 42, and 70, to vanish after about 1 hour. In those two patients, after the last injection at day 70, hand palm pruritus and transient erythema of the upper part of the trunk occurred more than 3 hours after LSP injection. There were no severe adverse events (life-threatening reactions).

A maintenance dose of 300 μ g LSP was initially injected into two patients. In one patient, the late occurrence (>2 hours) of local skin reaction and upper trunk flush at day 42 led to the interruption of the treatment. The other patient, for safety reasons, was subsequently allocated to the 100- μ g LSP treatment group, though the 300- μ g dosage was well tolerated.

DISCUSSION

We have shown in this study that a peptide-based allergen immunotherapy with LSPs derived from PLA2, a

major BV allergen, was able to induce T-cell anergy, immune deviation toward a T_H1 -type T-cell cytokine response, enhanced IL-10 secretion, and PLA2-specific IgG4 production. LSP immunotherapy was safe and did not induce severe systemic reactions, though dose cumulation appeared to induce mild, nonimmediate reactions in two patients.

The fact that LSPs could be injected without any local or systemic adverse events at day 0, though cumulative doses of each peptide were reaching >250 μ g (550 μ g in the two patients injected with 300 μ g LSPs), demonstrates the high safety profile of LSPs. Mild local reactions (pruritus and erythema) occurred in only two patients at days 14, 42, and 70 for >120 minutes after the injection and did not last for >1 hour. In the same patients, the ultimate peptide injection led to late (>3 hours) systemic reactions characterized by hand pruritus and a flash of the upper trunk. This presentation is not typical of anaphylaxis, since it occurred relatively late (>3 hours) as compared with usual anaphylactic reactions during conventional immunotherapy or rush protocols that are triggered within minutes. The delayed character of these reactions were suggestive of a late allergic reaction, as interpreted in previous allergen peptide trials,^{6,12,21} and might be related to the stimulation of specific T cells to produce T_H1 proinflammatory cytokines. These secondary events are dose-dependent,¹² which certainly suggests a need to adapt the dose of LSPs in further clinical evaluations of LSP immunotherapy. Reactions were all benign and self-limited. No life-threatening reactions occurred.

In *in vitro* dot blot assays, a nonsignificant trend toward an enhanced IgE binding to native PLA2, whole BV, or peptides was apparent after the third LSP injection. Taken together with the trend in serum anti-PLA2 IgE level increase, these data may suggest that in LSP immunotherapy, as in conventional BV immunotherapy, an increase in allergen-specific IgE may occur during the first weeks of treatment.^{4,22} This increase may essentially reflect the transient specific T-cell activation observed during the first 2 weeks of therapy. However, the number of patients in this study is too low to discuss the clinical relevance of this phenomenon; IgE binding activity to peptides was clearly limited and plateaued after day 42. It was not reflected by *in vivo* skin testing at initiation of the study. During the course of the trial, 4 patients had mildly positive ID test reactions to LSPs at 0.1 µg/mL, whereas the 5 others were still negative at 1 µg/mL. This difference was certainly significant because in the control group none of the patients had positive ID test reactions at 0.1 µg/mL concentration at the end of the trial. The clinical significance of these positive ID test reactions is difficult to appreciate: The two patients who had mild systemic reactions after day 70 injection were among those four patients. However, clinical tolerance to LSP injection was good in the two others. Longer-term studies on larger study populations will be necessary to assess the long-term safety of LSP-based immunotherapy.

One of the most prominent results of this study was the induction of a profound specific T-cell hyporesponsiveness at day 80. If at the screening visit, T-cell proliferation in response to LSPs was low, which essentially suggested a low number of BV-specific T-cell precursors, it peaked at day 14 in the peptide group before progressing to hyporesponsiveness. Although previously shown in murine models,²³⁻²⁵ this is one of the first demonstrations in human beings that anergy induction was preceded by T-cell activation. This observation is in agreement with the recent demonstration that the late asthmatic reaction induced by the first administration of allergen-derived T-cell peptides in asthmatic persons allergic to cats preceded the induction of antigen-hyporesponsiveness.¹² The progressive downregulation of T-cell response to LSPs was paralleled by enhanced IL-10 and IFN-γ secretion, peaking at day 42 to decrease thereafter. The pattern of T-cell proliferation over time was suggestive of T-cell anergy induction, though we cannot exclude that T-cell clonal deletion may have also contributed to the phenomenon, especially regarding the decrease in cytokine secretion occurring late in the course of therapy. Interestingly, the peak of IL-10 and IFN-γ secretion occurred about 4 weeks after the maximal T-cell proliferation, that is, at a time when T-cell anergy was installing. This situation is not incompatible with T-cell tolerance because *in vitro* anergic CD4+ T-cell clones are still able to differentiate into T_H1-like effector cells, to participate in T-dependent IgG2a antihapten responses and delayed-type hypersensitivity reactions.²⁶ Similarly, in allergy models to PLA2 in mice, we demonstrated the persistence of a strong IFN-γ production and antiallergen

IgG2a response despite tolerance induction by LSPs.^{13,27} IL-10 has been involved in T-cell anergy induction and appeared to be secreted by a subpopulation of T lymphocytes able to repress other CD4+ T-cell-specific activity, the so-called Tr1 subset.^{28,29} IL-10 also has prominent anti-inflammatory properties.³⁰ Though by itself an immune deviation to a T_H1-type cytokine production may be deleterious,³¹ a combination of an anti-inflammatory cytokine such as IL-10 and IFN-γ may reequilibrate a potentially detrimental cytokine secretion.

Specific anti-PLA2 serum IgG4 response was significantly stimulated. We and others have previously demonstrated a gradual rise in IgG4 during the incremental phase of conventional immunotherapy.^{4,32} Serum IgG4 levels may be predictive of effective protection in response to immunotherapy,^{33,34} though this notion, on an individual basis, may be controversial.³² It was recently shown that IgE and IgG4 levels obtained after 2 years of specific immunotherapy were specific and sensitive predictors of reactivity after hymenoptera venom challenge, a high IgG4 response being associated with protection and low IgG4 levels with anaphylaxis.³⁵ IgG4 may in part compete with IgE binding on allergen and thus contribute to clinical protection.³⁶ Although the demonstration of clinical efficacy was not an objective of this phase I trial, our findings may support this hypothesis. Definite demonstration of efficacy will need proper provocation with native PLA2.

In conclusion, the results of this placebo-controlled trial demonstrated that an LSP-based allergen immunotherapy was safe and able to induce specific T-cell hyporesponsiveness, immune deviation toward T_H1 cytokine secretion with parallel IL-10 secretion, and enhanced IgG4 production. As such, LSP immunotherapy reproduces the pattern of cellular and humoral events observed in rush and conventional immunotherapy without their inherent anaphylactic secondary events^{4,20,37,38} and may thus represent a novel and safe approach of specific immunotherapy.

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