

Modulation of T-cell response to phospholipase A₂ and phospholipase A₂-derived peptides by conventional bee venom immunotherapy

Regine Kämmerer, MD,^{a, b} Yolande Chvatchko, PhD,^{a, b} Alexander Kettner,^{a, b}
Nathalie Dufour,^a Giampietro Corradin, PhD,^b and François Spertini, MD^a
Lausanne, Switzerland

Background: Immunologic mechanisms of desensitization are still incompletely understood. Safer methods of immunotherapy with reduced risks of anaphylaxis need to be developed. **Objective:** To study the effects of conventional venom immunotherapy (VIT) on phospholipase A₂ (PLA₂)-specific T cells and on T-cell reactivity to short and long synthetic peptides that map the PLA₂ molecule.

Method: Proliferation of a CD4⁺ cell-enriched peripheral blood mononuclear cell fraction and cytokine secretion by T cell lines from patients hypersensitive to bee venom and undergoing VIT in response to PLA₂ and PLA₂ synthetic peptides were measured.

Results: T-cell proliferation in response to three synthetic peptides, 40 to 60 amino acids long and mapping the entire PLA₂ molecule with an overlap of 10 residues (1 to 59, 51 to 99, and 90 to 134) steadily increased during the first 14 weeks of VIT corresponding to the treatment period with incremental doses of antigen. These results are in contrast to the low proliferation indices obtained with short (15 amino acid-long) peptides, and the inability to characterize the immunodominant region of the molecule with short peptides. At the end of VIT (after 3 to 5 years), there was correspondingly, a marked decrease in T cell responsiveness to PLA₂ and to its long synthetic peptides. This response was paralleled by a shift in the pattern of cytokine secretion by T cell lines from a T_{H0}-type to a T_{H1}-type pattern.

Conclusion: After a transient increase in T-cell proliferation, late VIT was characterized by T-cell hyporesponsiveness to allergen and by modulation of cytokine secretion from a T_{H0}-type to a T_{H1}-type pattern. Because of their capacity to recruit multiple T-cell epitopes, long peptides mapping the entire PLA₂ molecule appear to be efficient T cell stimulators and may represent potential candidates for peptide immunotherapy. (*J Allergy Clin Immunol* 1997;100:96-103.)

Key words: Immunotherapy, desensitization, T cells, phospholipase A₂, peptides

Abbreviations used

BV:	Bee venom
CD8 ⁺ -PBMC:	CD8 ⁺ T cell-depleted PBMC fraction
IFN- γ :	Interferon- γ
LP:	Long peptide from PLA ₂
PBMC:	Peripheral blood mononuclear cell
PLA ₂ :	Phospholipase A ₂
TT:	Tetanus toxoid
VIT:	Venom immunotherapy

Allergy to bee venom (BV), which has an incidence of about 3% in the general population, is a frequently diagnosed hypersensitivity reaction that has its own epidemiologic characteristics, because it is usually not related to other atopic conditions.¹ Hyposensitization with BV extracts has been available only within the last 15 years and is currently recognized as the only successful long-term therapy for this allergy.²⁻⁵ It is still an empiric treatment with regard to the doses administered and the duration of therapy. The conventional schedule consists of monthly injections preceded by a period of weekly injections of incremental doses of BV.^{3, 6-8} Treatment is usually given for 3 years (up to 5 years for severe cases) and is time consuming and expensive.^{9, 10} The biologic criteria of successful immunotherapy usually consist of a decrease of intradermal skin test reactivity to BV, a decrease in the level of specific serum IgE, and a parallel increase in the levels of specific IgG and IgG4 against individual BV allergens.^{4, 10, 11} However, the association between these biologic markers and the degree of protection is controversial, except in patients with both negative intradermal tests and specific IgE to BV.^{9, 12-14}

The immunologic basis of desensitization therapy is still largely unknown. Various hypotheses have been proposed including the production of IgG blocking antibodies,¹⁵ an allergen-specific reduction of histamine release from basophils and mast cells,¹⁶ as well as the generation of antiidiotypic antibodies.^{17, 18} It appears that most of these immune manifestations are essentially markers of ongoing interventions on the immune system and may not represent primary protective mechanisms. For instance, there is no absolute correlation between the rise in antigen-specific IgG and a favorable clinical response to immunotherapy.¹⁹⁻²¹ Although the fine

From the ^aDivision of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois and ^bInstitute of Biochemistry, University of Lausanne, Lausanne.

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Reprint requests: François Spertini, MD, Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland.

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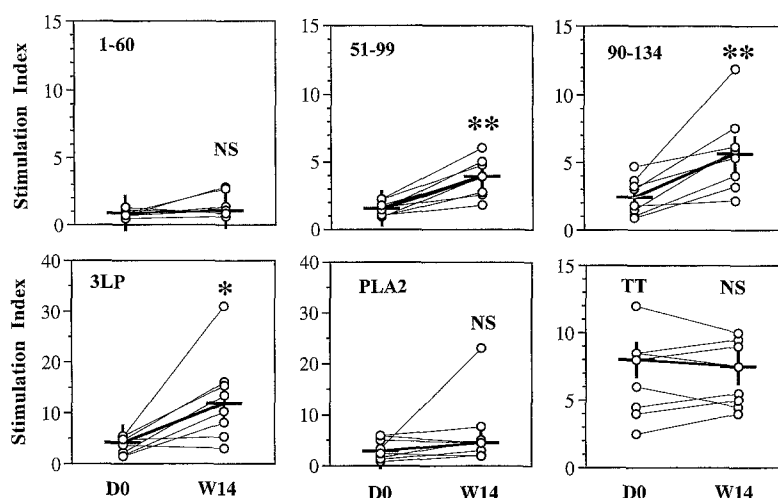


FIG. 1. CD8⁺ PBMC response to PLA₂ and its three LPs (1 to 60, 51 to 99, and 90 to 134) mapping whole PLA₂ (10 µg/ml each or as a mixture of three LPs) at initiation of VIT (D0) and 14 weeks later (W14). Results are expressed as individual stimulation indices (open circles) and as the median (black cross). Statistical analysis was performed with the paired Wilcoxon signed-rank test. Responses to the TT control showed no significant variation during this time period. NS, Not significant; *, $p < 0.05$; **, $p < 0.01$.

mechanisms of immunotherapy are unclear, these may be related to the principle of T-cell tolerance with supraimmunogenic doses of allergen.^{22, 23} Alternatively, specific immunotherapy may modulate the pattern of cytokines secreted by T cells and induce a shift in the IL-4: interferon- γ (IFN- γ) ratio.²⁴⁻²⁶

Thus, a logical strategy to mimic specific immunotherapy while decreasing its risks and potentially increasing its efficacy would be to administer the highest possible dose of T-cell epitope-containing peptides in order to render specific T cells anergic. This approach is theoretically feasible because peptides, in general, lack a proper three-dimensional structure, which results in little or no binding to IgE (Kämmerer et al. Unpublished data), and therefore does not expose patients to anaphylaxis. In this paper we show that conventional VIT is associated with an early enhanced T-cell response to phospholipase A₂ (PLA₂) or PLA₂ synthetic peptides, which is followed by a later state of specific T-cell hyporesponsiveness and a sharp decline in the IL-4:IFN- γ ratio, which correlate with a decrease in specific IgE and an increase in specific IgG and IgG4. Long peptides (LPs) that map the whole PLA₂ molecule, because of their capacity to recruit multiple T-cell epitopes, appear to be efficient T-cell stimulators and may represent potential candidates for peptide immunotherapy.

METHODS

Patients

Twenty-one patients hypersensitive to BV were enrolled in the study. Twelve patients (3 women and 9 men; age [mean $\pm$ SD], 42 $\pm$ 11 years) were enrolled at the initiation of conventional VIT and during the first 4 months of therapy (group I) and 9 patients (3 women and 6 men; age, 42 $\pm$ 12 years) were treated for 3 to 5 years (group II). Inclusion criteria for therapy and study were the

following: (1) grade III (generalized urticaria or angioedema together with respiratory symptoms [dyspnea, wheezing, stridor] or grade IV (any of the grade III symptoms together with at least two of the following conditions: fall in blood pressure, collapse, loss of consciousness) systemic allergic reactions after a honey bee field sting (according to the classification of Müller²⁷); (2) positive immediate intradermal skin test responses (occurrence of a wheal and flare reaction at a BV concentration of less than 0.1 µg/ml); and (3) specific IgE level greater than 0.35 kU/L against whole BV (as titrated by the CAP system [Pharmacia, Upsala, Sweden]). Measurements of specific serum IgE levels and intradermal skin tests were repeated in group II patients at the end of a 3- or 5- year course of VIT.

Conventional VIT

Patients were treated with aqueous whole BV extract (Pharmalgen venom; ALK, Denmark) that were administered subcutaneously in incremental doses up to a plateau dose of 100 mg, according to European Guidelines.⁵ The following regimen of an initial dose of aqueous whole BV extract followed 30 minutes later by a second dose was used. Week 1: initial dose of 0.01 µg followed by 0.1 µg; week 2: initial dose of 1 µg followed by 2 µg; week 3: initial dose of 4 µg followed by 8 µg; week 4: initial dose of 10 µg followed by 20 µg; week 5: 40 µg; week 6: 60 µg; week 7: 80 µg; week 8: 100 µg (plateau dose); weeks 9, 11, and 13: 100 µg; and subsequently, 100 µg once a month. Blood was collected before the start of therapy and, for patients undergoing VIT, between days 7 and 10 after the last venom injection. All patients gave informed consent for the study, and study protocols were approved by the Hospital Review Board.

Reagents

Whole BV and PLA₂ were purchased from Latoxan (Rosans, France). PLA₂ was detoxified by reduction and carboxymethylation, followed by cleavage with cyanogen bromide as described.²⁸ Tetanus toxoid (TT) (Swiss Serum Institute, Bern, Switzerland) was used as the control antigen. The following peptides were generated by using a multipetide synthesizer

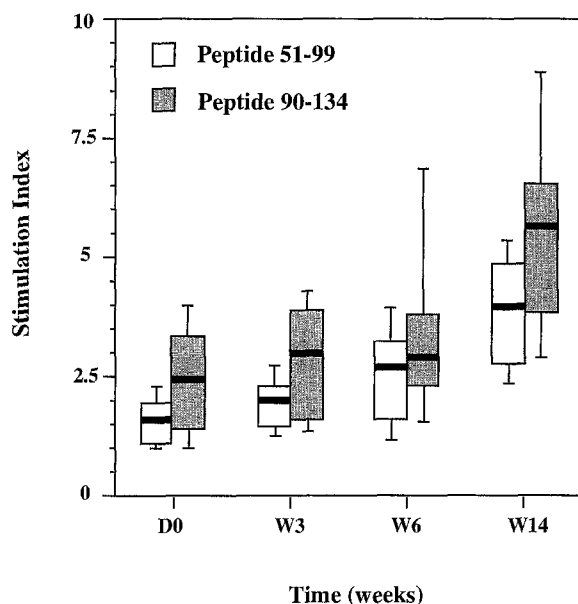


FIG. 2. CD8⁺ PBMC proliferation in response to peptides 51 to 99 and 90 to 134 at initiation of VIT (D0), at week 3 (W3), at week 6 (W6), and at the end of the incremental dose period (W14). Results show the distribution of stimulation indices from individual subjects as boxes (percentile 25, lower border; median, bold line; percentile 75, upper border) and whiskers (percentile 10, lower whisker; percentile 90, upper whisker). Statistical analysis between stimulation indices for each peptide at indicated time points was done with Kruskal-Wallis nonparametric analysis of variance tests ($p < 0.01$).

(Automated multiple peptide synthesizer, ABIMED, Germany): (1) peptides containing 15 amino acids and overlapping the preceding peptide by five residues and (2) three LPs consisting of 40 to 60 amino acids with an overlap of 10 residues (1 to 59, 51 to 99, and 90 to 134), mapping the entire PLA₂. F-moc, t-Bu strategy was used according to Atherton et al.²⁹ Peptides were purified by gel chromatography and used at a final concentration of 10 µg/ml. Amino acid analysis, analytical HPLC, and mass spectrometry were used to assess the composition and purity of peptides. Phorbol myristate acetate and ionomycin were purchased from Calbiochem (San Diego, Calif.).

Cell preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density gradient centrifugation over Ficoll-Paque (Pharmacia Biotech AB). For CD8⁺ cell depletion, PBMCs were suspended in phosphate-buffered saline containing 2% human AB⁺ serum (Swiss Red Cross, Bern, Switzerland) and 5 mmol/L ethylene diamine-tetraacetic acid, then incubated at a concentration of 10⁷ cells/80 µl with anti-CD8 monoclonal antibody-coated magnetic beads (Miltenyi Biotech GmbH, Bergisch-Gladbach, Germany) for 15 minutes. Cells were then separated in a magnetic cell sorter (MACS; Miltenyi Biotech). The CD8⁺ T cell-depleted PBMC fraction (CD8[−] PBMC) contained less than 1% CD8⁺ cells and more than 70% CD4⁺ T cells, as determined by flow cytometry (Epics Profile II; Coulter, Miami, Fla.).

PBMC proliferation assays

PBMCs (2×10^5 /well) from each donor were cultured in replicates of eight in 96-well round bottom plates (Nunc, Roskilde, Denmark) in RPMI medium (Gibco, Basel, Switzerland) containing 10% AB⁺ serum, 2 mmol/L glutamine, 1% sodium pyruvate, 1% nonessential amino acids, 1% kanamycin (all from Gibco) with the appropriate concentration of antigen. After 6 days of culture in 5% CO₂ at 37° C, cells were harvested after the addition of 1 mCi of tritiated thymidine (Du Pont NEN Products Boston, Mass.) during the final 18 hours of culture. Results were expressed as counts per minute of tritiated thymidine incorporated into DNA.

Short-term T cell lines

T cell lines were derived from the CD8[−] fraction of PBMCs (10^6 cells/ml) from hypersensitive donors stimulated in 24-well plates (Nunc) with a mixture of three LPs (10 µg/ml) and maintained in supplemented 10% AB⁺/RPMI 1640 medium as described earlier. T cell lines were restimulated after 7 and 14 days with autologous irradiated (2500 rad) PBMCs and adequate antigen (10 mg/ml). Recombinant human IL-2 (10 U/ml; Glaxo, Geneva, Switzerland) was added from day 9, twice a week. At day 14, to test for specificity, T cell lines were stimulated (1×10^5 cells/well in triplicate) with antigens and irradiated feeder cells. Seventy-two hours later, T cells were pulsed with 1 mCi of tritiated thymidine for 18 hours and harvested. Results of proliferation are expressed as counts per minute of tritiated thymidine incorporated into DNA.

Quantification of cytokines

At day 14, T cell lines (2×10^6 cells/well in 24-well plates) were washed three times and stimulated with PMA (25 ng/ml) and ionomycin (0.1 µmol/L), in the absence of feeder cells. Supernatants were harvested 24 hours later and stored at −80° C. IL-4 and IFN-γ were titrated by using commercially available ELISA kits (Cytoscreen Immunoassay Kit; Biosource International, Camarillo, Calif.), according to the manufacturer's recommendations. The limits of sensitivity of the ELISA were 8.7 pg/ml for IL-2, 2 pg/ml for IL-4, and 4 pg/ml for IFN-γ.

Quantification of specific serum IgE, IgG, and IgG4

Whole BV-specific IgE, IgG, and IgG4 were quantified with the Pharmacia CAP System Fluoroimmunoassay (Pharmacia Diagnostic AB) according to the manufacturer's recommendations. Calibration was performed with the World Health Organization standard for IgE between 0.35 and 100 kU/L. For quantification of specific IgE against PLA₂, CAP[®] beads were prepared by the manufacturer (Pharmacia Diagnostic AB) with purified PLA₂.

RESULTS

Because CD4⁺ T cells play a crucial role in IgE regulation via IL-4 production and appear to be one of the main targets of specific immunotherapy,^{24,30} we focused our experiments on the proliferation of CD8[−] PBMCs from patients hypersensitive to BV who were undergoing conventional VIT. We first examined the T-cell response to PLA₂ and PLA₂ peptides before VIT and during the first 14 weeks of treatment. CD8[−] PBMCs from patients in group I were incubated either with LPs mapping the whole PLA₂ molecule (1 to

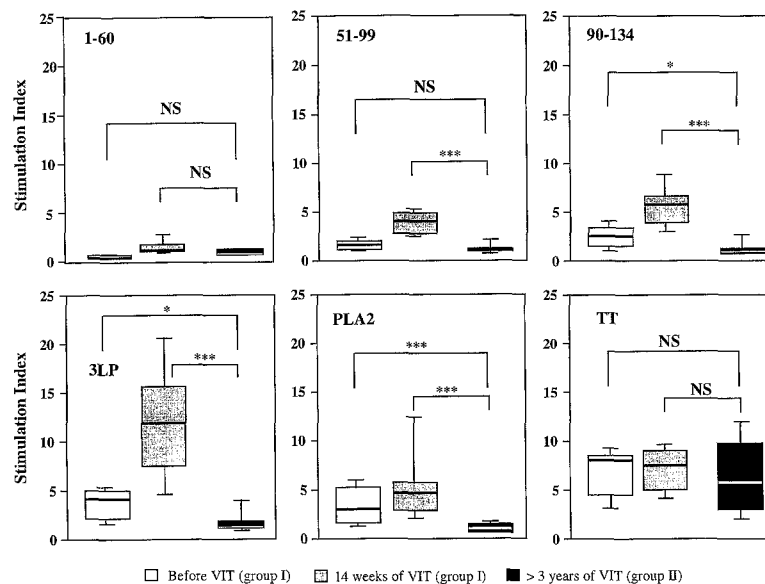


FIG. 3. CD8⁺PBMC responses from group I and group II patients to PLA₂ and its three LPs. Results are expressed as the distribution of individual data (stimulation indices) as described in Fig. 2. Statistical analysis between results from group I and II was done with the unpaired Wilcoxon signed-rank test. Responses to TT control showed no significant variation between groups. NS, Not significant; *, $p < 0.05$; ***, $p < 0.001$.

60, 51 to 99, and 90 to 134) or with short 15 amino acid-long overlapping peptides. When CD8⁺PBMCs were stimulated with PLA₂ LP (10 mg/ml), T-cell proliferation was in general weak before VIT. The strongest proliferation indices were obtained in response to carboxyterminal peptide 90 to 134, followed by peptide 51 to 99 (Fig. 1). Cells proliferated very poorly to N-terminal peptide 1 to 60. During the incremental dose period of VIT, proliferation to peptides 51 to 99 and 90 to 134 steadily increased (Fig. 2) and reached statistical significance after 14 weeks ($p < 0.05$), in comparison with the period immediately before start of VIT (Figs. 1 and 2). Proliferation to a cyanogen bromide-treated PLA₂ did not significantly increase, possibly indicating that methionine was within the epitope(s) recognized by T cells. In contrast to the response to LPs, CD8⁺PBMC responded poorly to 15 amino acid-long peptides (mean stimulation index, 2.1; range, 1.4 to 4.1), covering the whole length of the PLA₂ molecule, which did not allow definition of a particular pattern of dominant epitopes (data not shown). After 14 weeks of therapy for some patients, a trend toward an increase in T-cell proliferation was noticed for some 15mer epitopes, but this was highly variable among patients. CD8⁺PBMC proliferated vigorously to the TT control, and no significant change in CD8⁺PBMC response to TT was noticed during the first 14 weeks of VIT (Fig. 1). Thus, in contrast to an epitope-mapping strategy based on LPs, mapping PLA₂ T-cell epitopes with 15 amino acid-long overlapping peptides in an unselected group of patients provided inconclusive results.

In group II patients (those receiving 3 to 5 years of VIT), the proliferation response of CD8⁺PBMC to

PLA₂ and its LP was almost completely abrogated (Fig. 3). The decrease in cell proliferation was the most significant when compared to stimulation indices obtained after 14 weeks of VIT in group I patients for PLA₂, its LP 51 to 99, 90 to 134, or the three-LP mixture ($p < 0.01$). Although the mean proliferation response to PLA₂ and to its LP was low at initiation of therapy, the proliferation response of group II patients was even significantly lower in response to peptide 90 to 134, to the three-LP mixture, and to PLA₂. There was no significant difference between groups in the proliferation of CD8⁺PBMC in response to TT. Considered together, these results indicate that during the first 3 to 4 months of conventional immunotherapy, patients developed a progressive T-cell sensitivity to BV allergens, which was followed by T-cell hyporesponsiveness after 3 to 5 years of monthly administration of BV.

Although the variation of mean serum levels of anti-whole BV or anti-PLA₂ IgE did not reach significance during the first 14 weeks of VIT, individual IgE levels actually increased in six out of nine patients (Fig. 4, A and B). In contrast, T cell hyporesponsiveness observed in group II patients after 3 to 5 years of VIT was associated with a significant decrease in specific serum IgE levels against whole BV ($p < 0.05$) and PLA₂ ($p < 0.01$) (Fig. 4, C and D), as well as in intensity of the intradermal skin tests to BV (data not shown). There was no statistically significant change in serum levels of specific IgG and IgG4 during the first 14 weeks of VIT in group I patients (Fig. 5, A and B). However, serum levels of specific IgG and IgG4 increased ($p < 0.05$) in group II patients after 3 to 5 years of treatment (Fig. 5, C and D).

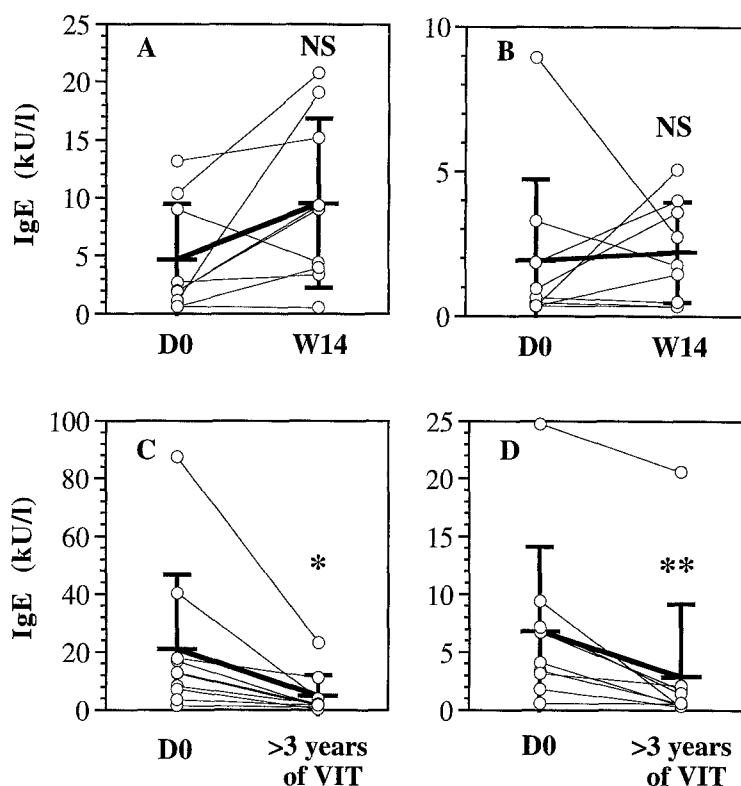


FIG. 4. Serum levels (in kU/L) of specific IgE against whole BV (A and C) and PLA₂ (B and D) in group I patients (A and B) at initiation of VIT (D0) and 14 weeks later (W14), and in group II patients (C and D) at initiation of VIT and after 3 to 5 years of VIT. L. Statistical analysis was done with a paired Student's *t* test NS; Not significant, *, *p* < 0.05; **, *p* < 0.01).

To better understand whether the initial increase (at week 14) in the specific T-cell response to PLA₂ peptides and the later hyporesponsiveness was accompanied by changes in cytokine secretion, cytokine production by CD8⁺ PBMC isolated from group I and group II patients was examined. However, as previously reported by others,³¹ stimulation of primary cultures at day 7 with PLA₂ peptides induced only low levels of cytokine secretion and did not provide reliable results. This was most probably due to the low frequency of specific circulating T-cell precursors, as suggested by low proliferation indices observed before VIT. Therefore, to obtain an enrichment in allergen-specific T cells and by using an approach described by others,²⁴ short-term T cell lines were raised against a three-LP mixture, stimulated twice with antigen and feeder cells, and then tested for cytokine production after stimulation with PMA or ionomycin for 24 hours. The specificity of T cell lines for PLA₂ was assessed by a proliferation assay to the three-LP mixture inducing a stimulation index ≥ 5 (data not shown). A clear trend of a decrease in IL-4 secretion was observed, whereas IFN- γ secretion significantly increased, when lines isolated from patients before VIT were compared with lines obtained from patients at the end of VIT (Fig. 6). Whereas at day 0 or after 14 weeks of VIT, the IL-4:IFN- γ ratio was 1.0 and 1.4 respectively, it decreased to 0.1 at the end of VIT (*p* < 0.05). These

results indicate that, in parallel to the changes in T-cell proliferation observed over the first 4 weeks of VIT and thereafter, the cytokine secretion pattern by short-term T cell lines was progressively modulated from a T_{H0}-like to a T_{H1}-like pattern, and was profoundly modified at the end of VIT.

DISCUSSION

In this paper we have examined the short- and long-term effects of conventional VIT on T-cell reactivity to PLA₂ or to peptides covering the entire PLA₂ sequence. As the first evidence of the effect of VIT on T cells, our results demonstrate that conventional VIT was associated with a steady increase in the response of the CD4⁺ cell-enriched T cell fraction to PLA₂ LPs 51 to 99 and 90 to 134 up to week 14 (Figs. 1 and 2). This early enhancement in T-cell proliferation was specific because no significant change in cell proliferation to TT was detected. Our observations are in sharp contrast with the very early decrease in PBMC proliferation noted during the first 50 days of ultra-rush VIT and suggest that these two immunotherapy protocols may alter T-cell responses by different mechanisms.²⁶

Because CD4⁺ T cells are critical for the induction of IgE synthesis,^{24, 32-34} we restricted epitope mapping to in vitro proliferation assays of aCD8-PBMC. Despite a selective enrichment in CD4⁺ T cells (greater than

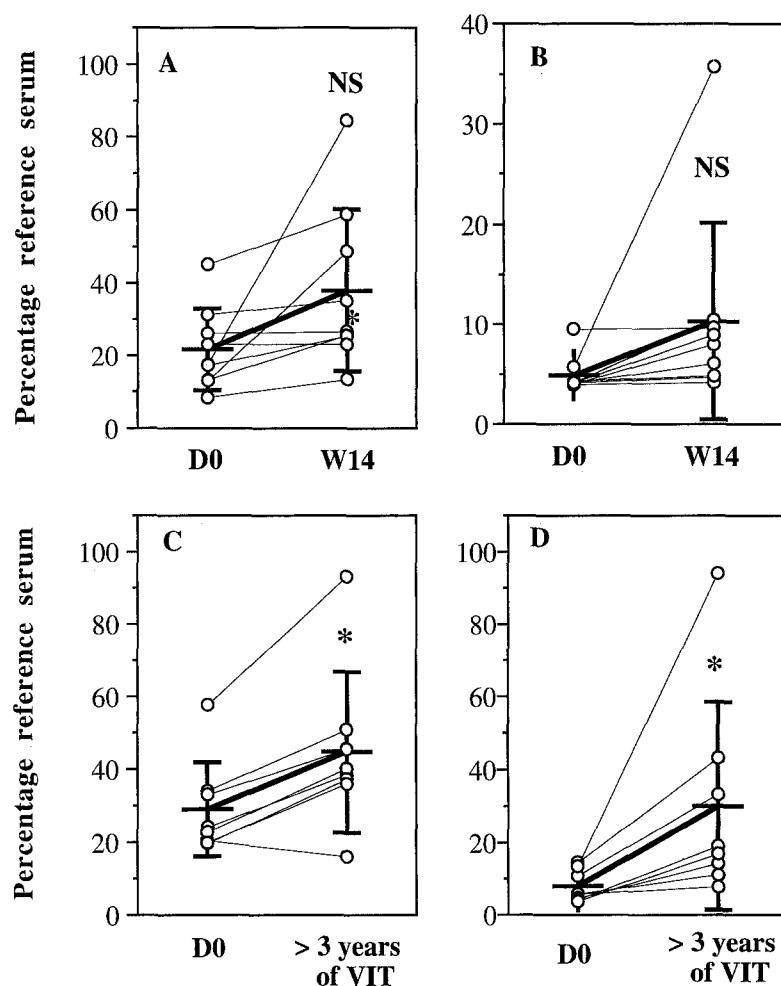


FIG. 5. Serum levels of specific IgG (A and C) and IgG4 (B and D) against whole BV in group I patients (A and B) at initiation of VIT (D0) and 14 weeks later (W14) and in group II patients (C and D) at initiation of VIT and after 3 to 5 years of VIT. Results are reported as percentage of a reference serum sample. Statistical analysis was done with a paired Student's *t* test NS; not significant; *, *p* < 0.05.

70%), the low stimulation indices obtained in some of our patients most probably reflect, at least before VIT, the low frequency of specific T cell precursors in patients who have been stung only rarely, sometimes weeks or months before therapy. However, results obtained at week 14 with PLA₂ LPs confirm that, because of their capacity to recruit multiple epitopes, LPs are efficient T-cell stimulators; this allowed characterization of the immunodominant region(s) of PLA₂. T-cell epitopes appeared to be encountered mainly in the C-terminal portion of PLA₂, in accordance with recent delineations of the minimal dominant T-cell epitopes of PLA₂ (45-62, 50-69, 83-97, 81-92, and 113-124) by using a short peptide mapping strategy.^{35, 36} In contrast to these reports, in our unselected group of patients, poor proliferation indices were obtained with 15 amino acid-long peptides, with a minimal increase in T-cell proliferation occurring for some epitopes only after 14 weeks of VIT, whereas a significant and steady increase in cell proliferation was detected using long overlapping peptides

(Fig. 2). This indicates that, in unselected patients, the use of short peptides (bearing minimal epitopes) does not adequately provide the sensitivity needed to delineate T-cell responses to allergens in all clinically proven cases of hypersensitivity, despite performing proliferation assays in replicates of eight.

The early increase in T-cell proliferation to PLA₂ and its three LPs in patients from group I contrasts with the significant decrease in the T-cell specific response after 3 to 5 years of VIT in group II. This suggests that after a period of T-cell sensitization to allergen (during the period of administering incremental doses of allergen), the monthly administration of the maximal tolerable dose of allergen results in T-cell hyporesponsiveness to allergen. Such an effect may be ascribed to a mechanism comparable to the *in vitro* tolerance observed upon administration of a supraimmunogenic amount of antigen, although the exact *in vivo* mechanisms are unclear.^{22, 37, 38} Indeed, the specific T-cell hyporesponsiveness correlated closely with a concomitant sharp decline

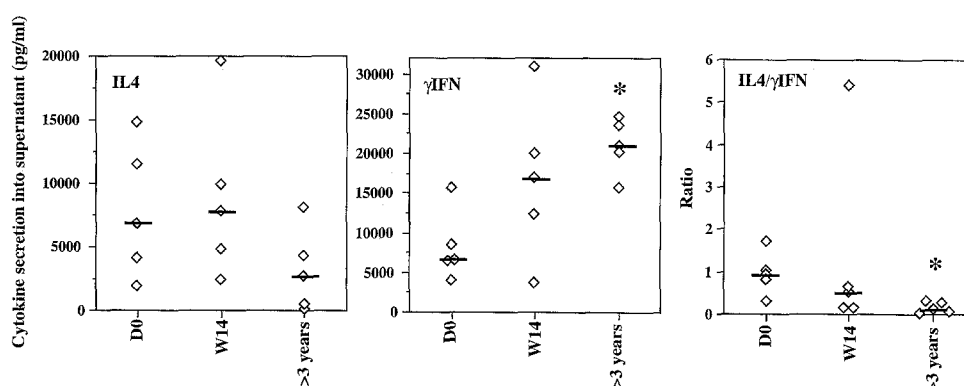


FIG. 6. Cytokine secretion by short-term T cell lines from patients hypersensitive to BV, which were stimulated with PMA/ionomycin for 24 hours: lines were raised against a mixture of three LPs mapping PLA₂, at initiation of VIT (D0), after 14 weeks of VIT (W14) (group I patients), and at the end of VIT (>3 years) (group II patients). Solid bars indicate the median of cytokine secretion or cytokine ratio from five individual lines. Statistical analysis was done with a nonparametric analysis of variance test * $p < 0.05$, D0 vs >3 years).

in serum levels of specific IgE (Fig. 4, C and D), and with a low IL4:IFN- γ ratio at the end of therapy. These data are reminiscent of recent in vitro and in vivo observations. In allergen-specific T_{H2} clones, nonresponsiveness to further antigenic stimulation or inability of T cells to secrete IL-4 or IL-13 was elicited by using high doses of allergen-derived peptides in the absence of antigen-presenting cells.²³ In vivo, in a murine model of allergy, IL-2 production in response to Fel d 1 was abrogated by prior treatment of animals with a high dose of Fel d 1 peptides.³⁹ Clinical trials based on specific immunotherapy with two 27 amino acid-long peptides derived from Fel d 1 in patients with allergy to cats are currently in progress. Although the long-term effects of VIT on T cells may represent one of the mechanisms contributing to allergen desensitization, this does not exclude effects on other cell populations, antigen-presenting cells in particular.⁴⁰⁻⁴³

The effect of VIT on serologic parameters has been investigated extensively. An initial increase in levels of specific IgE is usually reported, followed by a late decrease in IgE levels and in skin sensitivity, and an increase in specific IgG and IgG4 (see reference 4). Although the increase in mean serum anti-whole BV or anti-PLA₂ IgE, IgG, or IgG4 levels did not reach significance over the first 14 weeks of VIT in our limited number of patients, individual IgE, IgG, or IgG4 levels actually increased in several patients (Figs. 4, A and B and 5, A, and B), and this increase was paralleled by an increase in mean T-cell proliferation. Globally, serologic results from groups I and II are compatible with a relatively late shift from a T_{H0}-type IL4:IFN- γ ratio to a T_{H1}-type ratio, although a largely variable pattern of cytokine secretion was observed during the early weeks of therapy (Fig. 6). Thus, in comparison to ultrarush VIT in which an in vitro decrease in IL-4 and IL-5 secretion was already observed after 7 days,²⁶ the modulation of T

cell cytokines appeared to be slower during conventional VIT. The different pace of cytokine modulation and the steady enhancement in the T-cell proliferation response over the first 14 weeks of conventional VIT (Figs. 1 and 2) may be related to repeated T-cell stimulation with low amount of allergen. Though speculative, these differences in kinetics between ultrarush and conventional immunotherapy may possibly result in different effects not only on T cells, but also on other cells such as mastocytes, and even have different long- or short-term clinical implications.

In conclusion, our results indicate that in contrast to ultrarush VIT, conventional VIT is associated with an early enhancement of the T-cell response to allergen during the incremental antigen dose period and, later, with a state of specific hyporesponsiveness. Furthermore, a shift in the pattern of cytokine secretion by T cell lines from a T_{H0}-type to a T_{H1}-type pattern was observed. In parallel to new insights into the mechanism of conventional VIT, we also indicate in this paper that low T-cell proliferation response to short 15 amino acid-long PLA₂ peptides can be overcome by the use of LPs (containing 40 to 60 amino acids). Because they are linear, LPs do not bind IgE or do so only minimally, in contrast to native allergens (Kämmerer et al. Unpublished data). In addition, LPs simultaneously cover multiple epitopes, and their use in immunotherapy may overcome the problem of the genetic restriction inherent in short peptides. These advantages, in principle, make LPs ideal tools for immunotherapy based on the delivery of supraimmunogenic doses of peptides, in part mimicking conventional VIT, although the short- and long-term effects of this approach on T cells and other cell subpopulations remain to be further elucidated.

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