Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom

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Background: Specific immunotherapy with honeybee venom (BV) is highly effective, but allergic side effects can occur during treatment. Immunotherapy with peptides containing major T-cell epitopes of the relevant allergen or allergens provides an alternative strategy without these problems.
Objective: The study investigates the immunologic mechanisms and clinical effects of immunotherapy with T-cell epitope peptides of the major BV allergen, the phospholipase A2 (PLA).
Methods: Five patients with IgE-mediated systemic allergic reactions to bee stings were treated with a mixture of three T-cell epitope peptides of PLA. Ten patients allergic to BV receiving whole BV immunotherapy served as control subjects. Increasing doses of the peptide mixture, up to a maintenance dose of 100 μg, were administered subcutaneously within 2 months. The patients were then challenged with PLA and 1 week later with a bee sting. The cellular and humoral immune response was measured in vitro.
Results: No allergic side effects were caused by the peptide immunotherapy, and all patients tolerated the challenge with PLA without systemic allergic symptoms. Two patients developed mild systemic allergic reactions after the bee sting challenge. After peptide immunotherapy, specific proliferative responses to PLA and the peptides in peripheral blood mononuclear cells were decreased in successfully treated patients. The production of T_{H2} and T_{H1} cytokines was inhibited, and B cells were not affected in their capacity to produce specific IgE and IgG4 antibodies. Their levels increased after allergen challenge in favor of IgG4.
Conclusions: Immunotherapy of BV allergy with short T-cell peptides of PLA induces epitope-specific anergy in peripheral T cells and changes the specific isotype ratio in a fashion similar to that of conventional immunotherapy in successfully treated patients. (J Allergy Clin Immunol 1998;101:747-54.)

Key words: *Immunotherapy, T-cell epitopes, anergy, bee sting allergy, bee venom phospholipase A2*

Abbreviations used

BV:	Bee venom
BVIT:	Bee venom immunotherapy
EPC:	End-point concentration
IFN:	Interferon
mAb:	Monoclonal antibody
MHC:	Major histocompatibility complex
PBMC:	Peripheral blood mononuclear cell
PIT:	Peptide immunotherapy
PLA:	Phospholipase A2
PPD:	Tuberculin protein derivative
PRU:	Phadezym RAST units
SIT:	Specific immunotherapy
TT:	Tetanus toxoid

Systemic allergic reactions to Hymenoptera venoms, most often from honeybees and vespids, are observed frequently. Specific immunotherapy (SIT) with whole bee venom (BV) is effective in most patients, but allergic side effects can occur in up to 40% of patients.[1] Thus there remains a need for alternative strategies of specific therapy. Such may be provided by peptide immunotherapy (PIT) bearing most dominant T-cell epitopes of the major allergen or allergens.[2, 3]

In human beings T-cell peptides of the major cat allergen Fel d 1, were applied in SIT of cat allergy.[2, 4] Although the treatment was clinically successful, its efficacy could not be clearly related to defined immunologic parameters. However, in mice it was shown that therapy with peptides of Fel d 1[5] or the house dust mite allergen Der p 1[6] induces specific tolerance in T cells against the allergen.

The immune response to bee venom (BV) provides an especially suitable model to study the mechanisms of human allergen–specific isotype regulation and of SIT. In BV-sensitized individuals, phospholipase A2 (PLA) represents the major allergen and immunogen,[7, 8] and both IgE-mediated allergy and IgG4-related normal immunity are elicited by PLA.[7, 9-11] In PLA we have identified three T-cell epitopes corresponding to the amino acid sequences of PLA45-62 (PI), PLA82-92 (PII), and PLA113-124 (PIII)[12] and an epitope involving

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TABLE I. Clinical and diagnostic data of patients allergic to BL before PIT

Patient no.	Age (yrs)	Sex	Allergic reaction	Skin test EPC (gm/L)			Specific IgE (PRU)		
				BV	rPLA	PI-III	BV	nPLA	rPLA
1	20	F	U, AE, D	10^{-6}	10^{-8}	10^{-1}	19.8	16.5	8.8
2	39	M	U, AE	10^{-6}	10^{-8}	10^{-1}	8.5	5.8	3.2
3	18	F	U, AE, D	10^{-6}	10^{-8}	10^{-2}	20.4	17.1	5.1
4	25	F	U, AE, diz	10^{-6}	10^{-6}	10^{-2}	2.0	2.3	0.35
5	32	M	E, AE, D	10^{-8}	10^{-8}	10^{-1}	17.8	12.0	4.4

U, Urticaria; *E,* erythema; *P,* pruritus; *AE,* angiodema; *D,* dyspnea; *diz,* dizziness.

the carbohydrate side chain at position Asn13.[13] These epitopes are recognized by both allergic and hyperimmune nonallergic individuals.[12] Recently, we have demonstrated that BV immunotherapy (BVIT) induces peripheral T-cell anergy to the three PLA epitopes, as well as to the entire PLA allergen.[14] This state of anergy was characterized by suppressed proliferative and cytokine responses.[14] Here we present the results of five patients allergic to BV who were subjected to PIT with three peptides that contain the major T-cell epitopes of PLA. successful PIT was specifically associated with development of an anergic state against the epitopes of PLA in peripheral T cells and a change in PLA-specific IgE: IgG4 ratio in favor of IgG4.

METHODS

Antigens and reagents

Whole BV for skin tests and BVIT (Pharmalgen) was obtained from ALK (Horsholm, Denmark), and natural (n)PLA was obtained from Boehringer (Mannheim, Germany). Recombinant (r)PLA was expressed and refolded as described previously.[15] The three PLA peptides were synthesized by automated solid-phase synthesis, with subsequent high-performance liquid chromatography. For skin testing and PIT, allergens and peptides were dissolved in commercially available diluent (Pharmalgen, ALK). IL-2 was a gift from Sandoz Ltd. (Basel, Switzerland). IL-4 was produced by Ciba-Geigy (Basel, Switzerland). Tetanus toxoid (TT) and tuberculin protein derivative (PPD) were obtained from the Swiss Serum and Vaccine Institute (Bern, Switzerland).

Patients

Five adult patients (patient numbers 1, 2, 3, 4, and 5) with moderately severe systemic allergic reactions to BV were subjected to PIT. Before treatment, all five patients had positive skin test responses to 0.1 μg/ml BV and specific serum IgE antibodies to BV of RAST class 2 to 4. As shown in Tables I and II, they also showed positive skin reactions to an end-point concentration (EPC) of 10^{-6} to 10^{-8} gm/L BV or PLA. All had serum IgE antibodies to PLA in the range of 2.3 to 17.1 Phadezym RAST units (PRU) and BV of 2.0 to 20.4 PRU. They reacted less to rPLA, indicating IgE reactivity also against the carbohydrate portion of PLA. These data were confirmed by Western blot analysis, in which all patients showed specific IgE bands to PLA (14 to 16 kd). However, none of the patients reacted exclusively against PLA, and patients 1, 4, and 5 showed bands also to hyaluronidase (43 kd); patients 3 and 4 showed bands to a 35 kd protein (data not shown). A control serum from an anti-BV antibody–negative allergic patient showed no detectable IgE binding to any BV component.

In addition to the experimental group, 10 patients received conventional BVIT. Relevant clinical data of both groups are compared in Table II. The patients receiving BVIT had moderately severe systemic allergic reactions to BV similar to those had by the patients receiving PIT, and they had positive skin test responses and specific IgE antibodies against BV and PLA in the same ranges. A placebo or untreated control group could not be included for ethical reasons. Oral and written informed consent was obtained from all patients receiving PIT.

Skin tests

Intracutaneous skin tests were performed as previously described.[8] As a measure for skin sensitivity, EPCs in grams per liters of allergen, resulting in a wheal of 5 mm or greater in diameter with erythema, was used. An EPC of 10^{-4} gm/L was considered as indicating allergic sensitization to BV. At higher concentrations, nonspecific toxic skin reactions may occur.

Protocols of specific immunotherapy (PIT and BVIT)

PIT was started with 0.1 μg of an equimolar mixture consisting of the three PLA peptides (PI, PII, and PIII). The peptides were administered subcutaneously on the outer side of the upper arm. The treatment was continued by injecting, in successive doses, 1 μg, 3 μg, 6 μg, 12 μg, 25 μg, 50 μg, and then three times 100 μg in weekly intervals, resulting in a cumulative dose of 397.1 μg of the peptide mixture. At day 70, 1 week after the last peptide injection, a first challenge with 10 μg of subcutaneous nPLA was administered in the upper arm. After another week (day 77), the patients were subjected to a honeybee sting challenge. Thereafter, at day 84, treatment was switched for ethical reasons to conventional BVIT with 100 μg of whole BV given every fourth week.[1, 16] Blood samples for estimation of specific serum antibodies and cellular analysis were taken before (day 0) and after PIT (day 63, before PLA challenge) and after the sting challenge on day 84.

The BVIT-treated group of patients received a cumulative dose of 111.1 μg of BV on the first day. Then maintenance injections of 100 μg of BV were given at days 7, 21, 50, and thereafter at monthly intervals.[16] The study protocol was approved by the ethical committee of the Medical Faculty of the University of Bern, Switzerland.

Sting challenge

The sting challenge was performed under intensive care conditions. An intravenous infusion was applied, and patients were continuously monitored for blood pressure and electro-

TABLE II. Comparison of diagnostic data from five patients allergic to BV before PIT and 10 patients allergic to BV before BVIT

Treatment	Age (yrs)*	Sex (M/F)	Skin test EPC (geometric mean value)		Specific IgE (PRU)*		
			BV	rPLA	BV	nPLA	rPLA
PIT	24.6 (18-39)	2/3	$10^{-6.4}$	$10^{-7.6}$	13.7 (2.0-20.4)	10.7 (2.3-17.1)	4.3 (0.3-8.8)
BVIT	39.7 (18-54)	7/3	$10^{-5.2}$	$10^{-7.8}$	11.5 (2.3-27.7)	10.6 (2.4-26.1)	4.4 (2.0-19.2)

*Mean value (range).

cardiography. For sting challenges, adult honeybees (> 14 days after hatching) were obtained from the Swiss Institute for Agricultural Research (Bern) and placed on the volar side of the patient's forearm. The sting was left in the skin for exactly 1 minute. Patients were closely monitored for allergic symptoms during the following 2 hours.

T-cell cultures

As previously described in detail,[10, 12, 14] peripheral blood mononuclear cells (PBMCs) from patients were isolated by Ficoll (Biochrom, Berlin, Germany) density gradient centrifugation of peripheral venous blood. Cells were washed three times and resuspended in RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (Sera-Lab, Sussex, England) and supplemented as described.[10, 14] Cells (10^6/ml) were stimulated in a 24-well plate with PLA or 1 μmol/L epitope peptides. As controls, TT was used at 0.01 U/ml, and PPD was used at 1 mg/ml. Cultures were supplemented with IL-2 (25 U/ml) and IL-4 (25 ng/ml) at day 7. Five days later, cells were washed three times with phosphate-buffered saline, and 10^6 cells were restimulated in 1 ml medium with 1 μmol/L of the same antigens in the presence of 10^6 autologous 3000 Rad irradiated PBMCs. Supernatants were harvested 16 hours later for determination of IL-2 and IL-4 and 48 hours later for IL-5, IL-13, and interferon (IFN)-γ. It was previously demonstrated that primary response cytokine profiles in antigen restimulation are not affected by this expansion procedure.[10, 14] For determination of specific proliferation, 2×10^5 PBMCs were cultured in 200 μl medium for 6 days and pulsed with 1 μCi of tritiated thymidine (DuPont/New England Nuclear, Boston, Mass.). Labeled nucleotide incorporation was measured after 20 hours in an LKB Beta-Plate Reader (Pharmacia-Wallac, Uppsala, Sweden).

Measurement of cytokines

The solid-phase ELISA for IFN-γ was previously described.[10, 14] Briefly, microtiter plates (Maxisorb, Nunc, Denmark) were coated with monoclonal antibody (mAb) 43-11 to human IFN-γ and developed with biotinylated mAb 45-15 and extravidine peroxidase (Sigma). The chromogenic substrate was o-phenylene-diamine-dihydrochloride (Sigma) in citrate buffer (pH 5). The reaction was stopped with 0.5 N H_2SO_4. The sensitivity of the ELISA was 20 pg/ml of IFN-γ.

IL-4 and IL-5 were measured by chemiluminescence immunoassay as described.[14, 17] The mAbs 3H4 and TRFK5 (Novartis, Basel) were used for coating white plates (Microlite; Dynatech, Chantilly, Va.). For detection, mAbs 8F12 (Ciba Geigy, Basel) and JES 1-5A10 (Pharmingen, St Louis, Mo.) were labeled with 2,6-dimethyl-4-(N-succinimidyl-oxyl-carbonyl)-phenyl-10-ethyl-acridinium-ester according to the method of Weeks and Woodhead[17] and used at a final concentration of 0.3 μg/ml. The plates were incubated for 2 hours at room temperature, and chemiluminescence was measured in an LB 96 luminometer (Berthold, Wildbad, Germany) immediately after addition of 0.5% H_2O_2 in 0.1 N HNO_3 and 0.25 N NaOH and two washings with phosphate-buffered saline. The detection limit was 5 pg/ml for both IL-4 and IL-5.

The IL-2 activity was measured by tritiated thymidine uptake in cytotoxic lymphoid line cells as described.[18]

For detection of IL-13, the mAb JES 10-2F9 (provided by DNAX, Palo Alto, Calif.) was used for coating, and recombinant IL-13 (PeproTech) was used as a standard. Rabbit anti-IL-13 (PeproTech) and alkaline phosphatase–labeled goat antibody (Zymed, San Francisco, Calif.) were used for detection. The chromogenic substrate was 4-nitrophenyl-phosphate-disodium-hexahydrate (Merck, Darmstadt, Germany) in diethanolamine buffer (pH 9.8). The detection limit was 300 pg/ml of IL-13.

Measurement of PLA-specific antibodies

Specific IgE antibodies to BV and PLA were determined by Phadezym RAST (Pharmacia ABS, Uppsala, Sweden) as previously described.[1] Titers of allergen-specific IgE are given in Phadezym RAST units. Values higher than 0.35 PRU were considered as significant. In addition, IgE and IgG4 anti-PLA antibody contents in serum were measured by ELISA as described before.[10, 12, 14, 19, 20] Briefly, PLA-coated ELISA plates (Maxisorb) were incubated with diluted serum and developed with biotinylated anti-IgE mAbs 6-7 (Novartis, Basel) and peroxidase-labeled extravidine (Sigma) or with anti-IgG4 mAbs RJ4 (Oxoid, Basingstoke, U.K.) and peroxidase-labeled anti-mouse immunoglobulin (Tago, Burlingame). PLA-specific human IgG4 mAb BVA2[21] was used as a standard. Serum from a patient allergic to BV reacting only to PLA in Western blot and calibrated to RAST (Pharmacia) was used as a PLA-specific IgE standard.[10] These assays detected 0.05 U/ml IgE anti-PLA and 0.1 ng/ml of IgG4 anti-PLA, respectively.

Binding of specific IgE antibodies to BV components in 1:25 or 1:5 diluted serum samples was analyzed by Western blot. Whole BV (50 μg/cm) was separated onto an 8% to 16% density gradient sodium dodecylsulfate–polyacrylamide gel at 200 V for 45 minutes. Blots were developed with anti-human IgE mAb TN-142 (Novartis, Basel) and peroxidase-labeled goat anti-mouse immunoglobulin. Allergen bands were detected by the enhanced chemiluminescence system (Amersham Inc.).

Statistics

The geometric mean values of specific IgE and IgG4 anti-PLA before and during PIT and BVIT were compared by paired t test.

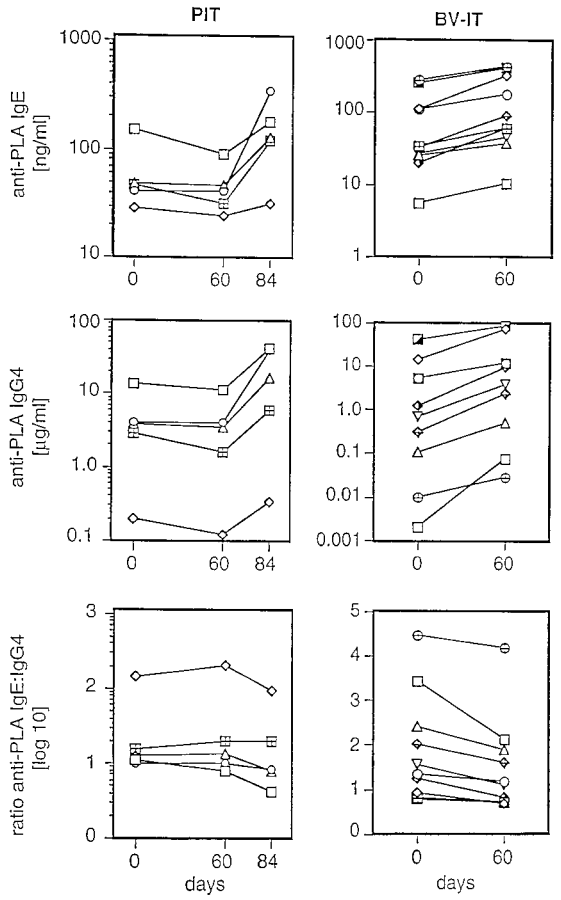

FIG. 1. IgE and IgG4 anti-PLA content in serum of patients allergic to BV before and after 2 months of PIT and antigen challenge *(left)* and during BVIT *(right)*. In BVIT specific isotypes increased within 60 days. In PIT, increase of specific antibodies was observed only after allergen challenge. In both PIT and BVIT ratio of PLA-specific IgE:IgG4 changed in favor of IgG4.

RESULTS

Clinical course

The five patients (numbers 1, 2, 3, 4, and 5) were subjected to PIT with an equimolar mixture of the three PLA peptides PLA PI, PII, and PIII, each expressing a single T-cell epitope. The results of PIT were compared with those of 10 patients receiving BVIT. The clinical course and the results of the provocation tests in patients receiving PIT are shown in Table III. During PIT, no local or systemic allergic symptoms or any other side effects were caused by the subcutaneously injected peptides in any of the patients (Table III). After 2 months of PIT, all patients tolerated a provocation test with 10 µg of subcutaneously injected PLA, corresponding approximately to the PLA content of a bee sting,[7] without systemic allergic symptoms. Patients 3 and 5 had an immediate local reaction at the injection site. After another week, patients were subjected to a live bee sting in the forearm. Three patients (1, 2, and 3) tolerated the sting challenge without any systemic symptoms. Patient 4 had some solitary wheals on the chest and mild swelling of the lips 15 minutes after the sting. Patient 5 had erythema of the face, mild angioedema of the eyelids, and a few solitary urticarial wheals on the chest and thighs 25 minutes after the sting. Both patients judged these symptoms as much less severe than those from the reaction before the treatment. After the sting challenge, all patients proceeded to a maintenance dose of 100 µg BV, as used in conventional BVIT, without any problems.

TABLE III. Results of provocation tests after 2 months of PIT

Patient no.	Reaction after 10 µg subcutaneous PLA	Reaction after live bee sting in forearm
1	None	Local wheal with erythema, no systemic symptoms
2	None	Local wheal with erythema, no systemic symptoms
3	Local wheal and erythema, delayed local swelling	Local wheal with erythema, no systemic symptoms
4	None	Local wheal with erythema; after 15 min, pruritus, solitary urticarial lesions on chest, mild angioedema of lip
5	Local wheal and erythema	Local wheal with erythema; after 25 min, erythema of face, single urticarial wheals on chest and thigh, mild angioedema of the eyelids

Skin test and specific IgE and IgG4 antibodies

Both BV and PLA skin tests in PIT and BVIT groups before treatment showed EPC values of 10^{-6} to 10^{-8} gm/L (Tables I and II), which did not change significantly during treatments (results not shown). As shown in Table I, the peptides did not induce any skin reaction up to an EPC of 10^{-1} to 10^{-2} gm/L in the five patients receiving PIT. Previously, similar results with the peptides were obtained in five patients allergic to BV who were not included in the study (not shown).

In Fig. 1 the serum levels of PLA-specific IgE and IgG4 antibodies determined by solid-phase ELISA are shown before and after 2 months of PIT and after the sting challenge in comparison with patients before and after BVIT. Both IgE and IgG4 PLA-specific antibodies slightly decreased during PIT or remained at their initial levels. However, after the sting challenge, a highly significant increase of PLA-specific IgG4 ($t = 6.15$, $p = 0.0035$) and IgE ($t = 3.39$, $p = 0.028$) was observed. Interestingly, the ratio of PLA-specific IgE to IgG4 changed in favor of IgG4 antibodies. The serum levels of total IgG4 and total IgE revealed no changes (results not shown). The patients receiving BVIT showed highly significant increases of serum levels of both PLA-specific

IgE (t = 8.16, p = 0.0001) and IgG4 (t = –5.86, p = 0.0002) after 2 months of treatment, and the ratio of specific IgE to IgG4 anti-PLA changed also in favor of IgG4.

Successful PIT induces specific suppression of proliferative response and cytokine production in peripheral T cells

Fig. 2 shows specific T-cell proliferation before and after PIT in response to PLA, the PLA-peptide mixture, and TT or PPD. In patients 1, 2, and 3 suppression of proliferative PBMC response to both the peptide mixture and PLA was induced. Patient 4 showed only slightly decreased proliferation to both PLA and the peptides after PIT. The specific proliferative response to the peptide mixture and PLA in PBMCs from patient 5 was already low before PIT and did not change significantly during treatment.

The cytokine response to PLA and the peptide mixture before and after PIT are shown in Fig. 3. After PIT, in parallel to the suppressed proliferative response, PBMCs of patients 1, 2, and 3 showed decreased production of all measured cytokines in response to both PLA and PLA peptides. In particular, the secretion of IL-4 and IL-5, but also that of IFN-γ, was decreased. IL-2 and IL-13, which were only measured in patients 3, 4, and 5, were also suppressed in the successfully treated patients. Only insignificant changes in cytokine responses were observed against TT or PPD. In patients 4 and 5 the decrease of cytokines after PLA or peptide stimulation was less pronounced (patient 4) or insignificant (patient 5).

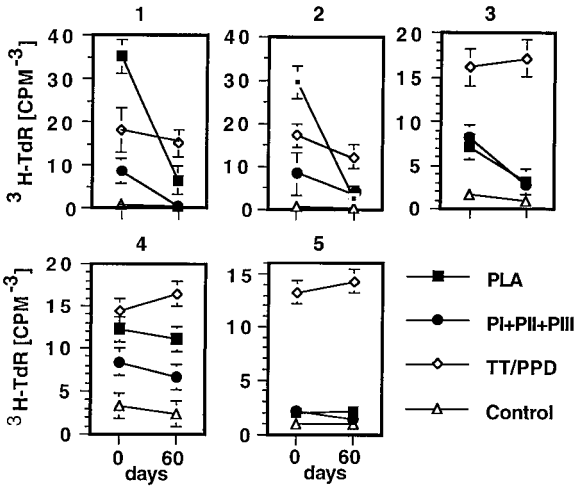

FIG. 2. Proliferative response of PBMCs to PLA, PLA-peptide mixture, and control antigens TT or PPD before and after PIT. Proliferative response to PLA and T-cell epitope peptides was decreased in patients 1, 2, and 3 after 2 months of therapy. *^{3}H-TdR,* Tritiated thymidine.

DISCUSSION

In this first study of PIT in BV allergy, we applied an equimolar mixture of three short peptides, each containing a T-cell epitope of the major BV allergen PLA. The total dose of peptides injected corresponded to a 40-times higher amount of PLA than normally applied in BVIT. This was possible because the peptides did not bind IgE antibodies, and the skin sensitivity to the peptides was at least 10^4 times lower than that to intact PLA. Successful PIT correlated with the induction of specific anergy in peripheral T cells against the same PLA epitopes to which anergy was induced by BVIT.[14] Induction of specific anergy by peptides in human T cells involves tyrosine phosphorylation and correlates with abrogated lck and ZAP-70 tyrosine kinase activities.[22] It requires high concentrations of peptides in the absence of costimulatory signals by antigen-presenting cells.[22-24] The proliferative response and T_{H1} cytokine synthesis were shown to be reactivated by IL-2 or IL-15, whereas the T_{H2} cytokine response could not be reestablished by IL-4.[14] This indicates that an SIT-induced shift in cytokine profile[25-27] involves different steps of T-cell activation and the participation of a particular microenvironment. IgE suppression was observed in earlier studies with pepsin-digested PLA fragments.[28]

Interestingly, immunodominant T-cell epitopes are also the most potent inducers of tolerance,[29] and both activation of T cells and induction of anergy can be achieved by the same epitopes. Although major histocompatibility complex (MHC) class II molecules of different individuals may recognize different peptides, promiscuous epitopes, which universally bind to human MHC class II, have been demonstrated.[30] Therefore it appears that the cross-reactivity among different MHC haplotypes is large enough that immunodominant epitopes can be recognized by most individuals. Indeed, recognition of the same PLA epitopes was found among patients allergic to BV and BV-sensitized nonallergic subjects,[12] showing also that allergenicity is not related to certain structural peculiarities of specialized allergenic epitopes that particularly stimulate T_{H2} cytokines and IgE antibody formation.[31, 32] Development of distinct cytokine patterns in T cells and establishment of MHC/peptide/T-cell receptor interactions may rather depend on the binding strength of antigenic peptides to the individual MHC class II molecules and antigen concentrations.[33, 34]

After bee sting challenge, three of the patients receiving PIT were completely protected, and two of them had mild cutaneous allergic symptoms. In contrast to the successfully treated patients, the specific proliferative responses in PBMCs before PIT and the decrease in PLA and peptide reactivity afterwards were less pronounced or even lacking in the other two patients. Therefore the major T-cell recognition sites for these patients may not have been contained in the peptides applied. In addition, these two patients showed substantial amounts of IgE antibodies to hyaluronidase and a 35 kd protein. Therefore in both patients the suboptimal efficacy of PIT could be attributed to a lack of PLA peptide–specific T-cell responses. On the other hand, this also demonstrates the specific action of PIT. How-

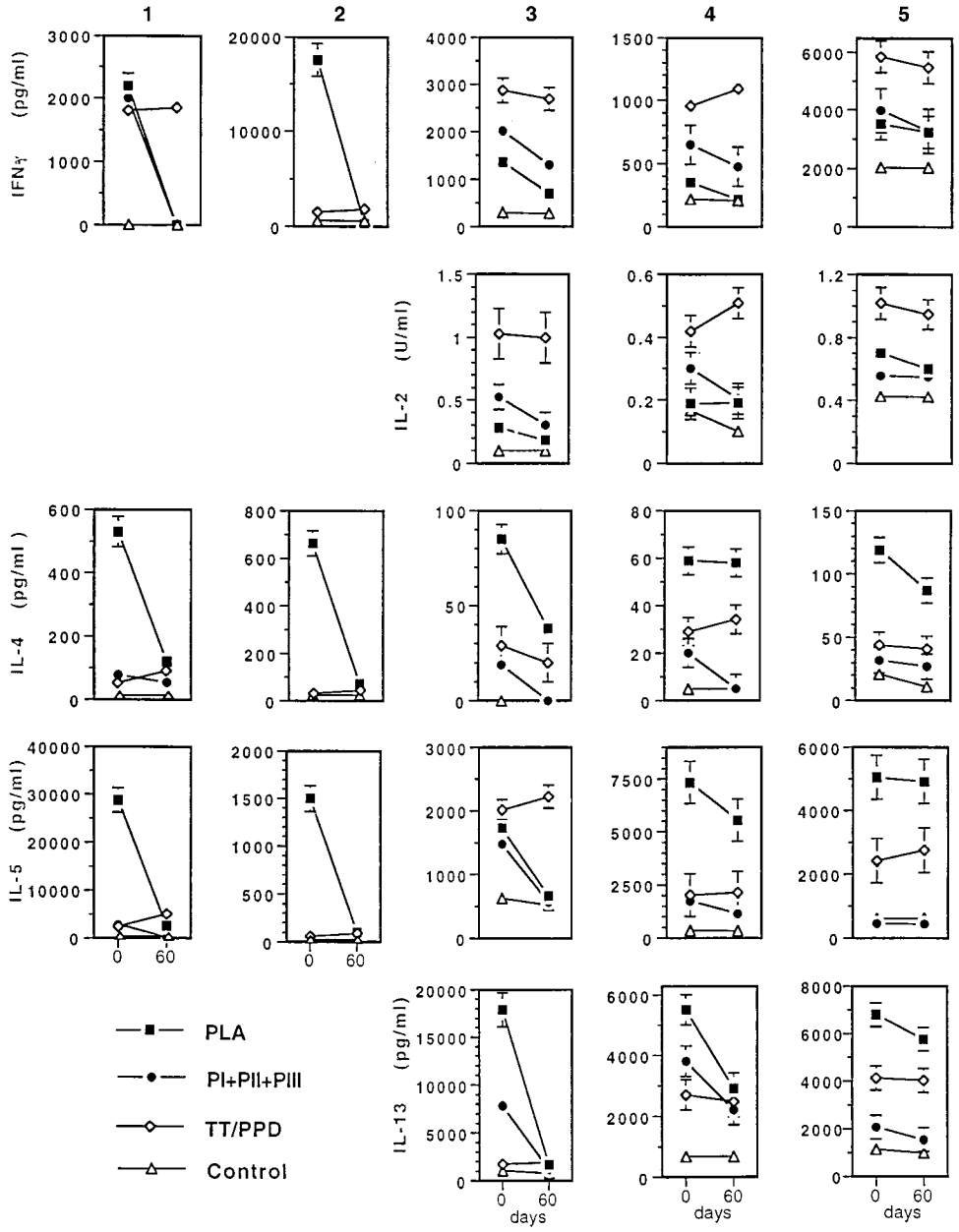

FIG. 3. Secretion of cytokines in PBMCs of patients stimulated with PLA, PLA-peptide mixture, and control antigens TT or PPD before and after 2 months of PIT. Cytokine production in response to PLA was decreased in successfully treated patients 1, 2, and 3.

ever, in this first study the amount of allergen applied and the schedule of application may not have been optimal. Nevertheless, all patients tolerated the full maintenance dose of BV after PIT without clinical problems, indicating that at least partial protection was induced.

Whereas specific T cells were anergized after both PIT and BVIT, B cells were still able to secrete antibodies, and a booster in PLA-specific IgG4 and IgE antibodies developed after allergen challenge. This indicates that the state of anergy included only the T-cell segment and was not manifest in B cells. Specific IgE and IgG4 antibody secretion by memory B cells is regulated differentially by IL-4 and IFN-γ,[10, 35, 36] respectively. It was shown that the ratio of specific IgE to IgG4 antibodies directly correlates with the ratio of IL-4 to IFN-γ in vitro.[10, 33-36] In PIT an increase of PLA-specific IgG4 relative to IgE antibodies has been observed only after allergen challenge. Because of the lack of conformational IgE binding sites in the peptides, stimulation of B cells and antibody synthesis was only possible after contact with the entire allergen and not by peptides.

After PLA contact, the ratio of specific isotypes in PIT changed in favor of IgG4, as observed also in BVIT.[14] As in this study, a definite decrease of both specific serum IgE antibodies and skin sensitivity to BV is usually not observed before 6 to 12 months of BVIT. Nevertheless, most patients are completely protected when restung during the first months of BVIT.[37-39] An explanation for this appears from the fact that release of mediators from blood basophils is strongly reduced already in the very early phase of BVIT.[40] The decrease in basophil releasability may be associated with anergy induction in T cells because effector cells in allergy require priming by T-cell cytokines for full activity and mediator release.[41, 42] Induction of allergen-specific IgG4, interfering with IgE binding to allergens,[9, 21] could further contribute to the protective state of patients after BVIT.

Because most allergens are composed of a variety of allergenic compounds, specific anergy to only one component may only suboptimally protect patients. However, it appears that suppression of the response to a dominant allergenic component may sufficiently reduce the reactivity against the entire allergen. Nevertheless, the application of peptide mixtures with additional epitopes of other allergens could increase the efficacy of the treatment. Accordingly, PIT offers an interesting possibility of allergen-specific treatment that is not hampered by IgE-mediated allergic reactions.

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