

Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN- γ production of T cells

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Background: Recent studies provide evidence that venom immunotherapy (VIT) alters the pattern of cytokine production by inducing an allergen-specific T-cell shift in cytokine expression from T_{H2} (IL-4, IL-5) to T_{H1} (IFN- γ) cytokines and also inducing the production of IL-10.

Objective: This study was carried out to analyze whether these changes in cytokine production of T cells already observed 1 week after the initiation of VIT in subjects with wasp venom allergy also influence the reactivity of effector cells, such as mast cells and basophils.

Methods: All subjects included in this study had a history of severe systemic allergic reactions to wasp stings and positive skin test responses with venom and venom-specific IgE in the sera. Peripheral blood leukocytes were isolated before and after the initiation of VIT (rush therapy reaching a maintenance dose of 100 μ g venom injected subcutaneously within 1 week) and preincubated with or without addition of IL-10, IFN- γ , IL-10 + IFN- γ , anti-IL-10, or anti-IFN- γ . After stimulation with wasp venom, histamine and sulfidoleukotriene release were assessed by ELISA and compared with spontaneous release and total histamine content.

Results: After the induction of VIT, venom-induced absolute and relative histamine and sulfidoleukotriene release were reduced. This was at least partially due to the induction of IFN- γ and IL-10 production, because (1) neutralization of IL-10 and IFN- γ by mAbs partially restored the release after the initiation of VIT and (2) the addition of exogenous IFN- γ and IL-10 caused a statistically significant diminution of the venom-induced histamine and sulfidoleukotriene release before VIT. Depletion of CD2⁺ T cells also restored the releasability after VIT.

Conclusion: These data indicate that T cells (producing IL-10 and IFN- γ after VIT) play a key role for the inhibition of his-

amine and sulfidoleukotriene release of effector cells. (*J Allergy Clin Immunol* 1999;103:326-32.)

Key words: Immunotherapy, wasp venom, histamine, leukotrienes, cytokines, sulfidoleukotrienes (LTC₄, LTD₄, LTE₄)

Allergies of the immediate type (type I, according to Coombs and Gell) are mediated by IgE antibodies specific for certain allergens. IgE is attached to basophils or mast cells by high-affinity Fc receptors. Crosslinking of cell-bound IgE by antigen is followed by mast cell or basophil degranulation with release of inflammatory mediators. These mediators include preformed mediators, such as histamine, which causes increased vascular permeability, vasodilatation, bronchial spasm, and mucous secretion. They also include newly generated mediators, such as leukotrienes, prostaglandin D₂, platelet-activating factor, and various cytokines.^{1,2}

IgE production is induced and enhanced by the T_{H2}-type cytokine IL-4 (and also by IL-13) and inhibited by the T_{H1} product IFN- γ , which favors the switch to IgG and inhibits simultaneously the switch to IgE.³⁻⁷ Stimulation of PBMCs from atopic and normal donors with IL-4 results in the production of both IgE and IgG, especially IgG₄.^{8,9} If there is a high IL-4 to IFN- γ ratio, as has been demonstrated for atopic persons,^{10,11} the production of IgE is stimulated, although IgG₄ production declines.¹²

Patients with severe allergy of the immediate type to bee or wasp venom are treated by venom immunotherapy (VIT; ie, subcutaneous injection of increasing doses of the respective venom).¹³⁻¹⁵ The clinical efficacy of VIT has been proved by several trials, but the mechanisms of protection against serious IgE-mediated systemic allergic reactions of the immediate type after stings of the respective insect are achieved are less well understood. In earlier studies the induction of "blocking" IgG antibodies, especially IgG₄, was held responsible for this protective effect.^{16,17} Indeed, a decline of allergen-specific IgE and an increase of allergen-specific IgG production can be observed after immunotherapy in the long run.^{18,19} More recently, we and others were able to demonstrate that VIT induces a shift in the dominance of

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Abbreviation used

VIT: Venom immunotherapy

allergen-specific T-helper cells from T_{H2} to T_{H1} and induces the production of the immunosuppressive cytokine IL-10.²⁰⁻²³ These changes in cytokine expression may be the reason for subsequent changes in the production of allergen-specific immunoglobulins.^{12,19} They may therefore explain the efficacy of VIT in the long run, leading to a termination of venom-specific IgE production, but they cannot explain the early protective effect of VIT, which can be observed after completion of the initial induction phase of rush therapy (after 1 week) when allergen-specific IgE is still present.

The purpose of this study was to analyze whether the observed changes in cytokine expression by T cells, which already occur after 1 week of VIT, also influence the histamine and sulfidoleukotriene release by effector cells such as basophils.

METHODS

Subjects

Forty-one patients who are allergic to wasp venom were selected on the basis of a history of severe systemic allergic reactions of grade II to IV, according to the classification of Mueller,²⁴ after a wasp sting (ie, generalized urticaria, angioedema, hypotension, or shock). Furthermore, all patients showed a positive skin test response to wasp venom (0.1 µg/mL venom injected intracutaneously) and had venom-specific IgE in the sera (RAST class ≥2). To evaluate the total number of basophils in the blood, cell counts were performed in all patients before and after induction of VIT. To ensure that the changes observed after induction of VIT were indeed induced by the treatment, patients who were not able to undergo VIT within 1 month were used as the untreated control group.

VIT

VIT was performed as rush therapy according to the manufacturers' dose schedule (ALK-lyophilized SQ; ALK-Scherax, Hamburg, Germany). The therapy began with a dose of 0.01 µg wasp venom and reached the maintenance dose of 100 µg venom injected subcutaneously within 1 week.

After the patients' consent was obtained, 15 mL EDTA or 10 to 40 mL of heparinized blood were obtained at the day of hospital admission (before VIT) and 1 week later at the day of discharge (after the initiation of VIT) when the maintenance dose of 100 µg was reached.

Preparation and stimulation of cells

For determination of spontaneous, venom-induced and IgE receptor-induced histamine release of basophils, heparinized blood was diluted 1:7 with histamine-release buffer. Then 100 µL of diluted blood was placed in 96-well round-bottom tissue-culture plates (Greiner, Frickenhausen, Germany) and stimulated with 5 µg/mL wasp venom (ALK-lyophilized SQ; ALK-Scherax), 0.55 µg/mL anti-IgE receptor antibody (DPC Biemann GmbH, Bad Nauheim, Germany) or incubated in buffer alone (spontaneous histamine release) at a final volume of 200 µL. After 30 minutes of incubation, supernatants were harvested and stored at -20°C until analysis. Total histamine content was determined after cell lysis with distilled water.

For determination of sulfidoleukotriene release, 250 µL dextran were added to 1 mL EDTA-blood and incubated for 90 minutes at room temperature to allow sedimentation of erythrocytes. Then, PBLs were centrifuged at 130g for 15 minutes and resuspended in 1 mL stimulation buffer (including 20 ng/mL IL-3). Afterwards, 200 µL were incubated in 96-well round-bottom tissue-culture plates (Greiner) at a final volume of 250 µL and stimulated as described earlier.

For some experiments, the following cytokines or mAbs were added to PBL 30 minutes before the stimulation with wasp venom: 1 to 100 U/mL rhIL-10 (PharMingen, San Diego, Calif), 1 to 100 ng/mL rhIFN-γ (PharMingen), 100 U/mL rhIL-10 + 100 ng/mL rhIFN-γ, 30 ng/mL blocking rat anti-human IL-10 mAb (JES3-19F1; PharMingen), 1 µg/mL blocking mouse anti-human IFN-γ mAb (Camon, Wiesbaden, Germany), or the same amount of isotype control antibodies (PharMingen).

T cell-depletion experiments

PBMCs were isolated from heparinized blood by Ficoll-Paque (1.077 g/cc; Biochrom, Berlin, Germany) density centrifugation and washed 3 times with PBS. Then CD2⁺ T cells were depleted from PBMCs with the anti-CD2 mAb OKT11 (mouse IgG₁; American Type Culture Collection [ATCC], Rockville, Md) and sheep anti-mouse IgG magnetic beads M-450 (Dyna, Hamburg, Germany). Depletion was controlled by flow cytometry. To evaluate unspecific influences of the depletion procedure on basophils, PBMCs were also incubated with the same amount of an isotype control mAb (PharMingen) and magnetic beads and used as a nondepleted preparation. Thereafter 2.5 × 10⁵ cells of T cell-depleted and nondepleted preparations were stimulated with venom or incubated in medium alone as described.

Determination of histamine and sulfidoleukotriene release of effector cells

Histamine and sulfidoleukotriene release were measured by histamine immunoassay (Coulter-Immunotech Diagnostics, Hamburg, Germany) or CAST (Cellular Antigen Stimulation Test; DPC Biemann GmbH), respectively, according to the manufacturers' descriptions. ELISA plates were analyzed at 405 nm in a microplate ELISA reader (Model 450; Bio Rad, München, Germany) with the use of the microplate manager software 2.0 (Bio Rad). The histamine and sulfidoleukotriene concentrations were calculated according to the standard curves. The detection limits were 0.2 nmol/L for histamine and 45 pg/mL for sulfidoleukotriene. The mean index of stimulation was defined as venom-induced (or anti-IgE receptor-induced) histamine or sulfidoleukotriene release/spontaneous release of the cells of an individual donor or venom-induced (or anti-IgE receptor-induced) histamine release/total histamine content, respectively.

Statistics

Student's *t* test was used to test the statistical significance of results; *P* values less than or equal to .05 were considered significant.

RESULTS

VIT decreases venom-induced and IgE-induced histamine and sulfidoleukotriene release

First, venom-induced histamine release, spontaneous histamine release, and total histamine content of the effector cells before and after the initiation of VIT were analyzed. Before VIT, all patients responded strongly to

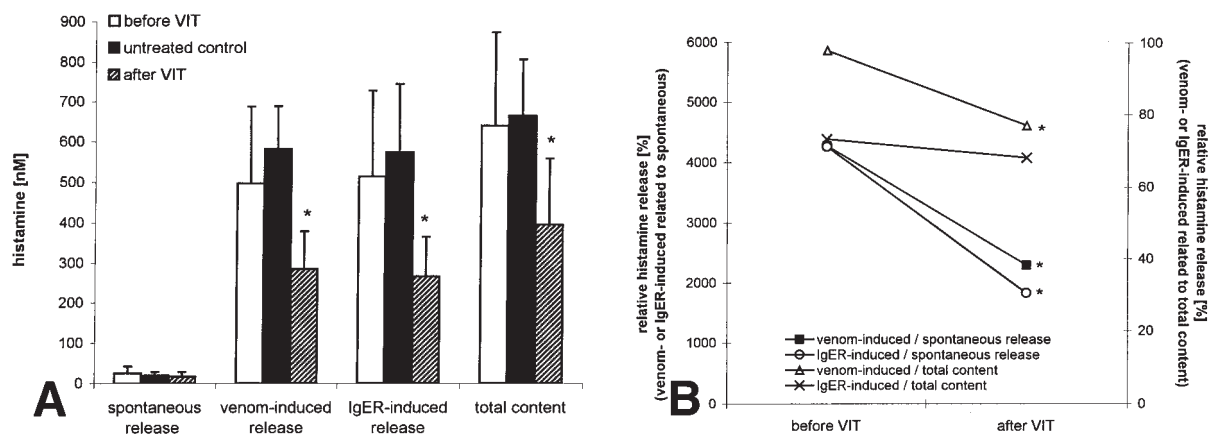


FIG 1. Spontaneous, venom-induced, and IgER-induced release and total histamine content of PBLs before and after initiation of VIT (1 week). Heparinized blood was diluted 1:7 with histamine-release buffer. Then cells were stimulated with 5 μ g/mL wasp venom, 0.55 μ g/mL anti-IgE receptor antibodies, or incubated in buffer alone as a negative control corresponding to the spontaneous histamine release. After 30 minutes, supernatants were harvested, and histamine release was measured as described in the Methods section. Total histamine content was determined after cell lysis with distilled water. The means $\pm$ SD from 20 patients are shown before and after induction of VIT and from 12 untreated patients as (A) nanomolars or as (B) mean index of stimulation related to spontaneous or total histamine release. The asterisk (*) indicates a result after initiation of VIT that differs significantly from the result before VIT ($P \leq .05$).

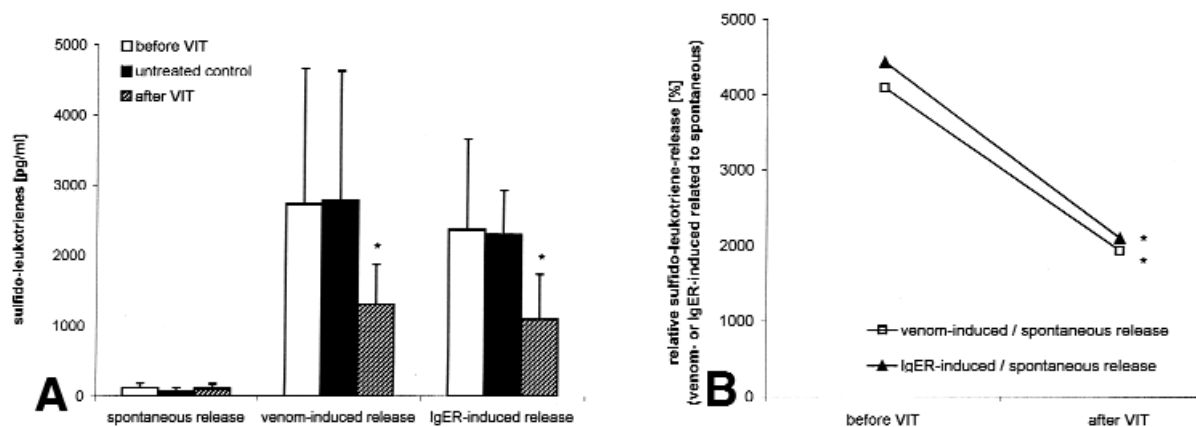


FIG 2. Spontaneous, venom-induced, and IgER-induced sulfidoleukotriene release before and after the initiation of VIT. After isolation of PBLs from EDTA blood by dextran sedimentation and resuspension in incubation buffer, cells were stimulated for 30 minutes with 5 μ g/mL wasp venom, 0.55 μ g/mL anti-IgE receptor antibodies, or incubated in buffer alone as negative control. Then supernatants were harvested, and sulfidoleukotriene release was measured as described in the Methods section. The data represent the means $\pm$ SD from 18 patients before and after the induction of VIT and from 12 untreated patients as (A) picograms per milliliters or as (B) mean index of stimulation related to spontaneous sulfidoleukotriene release. The asterisk (*) indicates a result after the initiation of VIT that differs significantly from the result before VIT ($P \leq .05$).

stimulation with wasp venom because the venom-induced histamine release was up to 20-fold higher than the spontaneous histamine release (496.88 ± 190.08 nmol/L [mean $\pm$ SD] versus 24.70 ± 17.18 nmol/L; $P \leq .001$; Fig 1, A). After the initiation of VIT, the venom-induced histamine release was significantly reduced by about 43% (284.14 ± 94.16 nmol/L; $P \leq .001$), although the spontaneous histamine release was not altered significantly (17.15 ± 11.06 nmol/L). Similar results were obtained after the stimulation of the effector cells with

anti-IgE receptor antibodies. After the induction of VIT, anti-IgE receptor-induced histamine release was significantly decreased from 514.75 ± 212.04 nmol/L to 266.34 ± 98.33 nmol/L ($P = .002$; Fig 1, A). However, VIT also significantly diminished the total number of basophils detectable in the blood, but only up to 32% ($0.65\% \pm 0.17\%$ before and $0.44\% \pm 0.19\%$ after VIT; $P = .0001$ [data not shown]); total histamine content was consequently reduced up to 39% from 639.41 ± 234.19 nmol/L to 396.04 ± 163.24 nmol/L ($P \leq .005$; Fig 1, A). Never-

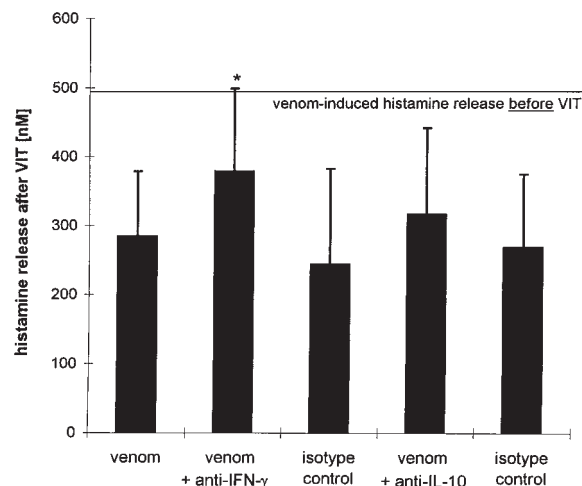


FIG 3. Influence of blocking anti-IFN- γ or blocking anti-IL-10 antibodies on venom-induced histamine release after the initiation of VIT. Heparinized blood was diluted 1:7 with histamine-release buffer. Then cells were cultured for 30 minutes with or without addition of 1 μ g/mL anti-IFN- γ mAb, 30 ng/mL anti-IL-10 mAb, or the respective isotype control antibodies. Afterwards, blood cells were stimulated with 5 μ g/mL wasp venom or incubated in buffer alone as negative control corresponding to the spontaneous histamine release. After 30 minutes, supernatants were harvested and histamine release was measured as described in the Methods section. The means $\pm$ SD from 18 patients after the induction of VIT are shown. The asterisk (*) indicates a result after the addition of an antibody that differs significantly from the result without addition of this antibody ($P \leq .05$).

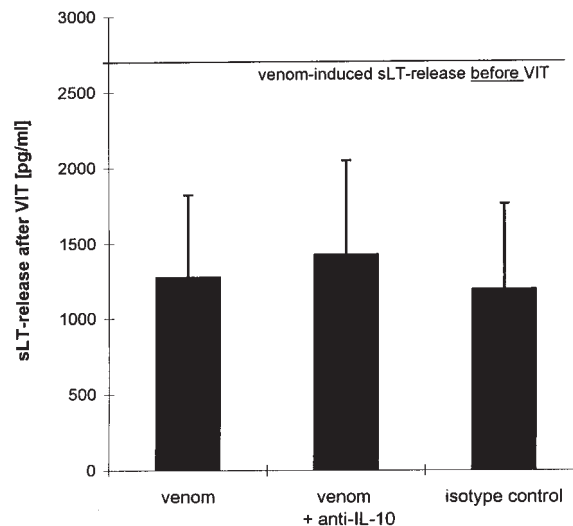


FIG 4. Influence of blocking anti-IL-10 antibodies on venom-induced sulfidoleukotriene release after the initiation of VIT. After isolation of PBLs from EDTA blood by dextran sedimentation and resuspension in incubation buffer, cells were cultured for 30 minutes with or without addition of 30 ng/mL anti-IL-10 mAb or the respective isotype control antibodies before stimulation with 5 μ g/mL wasp venom. Then supernatants were harvested, and sulfidoleukotriene-release was measured as described in the Methods section. The data represent the means $\pm$ SD from 18 patients after the induction of VIT. The asterisk (*) indicates a result after the addition of an antibody that differs significantly from the result without addition of this antibody ($P \leq .05$).

theless, the venom-induced histamine release related to the decreased total histamine content was still significantly decreased from 0.977 ± 0.35 mean index of stimulation to 0.768 ± 0.31 mean index of stimulation ($P \leq .05$, although the anti-IgE receptor-induced histamine release related to the decreased total histamine content did not reach statistical significance (Fig 1, B). The venom-induced and anti-IgE receptor-induced histamine release were also significantly reduced after VIT, compared with before VIT when related to the spontaneous histamine release (42.76 ± 32.4 mean index of stimulation to 22.93 ± 13.37 mean index of stimulation for venom induction ($P \leq .02$) and 42.59 ± 30.12 mean index of stimulation to 18.30 ± 8.58 mean index of stimulation for anti-IgE receptor induction ($P \leq .01$; Fig 1, B).

Parallel to the histamine release, we also measured the sulfidoleukotriene-release of PBLs before and after the initiation of VIT. Before VIT, addition of wasp venom increased the sulfidoleukotriene release from 111.24 ± 65.83 pg/mL to 2730.25 ± 1931.18 pg/mL ($P \leq .001$; Fig 2, A). After the initiation of VIT, venom-induced sulfidoleukotriene release was significantly decreased by more than 50% from 2730.25 ± 1931.18 pg/mL to 1310.98 ± 560.25 pg/mL ($P \leq .01$), although spontaneous sulfidoleukotriene release was not affected (Fig 2, A). The anti-IgE receptor-induced sulfidoleukotriene release was also significantly reduced from 2365.02 ± 1296.80

pg/mL to 1102.51 ± 638.69 pg/mL ($P = .004$; Fig 2, A). Again, the mean $\pm$ SD of the mean index of stimulation was significantly decreased from 40.92 ± 21.74 to 19.37 ± 14.59 for venom-induced and 44.32 ± 29.63 to 21.03 ± 8.24 for anti-IgE receptor-induced ($P \leq .001$; Fig 2, B).

In all cases the untreated control group showed no significant changes in histamine and sulfidoleukotriene release comparing the time point of diagnosis of wasp allergy and start of VIT (Fig 1, A, and Fig 2, A).

Addition of blocking anti-IL-10 or blocking anti-IFN- γ mAbs partially prevents the down-regulation of histamine and sulfidoleukotriene release after the initiation of VIT

Previously, we have shown that VIT induces a T_{H2} to T_{H1} shift and IL-10 production in subjects with allergic reactions to bee or wasp venom after completion of the initial induction phase within 1 week.²³ The levels of IFN- γ and IL-10 production of PBMCs from the patients included in this study were also determined and showed an increase after VIT: IFN- γ , 224.6 ± 153.9 pg/mL before VIT versus 531.1 ± 479.9 pg/mL after the initiation of VIT; and IL-10, 218.7 ± 184.4 pg/mL before VIT versus 445.5 ± 338.4 pg/mL after the initiation of VIT. To analyze whether this enhanced IFN- γ or IL-10 production after the initiation of VIT is responsible for the

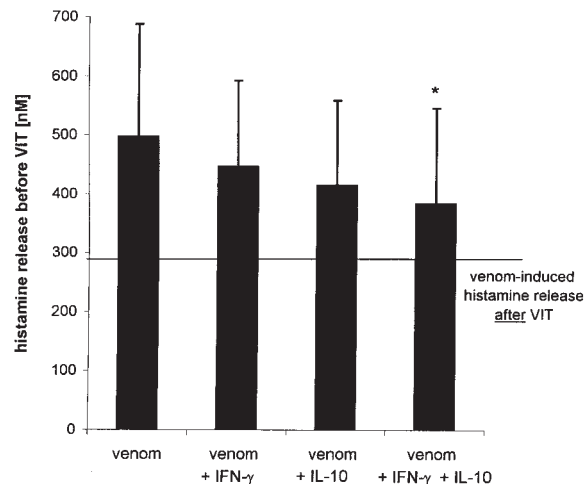


FIG 5. Influence of IL-10 and IFN- γ on the histamine release before VIT. Heparinized blood was diluted 1:7 with histamine-release buffer. Then cells were cultured for 30 minutes with or without addition of 100 ng/mL IFN- γ , 100 U/mL IL-10, or both cytokines together. Afterwards, blood cells were stimulated with 5 μ g/mL wasp venom or incubated in buffer alone as negative control corresponding to the spontaneous histamine release. After 30 minutes, supernatants were harvested, and histamine release was measured as described in the Methods section. The means $\pm$ SD from 19 patients before induction of VIT are shown. The asterisk (*) indicates a result after the addition of a cytokine that differs significantly from the result without addition of this cytokine ($P \leq .05$).

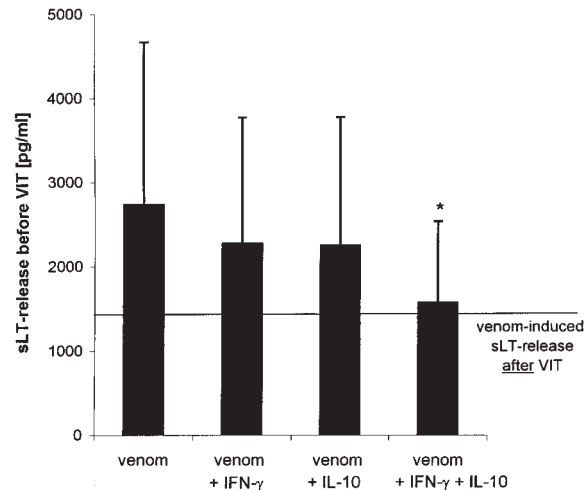


FIG 6. Influence of the cytokines IL-10 and IFN- γ on sulfidoleukotriene release before VIT. After isolation of PBLs from EDTA-blood by dextran sedimentation and resuspension in incubation buffer, cells were cultured for 30 minutes with or without addition of 100 ng/mL IFN- γ , 100 U/mL IL-10, or both cytokines together before stimulation with 5 μ g/mL wasp venom. Then, supernatants were harvested and sulfidoleukotriene release was measured as described in the Methods section. The data represent the means $\pm$ SD from 19 patients before the induction of VIT. The asterisk (*) indicates a result after the addition of a cytokine that differs significantly from the result without addition of this cytokine ($P \leq .05$).

decreased histamine and sulfidoleukotriene release described earlier and whether the addition of blocking antibodies can restore the releasability of effector cells, PBL from patients after the initiation of VIT were cultured with neutralizing anti-IL-10 mAb or anti-IFN- γ mAb 30 minutes before the stimulation with wasp venom. Preincubation of PBL with anti-IL-10 mAb resulted in a reproducible dose-dependent, but not significant, increase of the histamine release after the initiation of VIT from 284.14 ± 94.16 nmol/L to 315.94 ± 124.62 nmol/L. Addition of anti-IFN- γ mAb led to a significant increase of the histamine release after the initiation of VIT. The release rose from 284.14 ± 94.16 nmol/L to 378.24 ± 120.04 nmol/L ($P \leq .02$). The addition of isotype control antibodies had no effect on the releasability (Fig 3).

The sulfidoleukotriene release was also reproducibly (but not significantly) enhanced from 1273.17 ± 544.78 pg/mL to 1420.72 ± 623.22 pg/mL after the addition of anti-IL-10 mAbs 30 minutes before the stimulation with wasp venom. Again, the isotype control antibody did not lead to any change in sulfidoleukotriene releasability (Fig 4). Unfortunately, it was not possible to measure the sulfidoleukotriene release after the addition of neutralizing mAbs against IFN- γ , because all antibodies against human IFN- γ were mouse IgG₁ antibodies, which react with the capture antibody (rabbit anti-mouse IgG) used for the detection of sulfidoleukotriene in our assay (data not shown).

Preincubation of effector cells before VIT with IL-10 and IFN- γ decreases histamine and sulfidoleukotriene release

Because we have demonstrated that neutralization of IL-10 or IFN- γ can partially restore the releasability of histamine and sulfidoleukotriene after the initiation of VIT, we now examined whether, conversely, the addition of IL-10 and IFN- γ to PBLs before VIT leads to a decrease of the releasability. Therefore PBLs were preincubated with IL-10, IFN- γ , or IL-10 and IFN- γ 30 minutes before the stimulation with wasp venom. The addition of exogenous IL-10 (1 to 100 U/mL) or IFN- γ (1 to 100 ng/mL) alone resulted in a reproducible and dose-dependent (but not significant) decrease of the venom-induced histamine release from 496.88 ± 190.08 nmol/L to 413.43 ± 144.04 nmol/L for IL-10 and from 496.88 ± 190.08 nmol/L to 445.03 ± 146.32 nmol/L for IFN- γ . But if 100 U/mL IL-10 and 100 ng/mL IFN- γ were added together 30 minutes before the stimulation with venom, the histamine release was reduced significantly from 496.88 ± 190.08 nmol/L to 382.16 ± 162.24 nmol/L ($P \leq .05$; Fig 5).

Determination of sulfidoleukotriene release revealed similar results. Venom-induced sulfidoleukotriene release was significantly reduced from 2730.25 ± 1931.18 pg/mL to 1552.36 ± 969.08 pg/mL after the addition of IL-10 together with IFN- γ ($P \leq .02$; Fig 6); IL-10 alone resulted in a decrease from 2730.25 ± 1931.18 pg/mL to 2242.92

± 1523.62 pg/mL and the addition of IFN- γ alone in a decrease from 2730.25 ± 1931.18 pg/mL to 2266.37 ± 1502.02 pg/mL (not significant).

Depletion of T cells increases the histamine release after the initiation of VIT

Our experiments indicate that the decreased histamine and sulfidoleukotriene release after the initiation of VIT is partially due to IL-10 and IFN- γ production. Because T lymphocytes are the main producers of IL-10 and IFN- γ , we determined whether and how depletion of T lymphocytes influences the releasability of effector cells after VIT. Therefore venom-induced histamine release of T cell-depleted cells was compared with the release of control cells treated with the same amount of magnetic beads and isotype control antibodies to exclude influences of this preparation method on the releasability. After VIT, depletion of CD2⁺ T cells showed no significant effect on the spontaneous histamine release (20.01 ± 12.37 nmol/L before versus 22.16 ± 15.81 nmol/L after T-cell depletion), although venom-induced histamine release was significantly increased from 326.97 ± 64.24 nmol/L before to 471.36 ± 132.86 nmol/L after T-cell depletion ($P \leq .02$; Fig 7). This effect was not observed after the depletion of T cells before VIT.

DISCUSSION

These data clearly indicate that, in patients who are allergic to wasp venom, (1) total histamine content, (2) spontaneous, (3) IgE receptor-induced, and (4) wasp venom-induced histamine and sulfidoleukotriene release are reduced after the initiation of VIT. This effect is partially due to the induction of IL-10 and IFN- γ production of T cells described by us earlier²³; before VIT, the addition of exogenous IL-10 together with IFN- γ leads to a significant decrease of the venom-induced histamine and sulfidoleukotriene release, whereas, after the initiation of VIT, the neutralization of IL-10 and IFN- γ by mAbs or the depletion of CD2⁺ T cells restored the release of both histamine and sulfidoleukotriene partially. The observed effects are not due to spontaneous changes in the immune status of the patients because the described immunologic parameters did not change significantly in patients with allergic reactions who were not able to undergo VIT within a short period of time.

The decrease of total, spontaneous, IgE receptor-induced, and wasp venom-induced histamine and sulfidoleukotriene release after the initiation of VIT after 1 week may explain the rapid protection of the patients against wasp stings at this early time point. A decrease in numbers of basophils during the induction of VIT was also observed and may contribute to the protection of the patients, indicating that not only allergen-specific effects are responsible for the rapid efficacy of VIT. The decreased number of basophils after VIT is not the only reason for the reduced releasability because the venom-induced release of histamine and sulfidoleukotriene decreased more than the basophil counts, and the effects of T cell-derived cytokines IL-10 and IFN- γ could be demon-

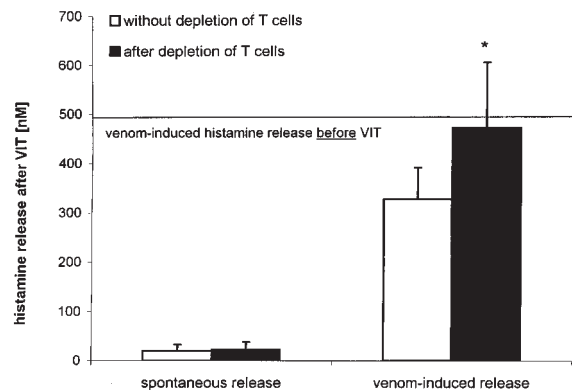


FIG 7. The influence of depletion of CD2⁺ T cells on the histamine release after the initiation of VIT. CD2⁺ T cells were depleted from PBMC before the stimulation with 5 μ g/mL wasp venom or incubation in medium alone. Then supernatants were harvested, and histamine release was measured as described in the Methods section. The data represent the means $\pm$ SD from 8 patients after the initiation of VIT. The asterisk (*) indicates a result after the depletion of T cells that differs significantly from the result before depletion ($P \leq .02$).

strated with the same preparations of PBLs containing the same amount of basophils.

Our results are in line with other studies, which also demonstrated a decrease of total histamine content after a few hours of treatment with wasp venom.^{25,26} In contrast to our study, only the absolute amount of histamine release was decreased but not the histamine release in response to venom, if expressed as percent release of total histamine. The discrepancy may be due to different preparation techniques of effector cells. In the study of Jutel et al,²⁵ leukocytes were obtained after dextran sedimentation of erythrocytes from EDTA blood, although in our study diluted heparinized blood was used for determination of histamine content.

The investigation of whether T cell-derived cytokines can influence the histamine and sulfidoleukotriene release of effector cells revealed that the addition of IL-10 and IFN- γ to PBLs before VIT before the challenge with wasp venom led to a dose-dependent diminution of venom-induced histamine and venom-induced sulfidoleukotriene release. Both cytokines added together had a synergistic effect. Because we and others have shown that VIT induces an increase of IFN- γ and IL-10–protein secretion in vitro,^{20,21,23} it is likely that this enhancement of IFN- γ and IL-10 production is, at least in part, responsible for the decline in venom-induced histamine and sulfidoleukotriene release. This presumption could be further supported by the fact that the addition of blocking anti-IL-10 and anti-IFN- γ antibodies after the initiation of VIT partially restored the histamine and sulfidoleukotriene release in a dose-dependent way, although isotype control antibodies had no effect on mediator release. The effect of anti-IFN- γ mAbs on the sulfidoleukotriene release could not be measured, because this mouse antibody reacted with the capture antibody

(rabbit anti-mouse IgG) used for the detection of sulfidoleukotriene in the CAST. But the finding that the histamine release could also be restored after depletion of in vivo-activated T cells from PBMCs after VIT provides further evidence for the great influence of T cell-derived cytokines (eg, IL-10, IFN- γ , or others) on the releasability of effector cells.

Our findings concerning the influence of cytokines on the releasability of basophils were not unexpected because it is well known that cytokines can modulate the effector phase of IgE-mediated allergic reactions. For example, the mast cell growth factor IL-3 enhances the release of histamine and leukotriene C₄.^{27,28} Furthermore, Radermecker et al²⁹ showed a dose-dependent enhancement of antigen-induced basophilic histamine release after the preincubation of leukocytes with IL-3, GM-CSF, or IL-5, but not with IL-8 and stem cell factor. Additionally, the immunomodulatory effects of histamine itself were described by Lagier et al,³⁰ who have shown that histamine inhibited IFN- γ production by T_{H1}-like cells but had no effect on T_{H2}-like cells; whereas, regarding T_{H0} clones, histamine inhibited IL-4 and only slightly inhibited IFN- γ production. Data on the effect of IFN- γ and IL-10 on the histamine and sulfidoleukotriene release have not been reported so far.

Taken together, the data presented in this study may explain how changes in cytokine production induced by VIT may also affect effector cells like basophils and may provide an additional explanation for the early protective effect of VIT before allergen-specific IgE production is inhibited. The data also provide additional evidence for the regulation of effector cells like basophils by T cells.

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