

Relevance of basophil histamine release changes during venom immunotherapy

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We investigated the effect of rush and long-term venom immunotherapy on histamine release parameters in bee venom allergic patients. Ten patients received rush venom immunotherapy, and histamine release data were obtained immediately before and after treatment. 17 patients were assessed by histamine release 24 to 63 months after termination of long-term venom immunotherapy. A control group of 10 non-allergic subjects was included in this study. Histamine released from whole blood was determined in a sensitive radio-enzymatic assay using a single isotope technique. Bee venom phospholipase A-induced histamine release from whole blood proved to be a test procedure of high specificity and sensitivity. Eight of 10 untreated patients and no control subject showed significant antigen-induced histamine release. Results obtained from patients immediately after successful rush venom immunotherapy showed an important decrease (mean 45.9%) of total histamine content of basophil leukocytes in all patients. Antigen-induced maximum histamine release was found to be increased in one, decreased in two and unchanged in seven patients. In patients who received long-term immunotherapy cell sensitivity to phospholipase A was significantly lower than in a group of untreated patients ($P \leq 0.002$). These results suggest that even years after discontinuation of immunotherapy, histamine release parameters reflect patients' protection from systemic sting reactions as assessed by sting challenges. Histamine depletion of basophils induced by rush immunotherapy may play an important role in patients' protection immediately after termination of the rush regimen.

Key words: allergy; basophil; bee venom; histamine release; immunotherapy.

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Since pure venom extracts have become available, venom immunotherapy is recommended as the treatment of choice for prevention of serious systemic reactions to insect stings. It is rapidly effective and an efficacy approaching 97% has been demonstrated (8). As its mechanism of action is incompletely understood, it has been difficult to describe and measure any objective parameters that define successful treatment in the individual patient. Immunotherapy is associated with an increase in specific IgG and a decrease of specific IgE-antibodies (7, 10), but both parameters do not show any significant variation in the course of a rush immunotherapy regimen (24). Histamine re-

lease from human basophils has been proven to be a valuable tool for diagnosis and *in vitro* studies of reaginic allergy. Previous studies on the effect of immunotherapy on antigen-induced histamine release demonstrated a decrease of cell sensitivity in patients with pollinosis (4, 12, 17, 23). However, reports on changes in leukocyte histamine release after 1 year of venom immunotherapy are not consistent (16, 18). To our knowledge no studies on histamine release in bee venom-allergic patients after termination of long-term immunotherapy have been reported.

The object of this study was to investigate the mechanism of therapeutic efficacy of rush ven-

om immunotherapy and its effect on histamine release. Results obtained before and immediately after successful rush immunotherapy are compared with data from bee venom-allergic patients 2 to 5 years after cessation of long-term immunotherapy.

PATIENTS AND METHODS

All 27 patients included in this study exhibited one or more of the following reactions after bee stings: 1) generalized urticaria or angioneurotic oedema, 2) respiratory difficulty due either to laryngeal oedema or bronchospasm, 3) vascular collapse with or without loss of consciousness. In addition, all patients presented a positive skin prick test reaction with purified bee venom and bee venom-specific IgE-antibodies (RAST classes 2 to 4).

Ten patients (mean age ($\pm$ SD) 13.4 ± 5.6 years; 4 females, 6 males) had never received venom immunotherapy. Four patients (Nos. 2, 6, 7, 9, in Figs. 2 and 3) were tested by sting challenge 1 day prior to immunotherapy and experienced generalized urticaria, nausea and/or vomiting and hypotension. All symptoms responded well to injections of β -adrenergics and antihistamines. The interval between the last sting and the *in vitro* study varied from 6 to 18 months in the other patients. All patients agreed to a venom immunotherapy following a rush regimen of 2 to 3 injections per day. The initial venom dose was $0.1 \mu\text{g}/\text{injection}$ and the maintenance dose of $100 \mu\text{g}$ was achieved in 7 to 10 days. Minor side effects in three patients (Nos. 2, 5, 8) during the first 2 days of the rush regimen were treated by antihistamines. All patients were challenged by an in-hospital bee sting before discharge without generalized or unusually extensive local reactions. 17 patients (mean age ($\pm$ SD) 18.7 ± 4.4 years; 5 females, 12 males) were successfully treated between 1978 and 1983. After initial rush immunotherapy a maintenance dose of $100 \mu\text{g}$ was subsequently administered at monthly intervals. The mean duration of immunotherapy was 46 months (range 28 to 89 months). The time interval after cessation of treatment ranged from 24 to 63 months. The efficacy of treatment was assessed

by annual bee sting challenges. 58 bee sting challenges were performed in 17 patients and 12 "wild stings" were reported. Two wild stings in two patients (both in the first year of immunotherapy) elicited generalized urticaria. No systemic reactions occurred in these patients after subsequent sting challenges. The minimum interval between the last bee sting and histamine release testing was 6 months in this patient group.

Patients of both groups did not differ in terms of severity of previous anaphylactic sting reaction or skin test reactions to bee venom prior to immunotherapy. Although these two patient groups were treated and diagnosed at different times, they are combined for statistical analysis.

The control group comprised 10 non-allergic individuals (mean age ($\pm$ SD) 17.2 ± 4.7 years; 3 females and 7 males). In each case absence of sensitization was verified by history, negative skin tests and RAST for bee venom.

Skin tests were performed with the purified venoms (Reless®, Pharmacia, Sweden) by a prick technique. Venom concentrations of 0.1, 1.0, 10 and $100 \mu\text{g}/\text{ml}$ were used. A wheal formation 3 mm or greater than the weal elicited by diluent after 15 min was considered as a positive reaction. Skin reactivity was tested with histamine ($1 \text{ mg}/\text{ml}$) and diluent (buffered saline with albumin).

Deliberate sting challenges were performed on the volar side of the arm under intensive care unit conditions in all 27 patients undergoing venom immunotherapy. The test was regarded as successful when no generalized or unusually extensive local reactions occurred.

Bee venom-specific IgE-antibodies were determined according to the Phadebas IgE RAST method (Pharmacia, Sweden) and expressed as RAST classes.

Blood was obtained in all patients immediately before sting challenges. Two ml of heparinized peripheral blood was drawn into a plastic syringe. Whole blood was added to the same quantity of a phosphate buffer and mixed with appropriate concentrations of phospholipase A2 (the major allergen of bee venom) and anti-IgE (Behring/Marburg, West Germany). Phospho-

lipase A/ (Sigma, USA) concentrations of 1, 10, 100 and 1000 ng/ml were used. One aliquot, without any addition, indicated spontaneous histamine release. All samples were incubated at 37°C for 20 min. The total quantity of intracellular histamine was determined from the supernatant of whole blood heated to 100°C for 10 min. After centrifugation at $600 \times g$ histamine present in the supernatant was assessed immediately.

Histamine was measured in a radio enzymatic assay using a single isotope technique as described by Shaff & Beaven (21) and modified by Fuhr & Kownatzki (5). Venom or anti-IgE-induced histamine release is given after subtraction of spontaneous release values. All results are expressed as percent histamine release of total histamine activity. Each assay was carried out in duplicate for the histamine standard curves and in triplicate for the experimental values.

Statistical analysis of phospholipase A-induced leukocyte histamine release before and after venom immunotherapy was performed using Fisher's exact t-test.

RESULTS

A relevant phospholipase A-induced histamine release (range 22 to 76% of total histamine activity) appeared in 8 of 10 untreated bee venom-allergic patients. Leukocytes of two pa-

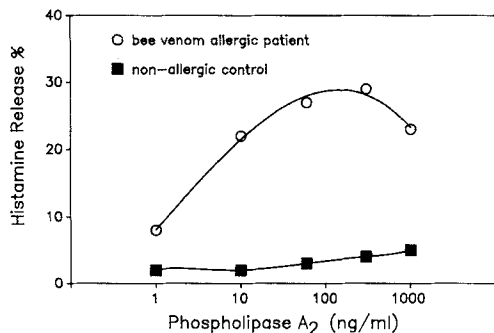


Fig. 1. Dose-response curve of phospholipase A-induced histamine release in a bee venom-allergic patient and a non-allergic control. Antigen concentrations from 1 to 1000 ng/ml were used for further investigation.

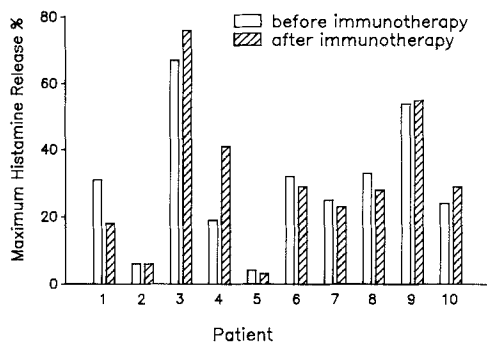


Fig. 2. Maximum histamine release induced by phospholipase A in 10 bee venom-allergic patients before and after rush immunotherapy (duration 7 to 10 days).

tients (Nos. 2 and 5 in Fig. 2) and one control donor showed no significant histamine release by phospholipase A or antigen-IgE. No antigen-induced histamine release was observed in any of the 10 normal controls. Fig. 1 represents a typical dose response curve in a patient allergic to bee stings in contrast to a control blood donor. Increasing histamine release values at 1000 ng/ml phospholipase A in non-allergic controls may be due to toxic cell degranulation.

Ten patients were treated by rush venom immunotherapy. Successful treatment was demonstrated by negative bee sting challenges before discharge in all patients. Histamine release data of Figs. 2 and 3 were obtained from blood

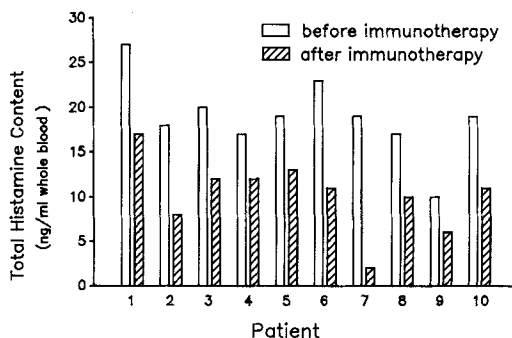


Fig. 3. Decrease of total histamine content ng/ml whole blood in 10 patients allergic to bee venom after rush immunotherapy. Success of treatment was assessed by subsequent negative bee sting challenges in all patients.

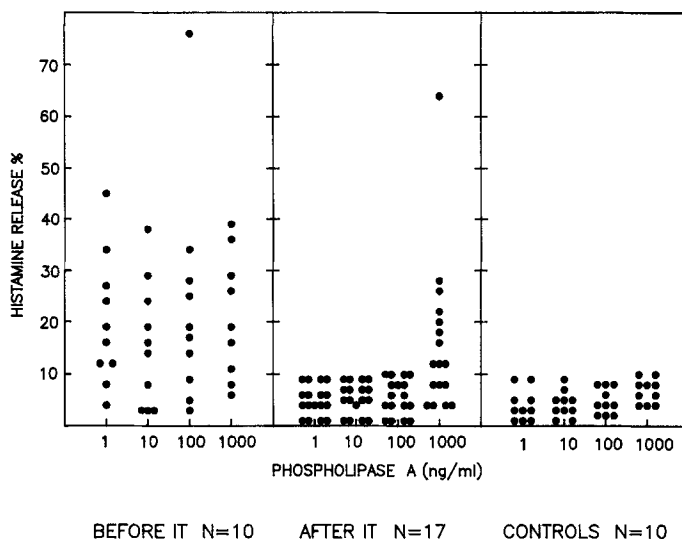


Fig. 4. Comparison of phospholipase A-induced histamine release in 10 bee venom-allergic patients before immunotherapy (before IT), 17 patients after successful long-term treatment (after IT) and 10 controls. Basophil histamine release at 1, 10 and 100 ng/ml phospholipase A was significantly increased in untreated patients ($P > 0.002$).

samples obtained immediately before and after successful immunotherapy. A slight decrease of cell reactivity to phospholipase A could be demonstrated in only two of 10 patients (Nos. 1, 10), reactivity increased in one (No. 4) and was considered essentially unchanged (maximum variation $\leq 10\%$) in seven individuals (Fig. 2). However, an important loss of total histamine content per ml whole blood (mean 45.9% range 89.1 to 30.5%) was found in all treated patients after rush immunotherapy (Fig. 3). Decrease of histamine activity in patients who received bee sting challenges before starting immunotherapy (Nos. 2, 6, 7, 9) tended to be more pronounced (mean 50.3%). In Fig. 4 phospholipase A-induced histamine release was compared in 10 untreated bee venom-allergic patients, 17 patients successfully treated by long-term venom immunotherapy and 10 controls. Histamine release from leukocytes was assessed at various antigen concentrations (1–1000 ng/ml) and maximum histamine release ranged from 0 to 76% in untreated, 0 to 64% in treated patients and 0 to 10% in controls. Considering histamine release at each different antigen concentration, significant differences could be demon-

strated between the three groups. At phospholipase concentrations from 1.0 to 100 ng/ml no patient treated by immunotherapy and no control subject showed histamine release exceeding 10%. In contrast, in untreated patients histamine release values ranging from 0 to 46% were found at these concentrations. There was a significant release of at least one antigen concentration in each of eight untreated patients.

Only at the highest concentration of 1000 ng/ml was it not possible to differentiate between untreated and treated patients on the basis of histamine release data. Controls, however, showed, even at the highest phospholipase concentrations, no significant histamine release. Statistical significance of histamine release differences between treated and untreated patients was $P \leq 0.002$ at 1.0, 10 and 100 ng/ml phospholipase.

DISCUSSION

The aim of immunotherapy is to normalize exaggerated host responses to allergen exposure by altering a number of effector and regulatory

mechanisms. Humoral changes include gradual decrease of specific IgE, blunting of seasonal specific IgE rises and increase of specific IgG (4). Cellular changes comprise decreased sensitivity of basophils, modulation of mononuclear responses and generation of antigen-specific suppressor cells (12, 17). We were able to demonstrate that histamine release parameters in bee venom-allergic patients can be a useful means of evaluating patients' allergic status following venom immunotherapy.

Our results confirm previous studies indicating the high specificity and sensitivity of histamine release procedures (6, 15, 23). Eight of 10 untreated patients (specificity 80%) and none of 10 controls (sensitivity 100%) showed significant histamine release from basophils incubated with phospholipase A. In two of 10 untreated patients and one of 10 controls histamine release could not be elicited either by stimulation with specific antigen or anti-IgE. This phenomenon appears to be genuine and is similar to previous results reporting about 15% unquestionably allergic patients failing to release histamine from washed leukocytes or whole blood (2, 11). However, each of the remaining eight patients was able to respond to Phospholipase A, thus confirming the major role of this antigen in bee venom allergy (9).

Our data obtained from patients immediately before and after rush immunotherapy showed an important loss of total histamine amount without relevant change of cell reactivity. This finding may be interpreted as a therapeutically induced histamine depletion as Diamant & Patkar (3) reported that total histamine content showed a good intraindividual reproducibility over years. In addition, plasma histamine levels are significantly elevated in patients receiving rush venom immunotherapy (1), thus indicating a depletion of mast cells and basophils. As the peripheral basophil count immediately after anaphylactic reactions is known to be low (22), decreased total histamine content could be due to basophil depletion. Our results suggest a role of degranulation of mediator secreting cells in patients' protection immediately after rush venom immunotherapy, since variations in concentrations of bee venom-specific IgE or IgG in

these patients were not observed (data not demonstrated). Maximum histamine release induced by phospholipase A showed no significant changes after rush immunotherapy. Therefore, this parameter was of no use for evaluating patients' allergic status after rush venom immunotherapy. Similar findings were recently obtained in a patient desensitized with increasing doses of benzyl-penicillin (19). Blood basophil histamine release, both by penicilloyl-human albumin and anti-IgE remain strong after desensitization. Total basophil histamine content was not reported in this study. *In vitro* studies with catecholamines and antihistamines suggested an inhibitory effect of these drugs on antigen and anti-IgE-induced histamine release from human basophils (13, 14). However, patients receiving drugs during rush desensitization presented no significant changes of antigen-induced histamine release in this study.

Before immunotherapy, venom induced histamine release varied widely but was, with the exception of the highest antigen concentration (1000 ng/ml), significantly higher in untreated patients than in patients after termination of immunotherapy and in the control group. These data indicate that basophil sensitivity to phospholipase A can be used as a predictive parameter for the clinical reactivity of patients, as histamine release was detectable at low antigen concentrations only in individuals reacting to bee sting challenges. In contrast, patients successfully treated by immunotherapy and showing normal local reactions after bee sting challenges presented increased histamine release values only at the highest antigen concentration. Thus even years after termination of immunotherapy, histamine release parameters were found to be nearly "normalized" compared with a control group. Persisting cell sensitivity to high phospholipase A concentrations in these patients was paralleled by remaining skin test sensitivity and persisting bee venom-specific IgE levels in a majority of these patients. However, these data are inconsistent with an earlier report (16) showing that immunotherapy in hymenoptera-allergic patients did not result in decreased cell sensitivity after 1 year of therapy. Our findings may be due to the much

longer duration of immunotherapy (mean 46 months) in our patients. Another study reported a loss of basophil reactivity to bee/wasp venom in all 17 patients tested after at least 1 year of immunotherapy (18). In contrast to our study, these patients seemed to include a very high percentage of donors unresponsive to anti-IgE where no correlation between histamine release data and clinical grade of sensitization can be expected.

In conclusion, our results suggest different mechanisms for patients' protection depending on duration and regimen of immunotherapy. Therapeutically induced histamine depletion was observed after 7 days of initial immunotherapy in all successfully treated patients and may contribute to patient protection after rush immunotherapy. In contrast, after termination of long-term immunotherapy significantly decreased cell sensitivity to phospholipase A was the most important parameter reflecting successful venom treatment.

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