

Modulation of venom-induced leukocyte histamine release by mononuclear cells: effect of venom immunotherapy

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Leukocyte histamine release and blood mononuclear cell proliferative responses to venoms were evaluated in nine patients with sting anaphylaxis. The effect of venom-activated mononuclear cells on the autologous mononuclear cell proliferative response and venom-induced leukocyte histamine release was also studied before and on maintenance immunotherapy. The mononuclear cell proliferative response was low (specific incorporations <2.0) and antigen nonspecific both before and during maintenance immunotherapy. Venom-induced in vitro leukocyte histamine release did not change significantly with immunotherapy. However, when patients were on maintenance immunotherapy, venom-activated mononuclear cells cocultured with autologous leukocytes significantly ($p < 0.05$) suppressed venom-induced histamine release. The suppressive effect of mononuclear cells was antigen specific and enhanced by in vitro mononuclear cell activation. Suppression of leukocyte histamine release by mononuclear cells extends the regulatory potential of these cells and may identify an additional mechanism by which immunotherapy protects Hymenoptera-sensitive individuals against systemic anaphylaxis. (J ALLERGY CLIN IMMUNOL 70:445, 1982.)

Hymenoptera sting sensitivity is a relatively common cause of serious allergic reactions, affecting 0.4% to 0.8% of the population.¹⁻⁴ These patients are at risk of experiencing anaphylaxis upon re-exposure, and the state of sensitivity persists for a variable period.^{3, 4} Venom IT has been shown to result in significant protection against serious anaphylactic reactions.⁵ This treatment is associated with a rise in venom-specific IgG (blocking-antibody).^{5, 6} However, the exact mechanism of therapeutic efficacy is unknown, as demonstrated by the persistence of detectable IgE antibody levels during the first year of IT at a time when the patients were clinically protected against a sting challenge.^{1, 5}

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Although the importance of mast cells, basophils, and IgE in atopic states is well recognized, the role of mononuclear cells has received attention only recently.^{7, 8} Enhanced in vitro proliferation⁹⁻¹² occurs after exposure to the specific allergen, which in a number of patients reverts toward normal after specific IT. This has been attributed in part to generation of antigen-specific suppressor cells during IT.⁷ Mononuclear cells, upon activation with mitogens, release soluble factors that induce or enhance leuko-

Abbreviations used

PBC:	Peripheral blood mononuclear cells
cpm:	Counts per minute
SI:	Specific incorporation
Con-A:	Concanavalin A
Tris A:	Tris buffer containing human serum albumin 0.3 gm/l
Tris ACM:	Tris buffer with albumin, 6×10^{-4} M calcium chloride and 10^{-3} M magnesium chloride
IT:	Immunotherapy
PHA:	Phytohemagglutinin

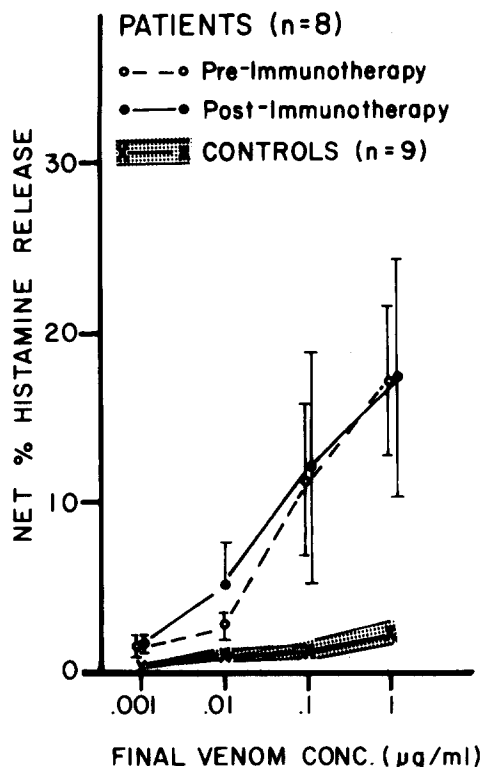


FIG. 1. Venom-induced leukocyte histamine release. Data shown are the means of net histamine release $\pm$ SEM. Note that although histamine release was greater from patients' leukocytes than from controls, there was no difference between pre-IT and post-IT (maintenance) values.

cyte histamine release.¹³⁻¹⁶ The role of these factors in the modulation of immediate hypersensitivity is unclear.

In this study, the venom-induced proliferative response of PBC and venom-induced leukocyte histamine release, together with their modulation by specifically activated autologous mononuclear cells, were studied prospectively in a group of patients undergoing venom IT. The results indicate that IT generates PBC with the capacity for further in vitro activation and development of the ability to suppress venom-induced histamine release from autologous leukocytes.

MATERIALS AND METHODS

Patients

Nine patients, ages 22 to 63 yr (mean 39 yr), who had experienced systemic anaphylaxis after Hymenoptera sting, volunteered to participate in the study. The interval between the last sting and in vitro study of their leukocytes varied from 1 to 39 mo and averaged 24 mo. There were five men and four women in the study group. Two of the patients had histories of atopic disease, manifested by seasonal allergic rhinitis, with perennial asthma in one and seasonal allergic rhinitis in the second. Neither had received IT for these symptoms. Three patients had received whole-body extract

IT prior to the study. Skin tests were positive for IgE antibody sensitization with one venom in four patients and to multiple venoms in five patients. All patients received venom IT (Pharmacia, Uppsala, Sweden), as dictated by positive skin tests. The initial dose was generally 10^{-4} µg and a maximal dose of 100 µg of venom was reached in about 8 wk. Patients were maintained on 100 µg of venom every 2 to 4 wk. Mean cumulative venom dose prior to repeat studies was 1252 µg.

Serial studies

Peripheral blood cells from patients were studied before and during maintenance IT for (1) proliferative response of PBC to skin test-positive and skin test-negative venoms, (2) blood mononuclear cell regulator function (helper or suppressor) by study of the venom-induced proliferative response of blood mononuclear cells cocultured with venom-activated mononuclear cells, (3) venom-induced leukocyte histamine release, and (4) modulation of venom-induced leukocyte histamine release by autologous mononuclear cells. The mononuclear cells were activated with Con-A and with specific or nonspecific venoms prior to coculturing with leukocytes. Cells from all patients, however, were not available for each of the studies.

Studies during IT were generally performed 5 to 12 mo (mean 9.5 mo) after a maintenance dose of venom had been achieved, and blood was drawn at least 2 wk after venom had been administered.

Collection and testing blood mononuclear cells. PBC were harvested from heparinized blood by the standard Ficoll-Hypaque density gradient centrifugation, washed three times with RPMI-1640 medium, and then resuspended in medium with 10% fetal calf serum (Kansas City Biologicals, Lenexa, Kan.). Mononuclear cells prepared in this fashion consisted of about 76% lymphocytes, 18% monocytes (nonspecific esterase positive), and 2% polymorphonuclear leukocytes and contained 148 ± 44 ng (mean $\pm$ SEM) of histamine/ 10^7 cells.

Proliferative response. Mononuclear cells suspended in RPMI-1640 medium containing 10% fetal calf serum were placed in a round-bottom microtiter plate (Linbro Scientific, Inc., Hamden, Conn.). Each well received 5×10^4 cells suspended in 200 µl. To this was added a 25 µl volume of culture medium containing 0.01, 0.1, and 1.0 µg of venom (final concentration of dialyzed venom protein; Pharmacia). Cell preparations were incubated for 5 days at 37° C in a humidified atmosphere of 5% CO₂ in air. Cells were pulsed with 0.25 µCi of ³H-thymidine 6 to 8 hr prior to harvesting, and incorporated radioactivity was counted by standard technique.¹⁷ Experiments were carried out in triplicate. In all experiments, cells from normal individuals served as controls for specific venom stimulation.

Proliferative response of cells to each dose of venom was calculated by absolute cpm or by SI, which is the ratio of cpm of cultures in the presence of venom to cpm in the absence of venom.

Venom-induced leukocyte histamine release. To determine optimal venom concentration for induction of histamine release in modulation experiments, histamine release

TABLE I. Proliferative response of blood mononuclear cells to venoms

Evaluation in relationship to IT	Dose of venom ($\mu\text{g/ml}$)	SI (mean $\pm$ SEM)*		
		Patients		Normal controls: nonspecific venom (n = 13)
		Specific venom (n = 8)	Nonspecific venom (n = 5)	
Pre-IT	0.01	0.87 $\pm$ 0.10	1.12 $\pm$ 0.10	0.98 $\pm$ 0.08
	0.1	1.09 $\pm$ 0.16	1.24 $\pm$ 0.10	1.07 $\pm$ 0.08
	1.0	1.30 $\pm$ 0.27	1.42 $\pm$ 0.11	1.08 $\pm$ 0.14
Maintenance IT	0.01	1.08 $\pm$ 0.16	1.00 $\pm$ 0.11	NA
	0.1	1.59 $\pm$ 0.33	1.27 $\pm$ 0.22	
	1.0	1.35 $\pm$ 0.17	1.25 $\pm$ 0.21	

NA = not applicable.

*Mean background cpm (mean $\pm$ SEM): patients before IT = 341 $\pm$ 110; patients on maintenance IT = 253 $\pm$ 47; normal control = 307 $\pm$ 47.

from dextran sedimented leukocytes was determined at multiple venom concentrations (0.001 to 1.0 μg) by previously described methods.¹⁸ In brief, dextran-sedimented leukocytes were washed twice with Tris A, resuspended in Tris ACM, and incubated for 60 min at 37° C with appropriate concentrations of venom protein. The latter was dialyzed for 16 to 18 hr before addition to leukocytes in order to remove histamine and other low-molecular-weight contaminants.

Histamine present in the supernatants and cell buttons (after boiling for 10 min) was measured by the enzymatic-isotopic method as described by Shaff and Beaven.¹⁹

Activation and testing regulator cells. Four million mononuclear cells were activated with Con-A as described earlier.^{8, 17} In brief, cells were placed in plastic tubes (Falcon Plastics No. 2054, Costa Mesa, Calif.) and incubated in 2 ml final volume with either culture medium (RPMI-1640-10% fetal calf serum) or after addition of 10 μg of dialyzed venom protein or 40 μg Con-A. After 20 hr of incubation the cells were washed three times with culture medium and counted, and viability was determined.⁸ The activated mononuclear cells were then used for modulation of either venom-induced mononuclear cell proliferative response or venom-induced leukocyte histamine release. For the former, 5×10^4 activated mononuclear cells were mixed with equal numbers of fresh autologous PBC and cultured for 5 days in the presence of 1 μg of specific venom. The proliferative response was evaluated by ³H-thymidine incorporation.¹⁷ Specific (venom)- and non-specific (Con-A)-activated regulator cell scores were calculated by the following formula: cpm of cocultures with Con-A or venom-activated regulator cells divided by cpm of cocultures in which regulator cells were not activated with Con-A or venom. Scores of >1 denoted helper activity and those of <1 indicated suppressor activity.

Modulation of venom-induced leukocyte histamine release by activated mononuclear cells was studied by means of autologous leukocytes freshly prepared by the dextran sedimentation method. The latter method yielded a leukocyte preparation consisting of a mean value of 89% neutro-

phils, 1% eosinophils, 0.5% basophils, 7% lymphocytes, and 2.5% monocytes. The leukocytes were suspended in Tris ACM and then cocultured with the activated mononuclear cells at a ratio of 10 leukocytes to one mononuclear cell for 15 min at 37° C. Dialyzed venom protein at a concentration that was predetermined for each patient to induce submaximal histamine release was added to the leukocyte-mononuclear cell mixture and the incubation was allowed to continue for further 60 min at 37° C. Spontaneous and venom-induced histamine release from leukocytes alone or from various types of activated mononuclear cells alone were simultaneously performed. Supernatant and cell-button histamine content was measured after the predetermined incubation period. All experiments were carried out in duplicate.

Statistical analysis

Statistical analysis of venom-induced leukocyte histamine release and mononuclear cell proliferation before and during maintenance IT was done using a t test for paired observations. Mononuclear cell modulation of leukocyte histamine release was analyzed by analysis of variance.²⁰ When paired observations were available, comparison of mononuclear cell modulation of leukocyte histamine release was made by Wilcoxon signed-rank test.

RESULTS

Mononuclear cell proliferative response

Proliferative response of blood mononuclear cells to the specific and nonspecific venoms at three doses (0.01, 0.1, and 1.0 $\mu\text{g/ml}$ final concentration) before and during maintenance IT are shown in Table I. Despite an apparent dose-response relationship, proliferative responses were not significant (SI < 2). However, occasional patients and even a few normal controls showed SI values of >2 . Higher venom concentrations (10 $\mu\text{g/ml}$) depressed proliferative response in cultures, while even higher concentrations (100 $\mu\text{g/ml}$) had a cytotoxic effect, causing decreased

TABLE II. Mononuclear cell modulation of venom-induced leukocyte histamine release

Dextran-sedimented leukocytes cocultured with:	Sting anaphylaxis patients*		
	Before IT (n = 6)	Maintenance IT (n = 10†)	Control subjects (n = 8)
None	10.26 ± 3.72	23.52 ± 6.90	2.51 ± 0.99
Medium-cultured mononuclear cells	8.18 ± 4.01	19.75 ± 7.46	0.64 ± 0.47
Con-A-activated mononuclear cells	16.86 ± 6.75	20.80 ± 8.64	1.23 ± 0.71
Venom-activated mononuclear cells	7.67 ± 3.29	15.29 ± 7.16‡	0.63 ± 0.28

*Data expressed as net percentage histamine release (mean ± SEM):

$$\frac{\text{histamine released by (leuk + mono + venom)} - (\text{mono} + \text{venom}) - (\text{leuk} + \text{mono} + \text{buffer})}{\text{total leukocyte histamine content}}$$

†Maintenance IT group contained five additional patients (not studied prior to venom IT for mononuclear cell modulation) explaining apparent group differences in net percentage histamine release in this part of the study, despite the almost similar release on venom dose response.

‡p < 0.05, compared with histamine release by leukocytes alone, using analysis of variance test.

TABLE III. Specificity of modulation by mononuclear cells of venom-induced leukocyte histamine release

Net percentage histamine release (mean ± SEM)* from leukocytes cocultured with:		
Buffer (control)	Specific venom-activated mononuclear cells	Nonspecific venom-activated mononuclear cells
31.25 ± 8.4	23.92 ± 10.6	31.26 ± 10.3
—	p < 0.05†	p > 0.95†

*Data represent a mean of experiments on six patients studied on maintenance IT.

†One-way analysis of variance for repeated measurements was performed to test the difference between the means. Dunnet's test was utilized to locate the significance between the venom-activated mononuclear cell cocultures and buffer coculture (control).

mononuclear cell viability and depression of the proliferative response at 5 to 7 days (not shown in Table I). Venom concentrations ranging from 0.001 to 1.0 µg/ml added simultaneously with PHA to normal blood mononuclear cell culture did not significantly inhibit the PHA-induced proliferative response in the 3-day cultures. This would indicate that the lack of proliferative response to venom was not due to its cytotoxic effects.

Venom-induced leukocyte histamine release

Fig. 1 shows histamine release by leukocytes from eight patients before and during maintenance IT. A dose-response effect by the specific venom is demonstrated. Cells from the ninth patient did not release any histamine after repeated measurements (not shown in Fig. 1). Leukocyte histamine release from patients varied widely but was clearly higher than that of the control group at all doses used. Except at the 0.01 µg/ml venom concentration, histamine release did not appreciably change after specific venom IT (Fig. 1). Maximal histamine release by nonspecific (skin test^{-ve}) venoms at concentrations up to 1 µg/ml

was only 3.2% ± 1.41% and 3.88% ± 1.55% before and during maintenance IT, respectively (not shown in Fig. 1).

Mononuclear cell modulation of leukocyte histamine release

Prior to IT, incubation with either venom-activated or medium-cultured mononuclear cells caused minimal but not significant suppression of leukocyte histamine release when compared with histamine release from leukocytes not incubated with mononuclear cells (Table II). Enhancement of histamine release was observed with Con-A-activated cells but was not consistent. None of the values of net percent histamine release, however, was significantly different from values of cultures in which leukocytes were incubated with the venom in the absence of mononuclear cells (Table II, "Before IT" column).

During maintenance IT leukocyte histamine release in one of the six patients originally studied fell to a degree that modulation of histamine release could not be interpreted in that individual. Modulation of leukocyte histamine release by autologous, specific

venom-activated mononuclear cells in the remaining five patients (studied both before and during maintenance IT, data not shown) showed significantly more suppressive effect when on maintenance IT compared with pre-IT values ($p < 0.05$ by Wilcoxon signed-rank test). To study this phenomenon, an additional five patients who had received comparable venom IT were included. Results from all 10 patients are shown (Table II, "Maintenance IT" column). When compared with values of histamine release from leukocytes not cocultured with mononuclear cells, leukocyte histamine release was suppressed after preincubation of the leukocytes with medium-cultured, Con-A- or venom-activated mononuclear cells. The suppressive effect was variable and of statistical significance only with venom-activated mononuclear cells ($p < 0.05$ by analysis of variance; Table II, "Maintenance IT" column). As shown in Table III, when mononuclear cells from patients on maintenance IT were activated with a specific venom, significantly more suppression of leukocyte histamine release was observed as compared with cells activated by a nonspecific (skin test-negative) venom in the six patients that were available for this comparison ($p < 0.05$ by one-way analysis of variance test).

DISCUSSION

IT may cause host responses by altering a number of effector and regulator mechanisms, including both cellular and humoral components. Humoral changes have been rather well studied and include a gradual decrease in specific IgE,^{6, 9, 21, 22} blunting of seasonal rise in specific IgE in response to seasonal allergens,^{22, 23} and rise in allergen specific IgG.^{5, 22} Cellular changes comprise a decreased mononuclear proliferative and secretory responses,⁹⁻¹¹ generation of antigen-specific suppressor cells,⁷ and decreased sensitivity of basophils.^{9, 22}

Before IT, venom-induced *in vitro* leukocyte histamine release in our patients was widely variable and rather low when compared with the results of previous studies.^{18, 24} This may be due to spontaneous decrease of leukocyte sensitivity over the months prior to study.³ In this study there was a long interval (mean 24 mo) between the last sting and the *in vitro* test. When patients were on maintenance IT, histamine release from leukocytes alone (in the absence of incubation with cultured PBC) as a group was unchanged compared with pretherapy values. Our data support earlier observations that venom IT, at least during the first year, does not cause a decrease in basophil sensitivity.³ However, wide variations in individual responses were observed, demonstrating heterogeneity of cellular response to IT.

We were unable to demonstrate in our patients with histories of sting anaphylaxis significant mononuclear cell proliferation upon incubation *in vitro* with the specific venom. Recent studies have shown that significant proliferation was seen only in patients with histories of large local reactions to Hymenoptera stings and not in those with systemic reactions.¹² The lack of significant proliferative response in the latter group could be due to presence of few clones of responding cells or presence of suppressor cells, or to requirement for different culture conditions. Although we have routinely used fetal calf serum in our experiments instead of autologous serum to evaluate mononuclear cell responsiveness independent of human serum factors, it may be that human or autologous sera are needed for optimal proliferation. A higher blastogenic response of blood mononuclear cells in fetal calf serum was noted and resulted in increasing the background counts in cultures from some patients. This may account for artificially decreasing an already marginal venom-induced proliferative response in those patients. The possibility of using inappropriate venom dosage is unlikely, since we used a wide range of venom concentrations with incremental response at the highest concentrations beyond which cytotoxic effects were observed. The lack of venom-induced mononuclear proliferation is in contrast to that seen with a number of other reported antigen systems, including ragweed, grass pollen, and house dust.^{7, 10, 11, 13}

The failure of mononuclear cells to proliferate *in vitro* in response to the specific venom prompted us to analyze whether mononuclear cells or a subset of mononuclear cells can modulate another immune mechanism, i.e., *in vitro* leukocyte histamine release in patients with systemic reactions to Hymenoptera sting. Our results indicate that venom IT induces a mononuclear cell subpopulation that upon further activation *in vitro* with the specific venom, renders them capable of suppressing venom-induced autologous leukocyte histamine release. To our knowledge, this observation is the first demonstration of modulation of histamine release by specifically activated mononuclear cells after IT. Possible mechanisms for such suppression of basophil histamine release include (1) the generation of suppressor cells or factors by the mononuclear cells, or (2) inactivation of histamine by the mononuclear cells, as was recently shown by alveolar macrophages with respect to suppression/inactivation of slow-reacting substance of anaphylaxis.²⁵ Carry-over of venom by the activated mononuclear cells could be another explanation for the observed results. Evidence from additional experiments, preliminary at present, indicate that these possibilities are an unlikely explanation of observed effect. Mono-

nuclear cells do not inactivate histamine when known amounts of the latter were added to the cells in vitro under conditions that simulate the experimental cultures. By means of radiolabeled venom, mononuclear cells were shown to carry over negligible amounts of added venom when processed identically to the experimental cultures.

When compared with histamine release by leukocytes alone, the observed suppression of leukocyte histamine release by medium-cultured PBC was statistically not significant (Table II). We believe that these cells, for a variety of reasons, are probably not an appropriate control. First, all these patients were receiving maintenance venom IT and consequently their cells had intermittent exposure to the specific venom. Second, separation procedures may cause activation of mononuclear cells.^{26, 27} In this context it is interesting to note that histamine release after incubation with nonspecific venom-activated PBC was comparable to leukocyte histamine release in the absence of any mononuclear cell incubation and significantly different from specific venom-activated PBC (Table III).

The interaction between mononuclear cells and cells responsible for mediator release has recently been studied in other systems. T cells were shown to modulate mast cell proliferation, differentiation, and mediator release.²⁸ T-inducer cells were shown to synthesize a factor that stimulated mast cell proliferation.²⁸ Activated T cells were shown to regulate histamine content of a specialized subset of mast cells.²⁹ Suppression of venom-induced leukocyte histamine release by venom-activated mononuclear cells after IT is an interesting new observation. Such modulation of basophil releasability could be important in the prevention or amelioration of systemic anaphylactic reaction in Hymenoptera-sensitive individuals after IT.

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