

Cytokine modulation of basophil histamine release in wasp-venom allergy

Radermecker M, Louis R, Leclercq M, Weber Th, Corhay JL, Bury Th. Cytokine modulation of basophil histamine release in wasp-venom allergy. Allergy 1994; 49: 641–644. © Munksgaard 1994.

We report the effect of interleukin-3 (IL-3) and of other cytokines on antigen-induced basophil histamine release in wasp-venom-allergic subjects. Leukocytes from 12 patients with documented anaphylactic sensitivity to wasp venom were preincubated in the presence or absence of IL-3, granulocyte/macrophage-colony stimulating factor (GM-CSF), IL-5, IL-8, or stem cell factor (SCF). Washed cells were then exposed to venom and to other secretagogues, and histamine release in the supernatant was measured fluorometrically. Preincubation of leukocytes with IL-3, GM-CSF, or IL-5 (0.02–2 ng/ml), but not with IL-8 and SCF, caused a dose-dependent enhancement of antigen-induced basophilic histamine release in all subjects tested. Mean maximum increase was about 100% for IL-3, IL-5, and GM-CSF. The priming effect of IL-3 was rapid, persisted up to 12 h, and was not accompanied by a change in cellular histamine. IL-3 had a comparable enhancing effect when basophils were triggered with anti-IgE or *N*-formylmethionylphenylalanine (FMP). By contrast, IL-3 had no effect on substance-P-induced histamine release. The significant enhancement of basophil releasability to antigen in wasp-venom allergy by very low concentrations of IL-3, GM-CSF, and IL-5 suggests that cytokines in the basophil (mast-cell?) microenvironment could be critical factors in determining the variability of sting reactions in Hymenoptera-venom-allergic subjects.

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Key words: cytokines; histamine release; venom allergy.

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Accepted for publication 30 December 1993

Anaphylaxis caused by Hymenoptera venom is a relatively pure model of type I allergy, involving mast cells and basophils, IgE antibodies, and the release of mediators. Elevated levels of IgE antibodies against bee or wasp venom are indeed found in the serum of Hymenoptera-venom-allergic subjects. Almost all patients show a wheal and flare reaction after intradermal injection of a nonirritating concentration of venom. Finally, the basophils of allergic subjects specifically release histamine *in vitro* in the presence of Hymenoptera venom at concentrations that are not effective in nonallergic controls (8).

This simple pathophysiologic concept can, however, be challenged by some frustrating clinical facts. For instance, it is impossible to predict the severity of clinical symptoms after reexposure to venom in allergic subjects (5). Secondly, there is no convincing correlation between the degree of immunologic sensitization, as measured by IgE antibody titers (RAST or skin tests), and the severity of the anaphylactic reaction (11, 12). These observations suggest that the degree of clinical hypersensitivity *in vivo*

could be better explained, at least to some extent, by the ability of mast cells/basophils to release their mediators.

Cytokines are potent auto- or paracrine polypeptides or glycoproteins secreted by several cell types in modulating the effector phase of IgE-mediated allergic reaction. One of the most important modulatory effects of cytokines on inflammatory cells is priming for more efficient release of mediators. For example, blood basophils markedly increase their capacity to release preformed histamine and to form *de novo* sulfidoleukotrienes (such as LTC₄) after brief incubation *in vitro* with interleukin-3 (IL-3), IL-5, and granulocyte/macrophage-colony stimulating factor (GM-CSF) (1, 4, 6, 7, 13).

The priming effect of cytokines on antigen-induced basophil histamine release in wasp-venom-allergic subjects has not been studied. We therefore compared the ability of IL-3 and other cytokines to modulate *in vitro* the release of histamine from basophils in patients sensitive to wasp venom. Our results show that IL-3, GM-CSF, and IL-5 in pico-

molar concentrations cause a significant enhancement of basophil releasability to antigen without changing the histamine content of the cells.

Material and methods

Twelve patients (mean age 37 ± 8 years, range 17–64, 7 men, 5 women) with a documented history of anaphylactic sensitivity to wasp venom (*Vespula germanica* or *V. vulgaris*) were studied 6–12 weeks after stinging anaphylaxis. All patients had a positive intradermal skin test with pure wasp venom (100 ng/ml) (Pharmalgen[®], Pharmacia, Uppsala, Sweden), positive RAST (Pharmacia), and histamine release from leukocytes to wasp venom (Pharmalgen, Pharmacia) at 10 and 100 ng/ml. Seven patients were atopic on the basis of their clinical history and positive skin tests and RAST for grass pollen, *Dermatophagoides pteronyssinus*, and animal proteins (Bencard, UK). Ten healthy subjects with no history of venom sensitivity were studied as controls. Allergic patients and controls received no treatment at the time of the study.

Leukocytic histamine release

Peripheral venous blood was collected into 10-ml heparinized Vacutainers[®] (Becton-Dickinson) containing 1 ml Plasmagel[®] (Bellon, Paris, France). The tubes were gently mixed and allowed to stand for 35–45 min. The upper plasma layer containing leukocytes and platelets was carefully decanted, and cells were washed three times in Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} . Cells were counted (Coulter) and resuspended in HBSS containing Ca^{2+} and Mg^{2+} to 2×10^7 cells/ml. To 1 ml of this leukocyte suspension was added the same volume of HBSS alone or containing a cytokine at concentrations ranging from 0.004 to 40 ng/ml. Cells were incubated for 20 min at 37°C . They were centrifuged, washed once, and then incubated in 2 ml of secretagogue. These included wasp venom (Pharmalgen, Pharmacia) at concentrations ranging from 1 ng to 10 $\mu\text{g}/\text{ml}$, polyclonal rabbit antihuman IgE (Behringwerke, Marburg, Germany), *N*-formyl-methionylphenylalanine (FMP, Sigma), or substance P (UCB-Bioproducts, Brussels, Belgium) at optimal concentrations. Previous studies from our laboratory have shown that the optimal concentrations for leukocyte histamine release are, respectively, 1/2000 (0.25 μg antibodies/ml) for anti-IgE and 10^{-4} for FMP or substance P (9). The leukocyte suspensions were incubated for 30 min at 37°C with the secretagogue and then centrifuged. Histamine was measured spectrofluorometrically in the supernatant fluid, as previously described (3). Histamine release was expressed in percentage of total histamine

content of the leukocyte suspension. Among the secretagogues used, only wasp venom showed a dose-related fluorescence at 1 $\mu\text{g}/\text{ml}$ and above.

Cytokines

Recombinant human IL-3 (Rh) produced in *Escherichia coli* was purchased from British Biotechnology Ltd (Oxford, UK). Recombinant human IL-8 (*E. coli*) and Rh IL-5, GM-CSF, and SCF (stem cell factor, C-kit ligand, Steel factor) produced in yeast were obtained from Genzyme (Cambridge, MA, USA). All the cytokines were of greater than 95% purity.

Statistical analysis

Student's *t*-test was used to compare mean values between groups. Results are expressed as mean $\pm$ SEM. Results were considered to be significant when $P < 0.05$.

Results

Venom-induced histamine release from leukocytes of 12 patients allergic to wasp venom and 10 non-allergic controls is shown in Fig. 1. We confirm that low concentrations of wasp venom (10^{-7} g/ml and below) release a significant amount of histamine only in allergic patients, and that venom at 10^{-5} g/ml may cause some nonspecific histamine release in non-allergic subjects. Incubation of leukocytes with the cytokines IL-3, IL-5, IL-8, GM-CSF, and SCF caused no significant histamine release. Fig. 2 shows the effect of these cytokines on the amount of histamine released by leukocytes of patients allergic to wasp venom with a suboptimal concentration of

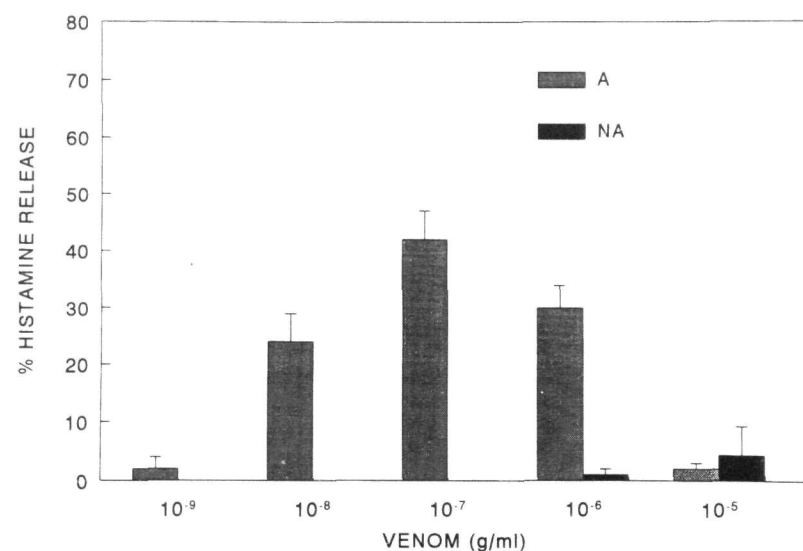


Fig. 1. Relationship between amount of histamine release from basophils and concentration of wasp venom (Pharmalgen) in 12 patients allergic to *Vespula* venom (A) and in 10 nonallergic healthy controls (NA).

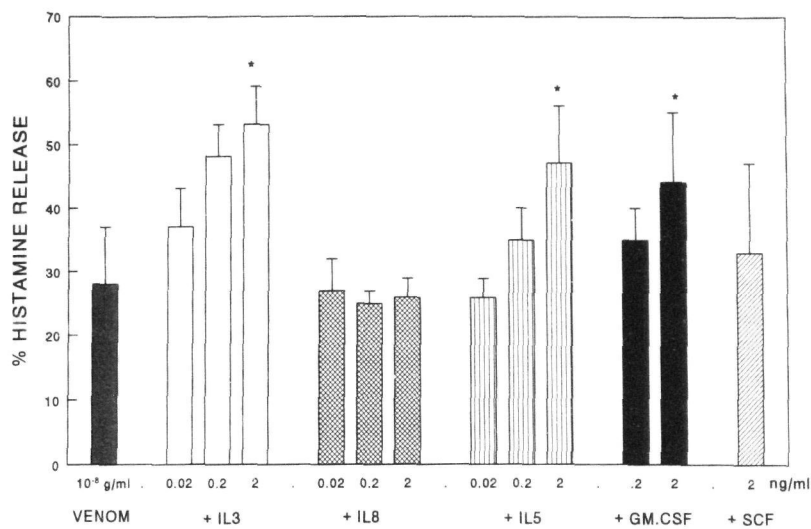


Fig. 2. Effect of cytokines on leukocyte histamine release induced by suboptimal concentration (10 ng/ml) of antigen in subjects allergic to *Vespa* venom ($n = 3-8$).

antigen. Preincubation of leukocytes with IL-3, IL-5, and GM-CSF caused a dose-related enhancement of histamine release, whereas IL-8 and SCF had no effect. On a molar basis, IL-3 was more potent than IL-5 and GM-CSF, but the three cytokines were nearly equally effective at their maximally effective concentration. At concentrations ranging from 0.02 to 2 ng/ml, IL-3 caused a dose-dependent increase of histamine release with a mean maximum of about 100%. A plateau was reached above 2 ng/ml. Interestingly, one allergic patient whose basophils did not respond to a suboptimal concentration of venom (10 ng/ml), and two whose basophils did not respond to anti-IgE (1/2000) converted from negative to positive after incubation with IL-3 (Table 1). The promoting effect of IL-3 was rapid, requiring no incubation time and persisted at least 12 h.

Incubation of leukocytes with IL-3 (2 ng/ml, 30 min, 37°C) had no significant effect on the histamine content of the cells (105% of control). The priming effect of IL-3 on venom-induced leukocyte histamine release was similar in wasp-venom-allergic patients whether the cell donors were atopic or not.

Fig. 3 shows mean percent histamine release to *Vespa* venom and to optimal concentrations of anti-IgE, FMP, and substance P when leukocytes from subjects allergic to wasp venom were preincu-

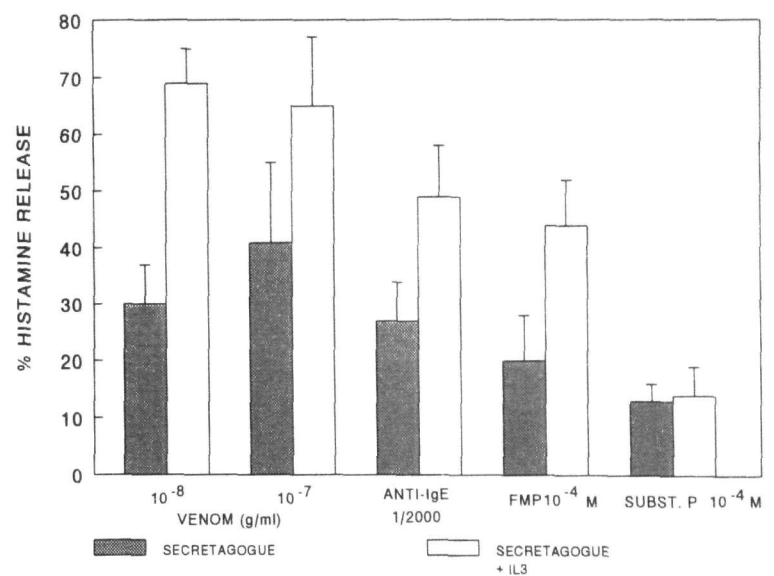


Fig. 3. Effect of IL-3 (2 ng/ml) on leukocyte histamine release to optimal concentrations of antigen, anti-IgE, *N*-formylmethionyl-phenylalanine (FMP), and substance P in 11 *Vespa*-venom-allergic patients.

bated in the absence or presence of IL-3 (2 ng/ml). In all patients, IL-3 caused a significant and comparable increase of histamine release, ranging from 20 to 300% of basal value with antigen, anti-IgE, and FMP. In contrast, IL-3 had no effect on substance-P-induced histamine release.

Discussion

This study shows that the cytokines IL-3, IL-5, and GM-CSF, but not IL-8 or SCF, in picomolar concentrations, significantly promote antigen-induced histamine release from basophils of patients allergic to wasp venom. This finding was not unexpected because these cytokines are known to enhance the release of histamine and leukotriene C₄ induced by IgE- and non-IgE-mediated mechanisms from leukocytes of normal and atopic subjects (1, 4, 6, 7, 13). A significant upregulating effect of IL-3 (mean maximum about 100%) was found in all subjects when basophils were triggered by antigen, anti-IgE, or FMP. The priming effect occurred rapidly, persisted at least 12 h, was not accompanied by a rise in cellular histamine, and was comparable in atopic and nonatopic subjects. Of particular interest was our finding that the basophils of certain subjects, initially unresponsive to antigen or anti-IgE, were rendered responsive by IL-3. It seems now to be established that the cytokines which are active on human basophils are not capable of priming human mast cells. To date, SCF is the only cytokine known to have *in vitro* upregulating effects on the release of mediators from dispersed lung mast cells (2).

The severity of allergic reactions to Hymenoptera venom may change with time, and it is impossible to predict the intensity of clinical symptoms after re-exposure to antigen (5, 11, 12). It thus appears that

Table 1. Effect of IL-3 on leukocytic histamine release in three subjects whose basophils did not respond to suboptimal concentration of wasp venom or to anti-IgE (1/2000)

Subjects	Secretagogue	Histamine release (%)	
		without IL-3	with IL-3
ME	Venom 10 ng/ml	0	20
	100 ng/ml	25	77
BR	anti-IgE (1/2000)	0	27
BI	anti-IgE (1/2000)	0	16

in Hymenoptera-venom allergy, as in atopic diseases in general, there is a poor correlation between the degree of allergen-specific hypersensitivity and clinical symptoms on allergenic challenge. There are also anecdotal observations showing that basophils from allergic subjects that release histamine in the presence of antigen may cycle from positive to negative in a few weeks, and vice versa. It is tempting to speculate that varying levels of cytokine production and *in vivo* priming may be responsible for the differences observed in mediator release and clinical hypersensitivity. However, it is not yet proved that priming by cytokines is as important *in vivo* as it appears to be *in vitro*. IL-3 and GM-CSF are currently used as cell growth factors in cancerous patients undergoing aggressive chemotherapy. Administration of Rh IL-3 in man causes an increase of leukocytes, mainly eosinophils and basophils, and a dose-dependent enhancement of anti-IgE-induced histamine release from basophils (10). Both the increase in basophil and eosinophil numbers and the enhanced releasability of basophils after IL-3 therapy support the concept of a modulating role of IL-3 in allergic diseases.

It thus appears that in wasp-venom allergy, the ability of basophils to release their mediators in the presence of antigen may show long-lasting changes in the presence of extremely low concentrations of IL-3, IL-5, and GM-CSF. It is possible that cytokines secreted by different cell types in the mast-cell/basophil microenvironment modify the releasability of mediators and other functions of inflammatory cells; this could modulate the clinical expression of the allergic reaction.

Acknowledgments

We thank M. Gobert and M. Henket for skillful technical assistance.

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