

Insect-sting challenge in 138 patients: Relation between clinical severity of anaphylaxis and mast cell activation

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One hundred thirty-eight patients with a previous anaphylactic reaction to a yellow jacket or a honeybee sting, as well as eight volunteers, were subjected to an in-hospital sting challenge. Plasma levels of histamine, tryptase, and prostaglandin D₂ (PGD₂) during sting challenge were studied in relation to clinical symptoms. Prechallenge levels (mean $\pm$ SD) of histamine, tryptase, and PGD₂ were 2 ± 1 nmol/L, 0.3 ± 0.3 U/L, and 320 ± 223 ng/L, respectively. In the volunteers and in none except for one of the nonreacting patients, these levels did not change significantly after challenge. In contrast, mean increases in the group of 18 patients with a mild reaction were significant for histamine and tryptase at one or more time points after the challenge. (Five patients demonstrated no increase in histamine; nine demonstrated no increase in tryptase.) Except for histamine levels in one patient, these increases were considerably more in all 17 patients with a severe reaction, starting from the first anaphylactic symptoms. Fifteen minutes later, peak values were reached of 1275 ± 2994 nmol of histamine per liter (range, 3 to 12800 nmol/L; median, 11 nmol/L) and 406 ± 1062 U of tryptase per liter (range, 1.8 to 4400 U/L; median, 17 U/L). This rise in levels inversely correlated with the mean arterial pressure. Plasma levels of PGD₂ in severely reacting patients did not differ significantly from those in patients with a mild or no reaction. In conclusion, only 28% of patients with a history of Hymenoptera anaphylaxis developed an anaphylactic reaction after an in-hospital challenge. The clinical severity of these reactions correlated with the level of systemically released, preformed histamine and tryptase, but not of PGD₂. (J ALLERGY CLIN IMMUNOL 1992;90:110-8.)

Key words: Anaphylaxis, immediate hypersensitivity, Hymenoptera venom, insect sting, mast cell, tryptase, histamine, prostaglandin D₂

The prevalence of anaphylactic reactions to Hymenoptera stings has been estimated to vary from 0.02% to 4%.¹⁻³ The pathophysiologic mechanism of these reactions, although they have been studied for nearly 90 years, has not yet been fully elucidated.^{2,4-6} In the past decade, research has focused on

Abbreviations used

PGD₂: Prostaglandin D₂
YJ: Yellow jacket
MAP: Mean arterial pressure
CV: Coefficient of variation

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the role of mast cells and basophils,⁶⁻¹⁰ which may be activated both by IgE-dependent, as well as by non-IgE-dependent mechanisms.¹¹⁻¹⁸ In anaphylactic reactions not concerning insect stings but, for instance, various drugs or Röntgen contrast media, it has already been demonstrated that the clinical severity of these reactions correlates with the extent of mast cell activation.^{6-10, 19-26} In these latter conditions, it is assumed that most of the symptoms are actually caused by mediators from activated mast cells and/or basophils. Schwartz et al.²² demonstrated increased plasma levels of histamine and tryptase in three patients after

a bee-sting challenge. Their limited number of patients, as well as the inconsistent rise in tryptase that they observed, preclude definite conclusions with regard to the exact role of mast cell activation in insect-sting anaphylactic reactions.

In The Netherlands, as well in some other European countries, it is a generally accepted practice to provoke patients with insect stings under strictly controlled intensive care conditions to select those individuals who may benefit from insect-venom desensitization. This selection criterion has been applied because several studies have demonstrated^{13, 15-18} the likelihood of unnecessarily treated and unnecessarily nontreated patients when the classic selection criteria of specific IgE in plasma or skin tests were applied. This approach allowed us to investigate the role of mediators during the development of insect-sting anaphylaxis. We prospectively studied the release of tryptase (mast cell-specific, preformed), PGD₂ (mast cell-specific, newly generated), and histamine (mast cell- and basophil-derived, preformed) in a group of 138 patients with a history of an anaphylactic reaction to a YJ or a honeybee sting, as well as in eight volunteers. Serial blood samples were obtained, both before and after an in-hospital insect-sting challenge. Our results demonstrate that the clinical severity of the acute symptoms of insect-sting anaphylaxis is related to the degree of systemically released, preformed, mast cell mediators.

MATERIAL AND METHODS

Patients

One hundred thirty-eight patients with a history of an anaphylactic reaction to either a YJ or a honeybee sting were subjected to a provocation test with the insect concerned. Careful insect determination occurred by the patient's identification of the doctor's description of the two predominant Hymenoptera in The Netherlands and, if this were necessary, supplemented by skin tests. Eight volunteers were also challenged, including five who had been stung by an insect of the same species in the past without experiencing anaphylactic symptoms. All subjects were in good health, none of the subjects used β -blockers, antihistamines, mast cell-stabilizing drugs or other medication, was pregnant, or had a cardiac disorder. Electrocardiogram, chest x-ray, and results of routine blood examination were normal in all of the subjects. The patients were first checked in the outpatient clinic, in which extensive oral and written information on the study protocol, "Anaphylactic Reactions after Insect-sting Challenges," was administered. After informed consent, the patients were entered into the study protocol, which had been approved by the Lichtenberg Hospital Medical Ethical Review Board.

Study protocol

The provocation procedure with living insects in the intensive care unit has been described previously.^{13, 18} Before the sting challenge, all subjects were placed on a constant

heart-rate monitoring device, and two intravenous lines were inserted. A YJ of the species *Vespula germanica* or a honeybee of the species *Apis mellifera* was induced to sting the lower left arm. The subject's blood pressure was recorded automatically every 1 minute in unstable, and every 5 minutes in stable conditions. MAP was calculated as two-thirds diastolic pressure plus one-third systolic pressure.

The severity of the anaphylactic reaction was graded according to the method of Mueller²⁹ in which the higher grades can be accompanied by symptoms of lower-grade reactions: 0, no systemic reaction; I, skin symptoms (generalized urticaria, itching, or erythema) or anxiety; II, gastrointestinal symptoms (generalized edema, stomach or chest pain, nausea, or vomiting); III, respiratory symptoms (shortness of breath, difficulty in swallowing, hoarseness, or stridor); and IV, cardiovascular symptoms (cyanosis, hypotension, collapse, arrhythmias, or angina pectoris).

In the text, we will refer to Mueller grade 0 as no reaction, Mueller grade I as mild, Mueller grades II and III as intermediate, and Mueller grade IV as severe reactions. The four patients with intermediate reactions to the insect-sting challenge were excluded from mediator analysis because the patients with these reactions were too few to permit statistical analysis.

An antihistaminic drug (clemastine, 1 mg/ml intravenously; maximum of 6 ml) was administered in all patients with intermediate or severe reactions, and in one of the 18 patients with mild reactions because of severe itching. Severe reactors also received fluid replacement (Haemaccel, intravenously; Behringwerke AG, Wardorf, Germany; maximum of 2.5 L). Epinephrine (0.1 mg/ml, intravenously; maximum of 5 ml) was administered in three of the 17 patients with a persistent severe reaction. Of these 17 patients, 15 recovered completely within 4 hours after the sting. For safety, they were kept overnight in the intensive care unit, but no later reactions occurred. Two patients were still not completely well 4 hours after the challenge but required no additional therapy. All 138 patients, including the 17 with a severe reaction, left the hospital in a good clinical condition. Only the patients with Mueller grade III or IV symptoms at challenge subsequently started receiving venom immunotherapy. The patients with Mueller grade II, I, or 0, did not receive additional therapy. When Mueller grade I- or II-reacting patients were uncertain about this regimen, they were offered either a rechallenge the following year, the use of an antihistaminic drug to be taken after a future sting, or venom immunotherapy. (None of these patients chose this last option.)

Blood sampling

Blood was sampled before insect-sting challenge (pre) and at 1, 5, 15, and 60 minutes after the moment that the patient indicated that the reaction had started. In subjects who did not experience an anaphylactic reaction, blood sampling was started at 15 minutes after the insect sting. The procedure of blood sampling has been previously described.¹⁸ Immediately before the provocation test, blood was collected from all 138 patients as well as from the eight volunteers. Serial samples after provocation, however, were obtained from 69 subjects only: from the eight volunteers, from all 39 patients with anaphylactic reactions after prov-

ocation, and from the first 22 of the 99 patients with no reactions. As the postchallenge data of the remaining 77 nonreacting patients were not expected to provide additional information, samples from these patients were not collected after the challenge. The samples were put on ice immediately after collection and were centrifuged and deep frozen at -70°C within 15 minutes after collection. All five samples of each individual patient were tested in a single batch.

Assays

Specific serum IgE and IgG4, as well as skin thresholds against the insect venom concerned, were determined as described previously.¹³ Briefly, the RAST classes related to the percentage of bound radioactive-labeled anti-IgE (2% to 5%, class 1; 6% to 10%, class 2; 11% to 20%, class 3; 21% and more, class 4). Skin thresholds were determined with increasing concentrations of 0.1 ml solutions with purified insect venom. A skin threshold was considered to be reached when an induration ≥ 5 mm and an erythema of ≥ 10 mm or an induration plus erythema of ≥ 20 mm was obtained.

Histamine was measured with an RIA (Amac, Westbrook, Maine). The assay was performed according to the manufacturer's instructions. The intra-assay CV was 25% at a level of 1.4 nmol/L and 10.3% at a level of 116 nmol/L. The interassay CV was 26% at a level of 1.9 nmol/L and 14.1% at 32.1 nmol/L. Cross-reactivity of the histamine antibody to methylhistamine (Pharmacia, Uppsala, Sweden) was $<0.007\%$. The detection limit in the assay was 0.5 nmol/L.

Tryptase was measured with a solid-phase sandwich RIA (Pharmacia) was performed according to the manufacturer's instructions. The intra-assay CV was 10.8% at a level of 1.0 U/L and 2.8% at a level of 10.0 U/L. The interassay CV was 15.8% at a level of 2.0 U/L and 3.6% at 35.0 U/L. Sera with a tryptase level 20.0 U/L were retested at dilutions prepared in diluent supplied by the manufacturer. The detection limit in the assay was 0.2 U/L.

PGD₂ was measured in plasma with an RIA (Amersham, Den Bosch, The Netherlands) according to the manufacturer's instructions. Plasma was first deproteinized by adding 800 μL of absolute ethanol to 100 μL of plasma. After centrifugation for 5 minutes at 4000g, the supernatant was dried with nitrogen, and the sample was reconstituted in distilled water. The detection limit of the assay was 20 ng/L, as assessed by adding purified PGD₂ at various concentrations to human plasma.

Statistical analysis

Except for the MAP, none of the parameters appeared to be normally distributed. In these cases, these data were log transformed. Paired Student's *t* tests were used to compare data between two groups. To assess relationships, data of all patients with anaphylactic reactions were taken together. Values were corrected for baseline values of the same patients, as well as for changes observed in the volunteers at the corresponding time points. Correlations were calculated with linear regression analysis. A *p* value of <0.05 was considered to represent a significant difference (Student's *t* test) or correlation (linear regression).

Results

Clinical course

Characteristics of the 69 subjects who were challenged and in whom the entire blood-sampling procedure was completed are presented in Table I. In agreement with previous studies,^{13, 18} no relation between the subject characteristics, the insect species, and the outcome of the reaction to insect-sting challenge was observed ($p > 0.05$). In the patients who had experienced a previous anaphylactic reaction to an insect sting, mean specific IgE and IgG4, as well as skin thresholds to venom, were higher than that in the volunteers ($p > 0.05$).

In the 39 patients who demonstrated an anaphylactic reaction after insect-sting challenge, the anaphylactic symptoms began 1 to 40 minutes (median, 10 minutes) after the sting. No significant relation was observed between this time interval and the clinical severity of the reaction. The anaphylactic symptoms of these patients are described in Table II. MAP decreased by <15 mm Hg or remained unchanged in the volunteers, as well as in the patients with intermediate, mild, or no reactions. In patients with a severe reaction on insect-sting challenge, however, MAP decreased to 65 ± 17 mm Hg ($63\% \pm 20\%$ of initial values) 5 minutes after the first symptoms of anaphylaxis.

Plasma levels of mast cell mediators

Histamine levels before provocation were 2 ± 1 nmol/L (range, 1 to 7; median, 1) in the three groups of patients with previous anaphylactic reactions (Fig. 1.). In the volunteers, initial histamine levels were comparable (1 ± 1 nmol/L; range, 1 to 2; median, 1). One of the volunteers, but none of the nonreacting patients, demonstrated a significant change in histamine after insect-sting challenge (up to 42 nmol/L at 1 minute). Mean histamine levels in the group of patients with a mild reaction were significantly higher (21 ± 39 nmol/L at 1 minute; range, 1 to 140; median, 4; and 11 ± 21 nmol/L at 60 minutes; range, 1 to 95; median, 5) than that in the volunteers and nonreacting patients ($p < 0.001$). Five of these mildly reacting patients, however, did not demonstrate a significant increase in histamine. All but one of the patients with a severe reaction demonstrated a considerable rise in histamine. Mean histamine levels were 1191 ± 3190 nmol/L at 1 minute (range, 2 to 13130; median, 122), 1275 ± 2994 nmol/L at 15 minutes (range, 3 to 12800; median, 117), and 22 ± 29 nmol/L at 60 minutes (range, 1 to 81; median, 10). Both the rise in histamine levels in the severely reacting patients as well as the differences with histamine levels at corresponding time points in the mildly reacting patients were highly significant ($p < 0.0001$ for all values). Individual histamine

TABLE I. Characteristics of 69 subjects who were challenged with a living insect and in whom the entire blood sampling procedure was completed

Group*	Volunteers	Mueller grade after sting challenge			
		0	I	II/III	IV
N	8	22	18	4	17
Insect†‡	3/5	9/13	4/14	2/2	5/12
Sex (M/F)‡	3/5	14/8	10/8	3/1	9/8
Age (yr)‡§	39	44	42	41	51
Previous reaction (I/II/III/IV)*‡	—	3/4/2/13	3/3/2/10	0/0/2/2	0/0/0/17
Spec IgG4 (U/L)‡§	0	3	4	15	3
Spec IgE (N)‡					
RAST					
0	7	6	3	—	3
1	—	2	3	—	4
2	1	6	5	1	5
3	—	5	6	3	3
4	—	3	1	—	2
Skin threshold (N)‡ (μg/ml)					
0.0001	—	1	2	—	2
0.001	—	4	1	4	2
0.01	1	9	12	—	6
0.1	—	4	2	—	5
1.0	—	2	1	—	1
10.0	1	2	—	—	1
100.0	6	—	—	—	—

Spec, Specific.

*Mueller grades of insect-sting anaphylactic reactions²⁹ as defined in the MATERIAL AND METHODS section.

†Honeybee/YJ.

‡No statistically significant differences between groups in Mueller grades 0, I, II/III, and IV ($p > 0.05$).

§Median values.

levels at 15 minutes after the first symptoms are illustrated in Fig. 2, A.

Tryptase levels before provocation did not vary among the four groups of subjects (0.3 ± 0.2 U/L; range, 0.2 to 2.0; median, 0.2; $p > 0.1$) (Fig. 1, B). Significant changes in tryptase levels were not observed in any of the volunteers or in any of the patients with no reaction after the insect sting ($p > 0.1$). One of the patients with a mild reaction demonstrated a significant rise in tryptase (to 11.3 U/L) at 5 minutes, but nine of these patients demonstrated no significant increase in tryptase. In this group of mildly reacting patients, however, tryptase levels were significantly higher than levels of nonreacting patients (0.4 ± 0.4 U/L at 15 minutes; range, 0.2 to 8.3; median, 0.3; $p < 0.05$; and 0.5 ± 0.5 U/L at 60 minutes; range, 0.2 to 7.0; median, 0.3; $p < 0.01$). In all but one of the patients with a severe reaction (the same patient who demonstrated no rise in histamine level), tryptase levels demonstrated a considerable rise. Mean levels were 123 ± 302 U/L at 1 minute (range, 0.2 to 1020; median, 6.8), 406 ± 1062 U/L at 15 minutes (range,

1.8 to 4400; median, 17), and 134 ± 369 U/L at 60 minutes (range, 0.9 to 1558; median, 6.6). This rise in tryptase levels in severely reacting patients as well as the differences with tryptase levels at corresponding time points in mildly reacting patients were both highly significant ($p < 0.0001$ for all values). Individual tryptase levels at 15 minutes after the first symptoms are illustrated in Fig. 2, B.

PGD₂ levels before provocation did not vary among the four groups of subjects (320 ± 223 ng/L; range, 90 to 1130; median, 340; $p > 0.01$; Fig. 1, C). In patients with a severe reaction after sting challenge, PGD₂ levels rose to 592 ± 489 ng/L at 1 minute after the first symptoms (range, 50 to 1800; median, 460; $p > 0.05$) and to 515 ± 354 ng/L at 60 minutes (range, 110 to 1100; median, 460; $p > 0.05$). Both this rise in PGD₂ levels in the group of severely reacting patients (only significant in three individuals of the 17 severely reacting patients), as well as the differences with the levels in the other three groups of subjects, were statistically not significant ($p > 0.05$).

TABLE II. Symptoms of anaphylaxis in 39 patients after an in-hospital insect-sting challenge

Symptoms	Mueller grade after sting challenge			
	I (N = 18)	II (N = 1)	III (N = 3)	IV (N = 17)
Generalized urticaria*	9	1	2	4
Generalized itching	13	—	3	11
Generalized erythema	11	—	3	11
Anxiety	9	—	2	14
Generalized edema		1	—	6
Stomach pain		1	1	7
Chest pain		—	—	4
Nausea		1	1	8
Vomiting		1	—	3
Shortness of breath			3	7
Difficulty in swallowing			1	6
Hoarseness			2	4
Stridor			2	3
Cyanosis				3
Hypotension†				17
Collapse				3
Cardiac arrhythmias				6
Angina pectoris				1

*One or more urticaria, not directly adjacent to the location of the insect sting.

†The maximum mean differences between MAP before and after the sting challenge for the volunteers, the nonreacting patients, the mildly reacting patients (Mueller grade I), and the severely reacting patients (Mueller grade IV) were -7.4 , -4.7 , -2.4 , and -39.8 mm Hg, respectively.

Relation of clinical symptoms and mast cell mediators

The change in MAP of all 39 patients with an anaphylactic reaction caused by the insect-sting challenge (Mueller grades I, II, III, and IV) inversely correlated with the change in levels of histamine and tryptase at 5 minutes after the first symptoms of anaphylaxis (Fig. 3, A and B, respectively; correlation coefficients of 0.77 and 0.86, respectively). Similar correlations between changes in MAP and in histamine and tryptase levels were observed at the other time points after the sting challenge (not presented).

DISCUSSION

The clinical picture of an anaphylactic reaction to a Hymenoptera sting may vary from mild (skin symptoms only) to severe (cardiovascular symptoms).^{2, 6, 8, 29} Although the exact pathophysiologic mechanism of anaphylactic reactions to insect stings is not clear, it is generally assumed that products released by activated mast cells and/or basophils, are essential.^{6, 9, 19-27} No prospective study on the release of mediators from these cells during the development of insect-sting anaphylaxis has been performed, al-

though observations in individual patients have been previously published.²²

In the present prospective study, 138 patients with a history of a previous anaphylactic reaction to either a YJ or a honeybee sting were challenged with the insect concerned, according to a strictly controlled protocol. Of these 138 patients, 99 did not demonstrate a systemic reaction, whereas 39 (28%) experienced anaphylactic symptoms of varying severity. The prevalence of repeated anaphylactic reactions to a new insect sting has been reported to vary from 16% to 80%.^{1-3, 5, 13-18} This varying prevalence rate may result from the different nature of the studies (i.e., prospective and retrospective) and from differences in patient selection. In agreement with our previous prospective studies,^{13, 18} we observed an approximately 30% chance of a repeated reaction. We are unable to explain this phenomenon. No difference in either prevalence or severity of repeated anaphylactic reactions was observed between honeybee- or YJ-sensitive individuals.

The clinical severity of an insect-sting anaphylactic reaction was assessed in the world wide-accepted Mueller grades.²⁹ We observed a relation between

these grades of severity and the increase in levels of histamine and tryptase, but not of PGD_2 (Fig. 1). Histamine and PGD_2 have been demonstrated to exert many effects, such as an increase in intracellular cyclic adenosine monophosphate and smooth muscle contraction, resulting in diverse symptoms, such as pruritus, erythema, edema, gastric secretion, mucus production, vasodilation, and increased vascular permeability.^{1, 10, 19, 21} The role of tryptase in mediating hypotension is not clear, but it may enhance the effect of histamine on the blood vessels.²⁴⁻²⁸ To investigate the role of preformed versus newly generated and mast cell versus basophil mediators, we studied the release of histamine, tryptase, and PGD_2 . The present study with the four groups of subjects (Fig. 1) strongly suggests that the preformed mediators, histamine and tryptase, are directly involved in the pathophysiology of the acute symptoms of Hymenoptera anaphylaxis. In addition, it is demonstrated that the extent of hypotension correlates with the level of circulating histamine and tryptase (Fig. 3). The severity of the clinical reaction in the individual patients did not completely match, however, with the rise in histamine or tryptase levels (Fig. 2). Five of the mildly reacting patients demonstrated no rise in histamine, and nine demonstrated no rise in tryptase levels at one or more of the time points after the challenge. Of the severely reacting patients, only one (who will be discussed later) demonstrated no rise in histamine. Plasma levels of the newly generated mediator PGD_2 did not change in the different groups of patients (although three of the 17 patients with a severe reaction demonstrated a moderate rise in PGD_2). These data do not suggest an important role of PGD_2 in the pathophysiology of acute insect-sting anaphylaxis. However, to appreciate the role of PGD_2 and related mediators more precisely, additional studies, including analysis of urinary levels, are needed.

One patient with a severe reaction demonstrated insignificant increases in two of the three circulating mast cell mediators studied (histamine rising from 1.5 nmol/L to a maximum of 3.0, PGD_2 from 150 ng/L to a maximum of 280, but tryptase from 0.2 U/L to a maximum of 3.5). The patient demonstrated a clinical reaction that was different from that of the other severely reacting patients; she lacked urticaria, erythema, edema, itching, and dyspnea but suffered from angina pectoris with signs of left ventricular ischemia on the electrocardiogram and a subsequent fall in MAP from 100 to 60 mm Hg, as well as a bradycardia to 40 beats/min. Within 2 hours after the sting, she had completely recovered, and the electrocardiogram had returned to normal. A coronary angiogram made the

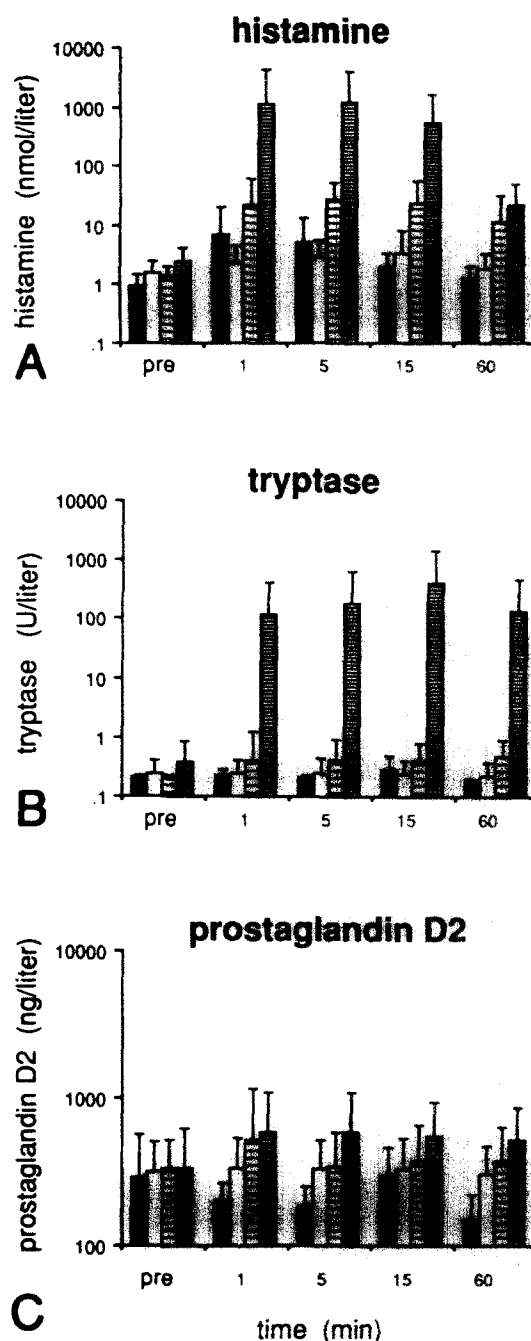


FIG. 1. Plasma levels of mast cell mediators during insect-sting anaphylaxis. **A**, Histamine (nmol/L), **B**, tryptase (U/L), and **C**, PGD_2 (ng/L) are depicted on a logarithmized scale as mean $\pm$ SD both before and after an in-hospital challenge with a YJ or honeybee sting. Four groups of patients are illustrated; eight volunteers (■); 22 patients who demonstrated no reaction (Mueller grade 0) (□); 18 patients with a mild reaction (Mueller grade I) (▨); and 17 patients with a severe reaction (Mueller grade IV) (▩); pre, levels before provocation; time points, time in minutes after patient had indicated onset of a reaction; 1-minute point in nonreacting patients and in volunteers, arbitrarily chosen as 15 minutes after insect sting.

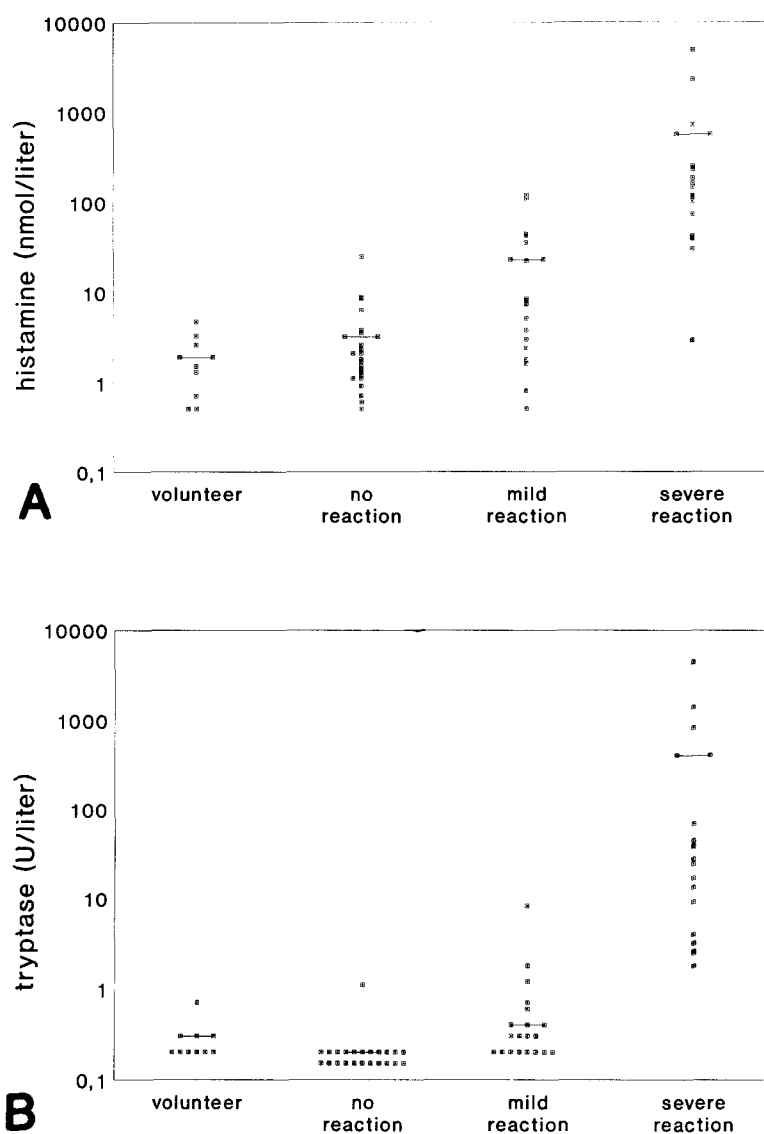


FIG. 2. Histamine and tryptase levels in individual patients and volunteers. **A**, Histamine (nmol/L) and **B**, tryptase (U/L) levels, as well as mean levels (*bars*), are illustrated on a logarithmized scale at 15 minutes after first symptoms of anaphylaxis (in nonreacting subjects, 29 minutes after insect sting). Four groups of individuals depicted are described in Fig. 1.

day after revealed no obstruction in any of the coronary arteries. Prechallenge RAST to YJ was 3, and her skin threshold to YJ venom was 0.001 $\mu\text{g}/\text{ml}$. She therefore had probably suffered from myocardial mast cell activation with subsequent localized histamine release and coronary artery spasm, as has been described previously.^{2, 6, 8, 9, 19, 20, 30}

Basophils contain histamine but no tryptase or PGD_2 .^{7, 8, 11, 20, 23-27} Although some activation of basophils cannot be ruled out, our results indicate that the release of preformed mast cell rather than basophil products is the central event in acute (severe) insect-sting anaphylaxis. It is not clear which type of mast

cells is activated and whether histamine and tryptase alone account for all anaphylactic symptoms. The role of other preformed mast cell and possibly basophil products,^{9, 10, 30} and other mediators such as complement factors,^{18, 31, 32} endothelial-derived factors,³³ cytokines,³⁹ coagulation, and/or fibronolytic cascade products³⁵ that may also be involved,^{9, 10, 18, 30-35} remains to be established.

The release of histamine and tryptase, which were started within the first minute after the onset of the first symptoms, was frequently indicated by the patient as "something was happening to him/her," without any objective clinical symptoms. In the three patients

described by Schwartz et al.,²² the time interval between the bee-sting challenge and the rise in plasma tryptase varied, whereas a rise in histamine levels was observed within 15 minutes from the sting. In contrast with our study, Schwartz et al.²² used a time scale with the insect sting as a starting point, whereas we used a different scale, with the onset of the patient's first symptoms as a starting point. We chose this scale to correct for the varying time intervals that elapse between the insect sting and the first symptoms (1 to 40 minutes; median, 10 minutes) and to facilitate analysis of these data. Our data are therefore not necessarily in contrast with those of Schwartz et al.²² The reason for this varying time interval between the sting and the clinical symptoms is not clear. It may be related to whether or not the venom is injected intravascularly or to a variable activation time of mast cells in individual patients. It is striking, however, that in some patients a very short time interval (e.g., 1 minute) was observed between the sting on the one hand and the appearance of systemically released, preformed, mast cell mediators and the clinical symptoms of anaphylaxis on the other hand.

Insect venom itself is known to contain a number of vasoactive substances, including histamine but not tryptase.²⁻⁶ We therefore measured the histamine levels in bee venom and YJ venom used for desensitization procedures (Pharmacia). Levels of histamine were 0.4 nmol/L and 2.1 nmol/L in honeybee and YJ venom, respectively. This observation in purified venom indicates that the rise in plasma histamine was not due to injected histamine. Moreover, plasma levels of histamine in our study did not change in either the volunteers or in the nonreacting patients, although each of these patients had a histamine-mediated local reaction at the site of the insect sting.

Our data provide firm evidence that mast cell activation does occur in insect-sting anaphylaxis, but these data do not provide insight into the mechanism of this activation. In agreement with previous studies,¹²⁻¹⁸ we observed no clear correlation between prechallenge insect venom-specific skin thresholds, serum IgE or IgG4 levels, and the clinical reaction to a new insect sting (Table I). Anaphylaxis to insect stings has even been described in patients without detectable insect-specific IgE and in patients who developed allergic reactions on first insect-sting exposure.^{12, 17} In our study, two of 17 patients who demonstrated a renewed severe (cardiovascular) reaction had negative skin thresholds ($>0.1 \mu\text{g/ml}$) to the insect concerned, and seven of these 17 patients had RAST classes 0 or 1 of specific IgE against the insect concerned. Thus, a prediction of future anaphylactic reactions to either a YJ or a honeybee sting cannot be derived from skin

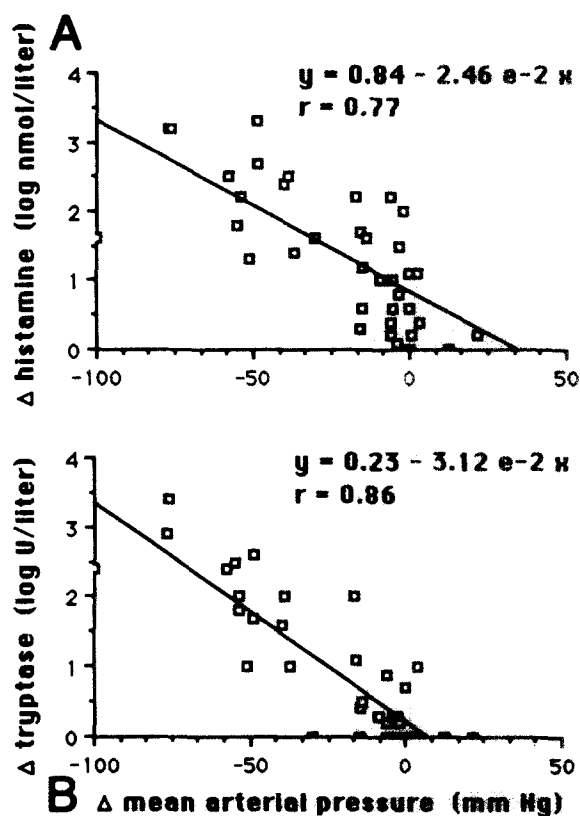


FIG. 3. Relation between changes in plasma histamine and tryptase and changes in MAP of 39 patients with anaphylactic symptoms after insect-sting challenge. **A**, Histamine and **B**, Tryptase levels at 5 minutes after onset of anaphylaxis were corrected both for individual baseline values and for changes in volunteers at corresponding time points and were log transformed. Values for MAP, corrected in same way, were not log transformed.

thresholds to purified venom of the insect concerned or from levels of insect-specific IgE or IgG4. Possible explanations for this phenomenon have been described previously.¹³

In conclusion, in this prospective study, we have demonstrated that 39 (28%) of a total of 138 patients with a history of an anaphylactic reaction to a honeybee or a YJ sting developed renewed anaphylactic symptoms after an in-hospital sting with an insect of the same species. The clinical severity of these acute reactions correlated with the degree of systemically released preformed products (histamine and tryptase) of activated mast cells. Further studies should identify the specific route of mast cell activation in this condition.

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