

In vivo histamine release during the first minutes after deliberate sting challenges correlates with the severity of allergic symptoms

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In this study, deliberate sting challenge was investigated as a method for estimating the severity of anaphylactic reactions in bee venom-sensitized subjects. Twenty-one patients with previous anaphylactic reactions to field bee sting were subjected to a deliberate sting challenge ($n = 32$). To document anaphylactic reactions, plasma histamine levels were measured before, and then 1 and 2 min after, bee sting challenge. Eleven patients were re-challenged after 3–5 weeks. On 18 occasions, sting challenges caused no systemic reactions, in seven cases reactions were mild, in five moderate and in two severe. In all children showing systemic reactions, significant increases of plasma histamine were measured after 2 min. The results correlated significantly with clinical scores but not with skin prick test or with specific immunoglobulin E (IgE) and immunoglobulin G (IgG) antibodies against bee venom. In patients developing local reactions only, no increase of plasma histamine was detected. The relative amount of released histamine correlated significantly with the severity of clinical symptoms. Significant histamine release occurred during the first 2 min after sting challenge in children with subsequent systemic reactions and the severity of these subsequent anaphylactic reactions correlated with plasma histamine concentrations. The measurement of plasma histamine levels in the first minutes after challenge test may therefore be used as an objective marker of a potential systemic reaction.

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Bee venom allergy has a potentially fatal course. Great efforts have been made to classify the grade of sensitization. To date, assessment of specific immunoglobulin E (IgE) and immunoglobulin G (IgG) antibodies (1), as well as skin prick or intradermal testing (2), are well-accepted diagnostic methods. However, it is agreed that these tests cannot be used to predict the severity of future reactions to bee stings. Therefore, provocation tests with live honey bees have been established (3) to ascertain the clinical severity of allergic

reactions to bee venom. Although single diagnostic sting challenges give additional information, there is increasing concern about the possible booster effect. From the natural history of bee venom allergy, we know that one sting followed by another 2–4 weeks later will result in the highest incidence of systemic reactions. In a previous study we attempted to mimic this naturally occurring event by subjecting test subjects to sequential sting challenges in order to detect the patients at highest risk (4).

Table 1. Grade of systemic reactions according to Mueller after field sting and deliberate sting challenge

Grade of reaction	Signs and symptoms	IgE	IgG	SPT (mm)	Field sting (n)	Deliberate sting (n)
Grade 0	–	2.0 (2.0–3.0)	200 (50.0–400)	2.0 (1.25–3)	–	18
Grade I	Generalized urticaria, itching, malaise, anxiety	3.0 (2.0–3.0)	125 (25.0–400)	2.0 (2–3)	5	7
Grade II	Symptoms of grade I plus two of the following: angio-edema, dizziness, constriction of chest, abdominal pain, nausea, vomiting, diarrhea	2.0 (1.5–2.5)	25 (12.5–62.5)	2 (2–2)	5	5
Grade III	Symptoms of grade II plus two of the following: dyspnea, dysphagia, hoarseness or thickened speech, confusion, feeling of impending disaster	2.5 (2.0–3.0)	25 (0–50)	3.5 (2–5)	11	2
Grade IV	Symptoms of grade III plus two of the following: cyanosis, fall in blood pressure, collapse, incontinence, unconsciousness	–	–	–	–	–

IgE results are presented as RAST classes.

IgG results are reciprocally expressed as the highest serum dilution giving a positive reaction.

Results are presented as median (quartile 1–quartile 3).

SPT, skin prick test.

For histamine levels in the circulation to be used an indicator of basophil and mast cell degranulation, the time course of the appearance of raised levels of plasma histamine needs further investigation. Plasma histamine levels have been shown to be elevated 5–10 min after bee sting challenge – associated with systemic anaphylactic symptoms – and to decline rapidly to baseline after 15–60 min (5, 6). Therefore, determination of plasma histamine or its metabolites was considered to be a measure of allergic reactions after allergen exposure (3). The assessment of plasma histamine, however, is complicated owing to its rapid clearance from the circulation (7). In addition, urinary N-methylhistamine increases in severe systemic reactions only (8).

The aim of this study was to examine if plasma histamine concentrations increase immediately after deliberate bee sting, and whether plasma histamine levels could be used as a parameter to determine the severity of subsequent systemic reactions at such an early stage of reaction. We therefore monitored plasma histamine levels during the first 2 min after bee sting to determine the occurrence of *in vivo* histamine release prior to histamine clearance or degradation from the circulation.

Subjects and methods

Patients

Twenty-one children with a history of allergic reactions after honey bee sting (six female, 15 male; age (mean $\pm$ SD) 9.7 ± 3.38 yr) were enrolled in this study. All individuals were questioned carefully to determine their reactions to previous field stings, according to the criteria of Mueller (9). Patients' and parental agreement was obtained in all cases, and the study design was approved by the Ethical Committee of the University Hospital in Freiburg.

Sting challenge protocol

An intravenous cannula was inserted into the cubital vein prior to sting challenge, and equipment and drugs for resuscitation were immediately available. For each challenge test a newly captured honey bee obtained by a bee keeper was used for sting challenge on the forearm of the patient. Eleven out of 21 patients were re-challenged after 3 to 5 weeks in order to obtain additional information about a possible booster effect, as described above. The severity of systemic reactions to bee stings was classified by using the Mueller score (Table 1).

Skin tests

For skin prick tests (SPT), commercially available bee venoms (Pharmacia, Uppsala, Sweden) were used at concentrations of 100 µg/ml. Mean wheal diameters were recorded 15 min after performing SPTs.

Serum measurements of IgE and IgG antibody levels

Serum IgE concentrations were determined by using a radioallergosorbent test (Phadebas IgE RAST, Pharmacia, Uppsala, Sweden) following the manufacturer's instructions (10). Serum concentrations of IgG were measured by using sensitive and specific enzyme-linked immunosorbent assays (ELISAs) (11), as previously described. The results were reciprocally expressed as the highest serum dilution giving a positive reaction. All of these tests were performed before sting challenge.

Serum measurements of plasma histamine concentrations

Blood was drawn in all cases from the contralateral arm on which sting challenge was performed, immediately before starting sting challenge and 1 and 2 min after the sting. A solution (25 µl) containing 76 mg/ml ethylene glycol-*bis*-(beta-amino-ethyl ether) N,N,N',N'-tetra-acetic-acid and 48 mg/ml glutathione (Sigma Chemicals, Munich, Germany) was immediately added per ml of whole blood. The above mentioned solution – which had no effect on histamine plasma concentrations compared with histamine levels in EDTA plasma – was used to inhibit degradation of catecholamines, as previously reported (12). After centrifugation at $500 \times g$ for 10 min (at 4 °C) plasma was immediately frozen at –20 °C. Blood samples were collected in polypropylene syringes to avoid histamine absorption on the syringe wall, as observed for glass tubes. Histamine determinations in plasma were performed (in duplicate) by using a radioimmunoassay (Immunotech, Marseille, France). Assessment of histamine was performed by competition between acetylated histamine in the sample and iodinated histamine tracer for the binding to the antibody coated on tubes. Plasma samples from the same patients were tested within the same assay. This assay was shown to be sensitive (sensitivity of 0.02 pg/ml) and specific in previous studies (13, 14). The intra-assay variability was less than 5%.

Statistical analysis

Results are presented as median (quartile 1–quartile 3) unless indicated otherwise. Non-parametric tests were used in the comparative analysis. The Kruskal–Wallis analysis of variance procedure was applied first to the data of more than two groups. When this was significant, each pairing was examined by the Mann–Whitney *U*-test. Coefficients of correlation were obtained by means of the Kendall Tau B procedure. The level of significance was considered at the fifth percentile ($p < 0.05$).

Results

After deliberate bee sting ($n=32$) in 21 children (11 children were challenged a second time 3–5 weeks later), 18 sting challenges caused no systemic reactions, on seven occasions grade I systemic reactions were observed, in five grade II, and in two grade III (grading according to Mueller; Table 1). In the 14 challenges where symptoms of systemic anaphylaxis were observed, reactions occurred within 2–60 min (Table 1). Patients were monitored for symptoms every 5 min. No relationship was found between the time of occurrence of anaphylactic reactions and the severity of symptoms. Furthermore, no relationship was observed between the severity of anaphylaxis after field bee sting and the severity of reactions after sting challenge.

After field sting, five of 21 patients experienced grade I systemic reactions, five grade II systemic reactions, and the remaining 11 patients had grade III allergic reactions (Table 1). No correlation was seen between severity of reaction and prechallenge SPT results (range of mean wheal diameter: 0.5–5 mm), bee venom-specific IgE antibody levels (RAST class: 1–4) and specific IgG antibody levels (range: 0–1600). Moreover, discrepancies between anamnestic-evaluated severity of reactions to previous field stings and results obtained during sting challenges were observed (Table 1).

During all the systemic allergic reactions ($n=14$) a significant increase of plasma histamine concentrations were found 1 and 2 min after sting, compared to those with local reactions only (Fig. 1). No relationship was detected between the time of the start of systemic allergic reactions and the rise of plasma histamine. Measurement of the percentage of histamine liberated *in vivo* allowed a classification according to the scoring system used.

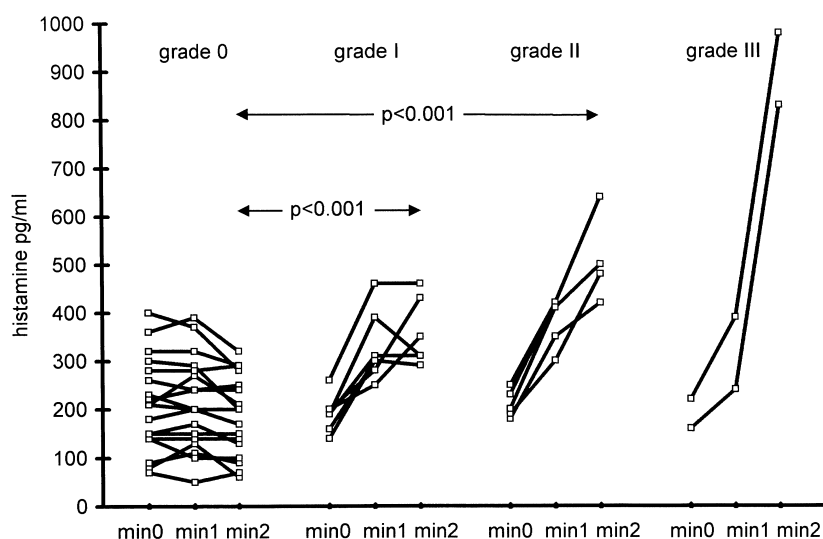


Fig. 1. Plasma histamine concentrations before sting, and 1 and 2 min after sting, in patients sub-divided on the intensity of their reactions after bee sting (Mueller score system, $n=32$). Histamine concentrations before bee sting: grade 0, 210 pg/ml (140–280); grade 1, 190 pg/ml (160–200); grade 2, 200 pg/ml (190–230); grade 3, 190 pg/ml (160–220). Histamine concentrations 1 min after bee sting: grade 0, 200 pg/ml (140–280); grade 1, 300 pg/ml (280–390); grade 2, 410 pg/ml (350–420); grade 3, 315 pg/ml (240–390). Histamine concentrations 2 min after bee sting: grade 0, 200 pg/ml (130–250); grade 1, 310 pg/ml (290–430); grade 2, 500 pg/ml (480–640); grade 3, 905 pg/ml (830–980).

Discussion

Until now, substantial increase of plasma histamine levels in children have been reported only during severe anaphylactic reactions (15) with a peak 5–10 min after challenge (5). Our findings demonstrated *in vivo* histamine release in children with systemic reactions to bee venom, after challenge, during the first 2 min after bee sting (measurements were taken at 1 and 2 min after challenge), indicating significant histamine liberation before symptoms of anaphylaxis are evident. The patients who subsequently suffered from more severe systemic reactions demonstrated higher histamine plasma concentrations than children with less serious reactions (Fig. 1). This may be caused by a more rapid histamine liberation in these patients. One may speculate that a more rapid increase of histamine levels could be related to an earlier start of systemic reactions. This, however, was not observed in our patients. Activation of a higher number of histamine-releasing cells as mast cells and basophil granulocytes might be an alternative explanation. In patients without systemic reactions no increase of plasma histamine concentrations occurred, even if they presented large local reactions. As previously demonstrated, localized release of histamine generally leads to little or no detectable increase in serum histamine levels. This appears to be caused by the fact that histamine is rapidly cleared from the circulation and across vascular beds (8). Nevertheless, at present no diagnostic tests are available to adequately predict the clinical outcome of future bee stings. Considerable health risks exist, and in one patient subsequent intensive care treatment, including artificial ventilation, was required (16). However,

there are controversies among allergologists, for ethical reasons, about indications for performing sting challenges (17, 18). In Freiburg, every patient with a history of bee sting allergy is given a provocation test with the sting of a living insect before specific immunotherapy (SIT), treating only those who react to this provocation test. This is associated with high expenses for the patients and their medical insurance because this sting exposure needs to be performed where intensive care facilities are available.

Additionally, a threatening protracted anaphylactic reaction (15) can be predicted, as plasma histamine levels after sting challenge correlated significantly with subsequent anaphylactic symptoms. Therefore, levels of plasma histamine 2 min after sting might be used as a parameter for subsequent anaphylactic reactions.

In conclusion, our findings demonstrate substantial *in vivo* histamine release during the first 2 min after bee sting challenges and before allergic reactions occur. The clinical severity of anaphylactic symptoms after insect sting was significantly related to the degree of *in vivo*-released histamine during the first 2 min after sting and may therefore be used as an objective marker of the severity of anaphylactic reactions during sting challenge.

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