Laboratory markers of mast cell and basophil activation in monitoring rush immunotherapy in bee venom-allergic children

Aim: To evaluate markers of mast cell and basophil activation in children undergoing the initial phase of honeybee venom immunotherapy (VIT). **Patients & methods:** Five children (four boys and one girl) aged 9.5–18 years with severe systemic bee sting reactions and confirmed IgE-mediated allergy were enrolled. Plasma and urine concentrations of 9α,11β-PGF2 and serum tryptase levels were measured at four time points and peripheral blood basophil count and CD63 expression were measured at three time points in the course of VIT, including 5-day rush initial immunotherapy (cumulative dose of 223 µg of bee venom allergen) and two subsequent maintenance doses of 100 µg. **Results:** In the first 40 days of VIT, there was a decrease in mean plasma levels of 9α,11β-PGF2 (from 41.5 to 27.9 pg/ml; $p < 0.05$), accompanied by an increase in baseline basophil activation (from 2 to 15%; $p < 0.05$). The median serum tryptase levels increased from 3.45 to 4.40 ng/ml during rush phase and subsequently returned to initial values (statistically not significant). In four patients, the basophil activation test in response to bee venom allergens remained positive throughout the study. The fifth patient was basophil activation test-negative at all three measurements, and a *post hoc* analysis revealed clinical peculiarities that are discussed in the paper. **Conclusions:** Our preliminary results indicate that plasma levels of 9α,11β-PGF2 decrease while numbers of activated basophils increase during the initial phase of bee venom rush immunotherapy in children.

KEYWORDS: allergy ▪ basophil activation ▪ children ▪ honeybee venom ▪ immunotherapy ▪ mast-cell activation

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Mediators of mast cells and basophils are considered to be interesting as potential markers of anaphylaxis that could be useful in both diagnosis of venom allergy and monitoring venom immunotherapy (VIT). In adults, elevated baseline serum tryptase was recently indicated as a potential predictor of severe post-sting reactions, as well as a marker suitable for monitoring the safety of VIT [1,2]. Urinary 9α,11β-PGF2, a metabolite related to mast cell activation, was previously suggested by Ono *et al.* as a sensitive marker of anaphylactic reaction [3]. Bochenek *et al.* suggested that in asthmatic adults, plasma 9α,11β-PGF2 may be more sensitive than urine measurements in detecting mast cell activation during bronchial challenge with allergens [4]. Flow-assisted monitoring of basophil activation proved helpful in detecting hymenoptera venom allergy – even in IgE-negative cases and in predicting the side effects during VIT [5]. The aim of the present study was to analyze levels of mast cell activation markers (tryptase and 9α,11β-PGF2) and basophil activation markers (peripheral blood basophil count and CD63 expression) in honeybee venom-allergic children undergoing initial rush VIT and the subsequent 1-month maintenance immunotherapy.

Patients & methods

Five patients: four boys and one girl aged from 9.5 to 18 years with history of systemic reactions after honeybee stings participated in the study. The patients were qualified for honeybee venom (BV)-specific immunotherapy based on the following criteria: history of severe systemic reactions (Mueller's grade III or IV) after honeybee stings; confirmed IgE-mediated hypersensitivity to BV (positive skin tests or specific IgE); and lack of skin symptoms of mastocytosis. Informed consent was obtained from the children's guardians following recommendations of the local bioethics body. Clinical characteristics of the patients along with results of allergy tests are shown in Table 1. The study covered 40 initial days of immunotherapy, including the 5-day rush build-up phase with cumulative dose of 223 µg BV allergen (Pharmalgen, ALK Abello, Denmark), and two consecutive maintenance doses of each 100 µg BV (approximately 2 and 4 weeks after rush VIT). Plasma and urine concentration of 9α,11β-PGF2 and serum levels of tryptase were monitored to assess mast cell activation, while peripheral blood basophil count and expression of CD63 on basophils served as basophil activation markers. All medications were withheld 24 h prior to the measurements.

Table 1. Characteristics of the study participants.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender; age	M; 14 years	F; 18 years	M; 15.5 years	M; 15 years	M; 9.5 years
Mueller's grade	4	3	4	3	3
Number of bee stings before SSR	4	1	1	4	0
Total IgE IU/l	133	97	374	55	27
Serum sIgE to honeybee venom (class)	47.7 IU/l (CAP 4)	67.0 IU/l (CAP 5)	100.0 IU/l (CAP 6)	10.0 IU/l (CAP 3)	11.3 IU/l (CAP 3)
sIgE/total IgE	0.359	0.691	0.267	0.183	0.417
SPT results to bee venom at 100 µg/ml	Negative	Negative	Negative	Negative	Negative
IDT with bee venom (titration point)	Positive at 0.01 µg/ml	Positive at 0.01 µg/ml	Positive at 0.01 µg/ml	Positive at 0.01 µg/ml	Negative at up to 1 µg/ml

F: Female; IDT: Intradermal test; M: Male; sIgE: Specific IgE; SPT: Skin prick test; SSR: Severe systemic reaction.

Blood samples for the determination of mast cell activation markers were taken 1 h after injection of allergy vaccines, corresponding with the knowledge that during bee sting-induced anaphylaxis, the blood levels of tryptase peak within 1 h and then decrease with a half-life of approximately 2 h [6]. Urine samples for determining 9α,11β-PGF2 were collected upon first spontaneous urination after the vaccination, with measurements standardized to creatinine. Blood and urine samples for 9α,11β-PGF2 were collected on internal deuterated standard (Cayman Chemicals, Ann Arbor, MI, USA) and analyzed by means of gas chromatography negative-ion chemical ionization mass spectrometry. Serum tryptase was measured with ImmunoCAP 100 (Phadia, Sweden). Blood samples for basophil analyses were taken before each administration of the VIT, as we could not find any reliable information regarding the time of recovery to the baseline after basophil activation, and suspected that subcutaneous allergen injections would cause degranulation of basophils in circulating blood, disabling in this way a reliable interpretation of the basophil activation test (BAT). The peripheral blood basophil count was calculated as the percentage of basophils (defined as $SSC^{low}CCR3^{+}$ cells) among all $CD45^{+}$ cells (peripheral blood leukocytes). The spontaneous and allergen-induced basophil activation was measured as percentage of basophils expressing the surface marker CD63 [7,8]. The CD63 expression analyses were done without stimulation (baseline activity), after stimulating the cells with BV allergens at five concentrations (a tenfold dilution series with final incubation concentrations ranging from 11.5 ng/ml down to 1.15 pg/ml) and two positive controls (anti-FcεRI and fMLP). The analyses were carried out in samples of whole blood on FACS Calibur cytometer (BD, CA, USA), using reagents from the Flow2CAST kit and bee venom allergen BAG2-I1 (Bühlmann, Switzerland), following the manufacturer's instructions. An increase of $CD63^{+}$ basophils in response to the allergen by at least 10% was considered as positive BAT result. The Wilcoxon rank test for small groups was used in statistical analyses.

Results

None of the patients demonstrated any systemic side effects during the course of VIT. Figure 1 shows trends of the markers measured in the blood. The concentrations of urinary 9α,11β-PGF2 greatly varied with no consistent trends (data not shown). In four patients, the results of BAT with venom allergen remained positive throughout the whole observation period: all reacted to the highest allergen concentration (11.5 ng/ml), and two patients also to the tenfold dilution (1.15 ng/ml). The fifth patient remained BAT negative on all three measuring points; nevertheless, he was not excluded from the present analyses, as he had fulfilled the predefined inclusion criteria for the study.

Discussion

To the best of our knowledge, there are no published data on the change in levels of PGD2 metabolites during rush VIT in children. The present study was aimed at filling this gap, however, the results should be taken with due caution due to limited sample size. As there were no systemic side effects to VIT in the treated group, our results may serve as a provisional reference for future studies of plasma 9α,11β-PGF2, serum tryptase and basophil activation in the course of VIT in children. The serum levels of tryptase (range: 1.94–5.06 ng/ml) observed in our pediatric patients are comparable with the

values previously observed in adults undergoing VIT (median: 4.25 ng/ml) [1]. There were no significant changes in tryptase levels during VIT in our patients, which is consistent with previous study of serum tryptase during allergen provocation test in patients with history of anaphylaxis [3]. On the other hand, there was a significant increase of spontaneous basophil activation during the VIT, expressed as a percentage of $CD63^+$ basophils in samples of peripheral blood without *in vitro* stimulation from BV allergens (baseline CD63 expression). As the addition of BV allergens caused further increase in numbers of activated basophils, BAT results remained interpretable and consistent throughout the study. This observation reinforces the opinion of BAT being a robust test for the detection of BV allergy [9]; in our patients, BAT results remained consistent throughout the course of VIT in spite of the increasing baseline basophil activation. One of our patients (patient 5; see TABLE 1) – a 9.5-year-old boy remained BAT negative at all three measuring points. A *post hoc* analysis revealed that his anaphylactic reaction occurred reportedly after a first bee sting (no previous bee stings noticed), his intradermal tests to BV were negative, his total IgE was lowest in the group, moreover, he had specific IgE to both bee venom (11.3 IU/l, CAP class 3) and wasp venom (3.0 IU/l, CAP class 2). Possible explanations to this finding might be a true double sensitization to both bee and wasp venom, or a cross-reactivity due to venom hyaluronidases or carbohydrate determinants [10]. Ebo *et al.* reported on a decrease in specific basophil reactivity to submaximal doses of allergens after 6 months of wasp venom immunotherapy, which was still present 1 year after concluding the VIT [11]. It seems that this phenomenon may appear later in the course of VIT, as we have not seen such tendency in our small group during the first 40 days of VIT. Instead, we have observed a decrease in the percentage of circulating basophils accompanied by a significant increase of $CD63^+$ basophils.

When looking at the results, one should be aware of the different time points of taking samples for mast cell and basophil analyses: Blood samples for assessing mast cell activation were taken 1 h after allergen injections, urine for 9α,11β-PGF2 determination was collected upon the fist spontaneous urination after the injections. Thus the results may be considered as an '*ex vivo*' provocation. By contrast, blood samples for basophil testing were taken before administering the subsequent allergen dose – in this way, we tried to avoid the basophil stimulation with allergen in the vaccine before adding the allergen to the cells during BAT. With this respect, the BAT may be viewed as an '*in vitro*' allergen provocation. On the other hand, the allergen vaccines also turned out to be a long-term stimuli for circulating basophils, which becomes apparent when looking at the baseline percentage of activated basophils that increase throughout the course of the study (FIGURE 1).

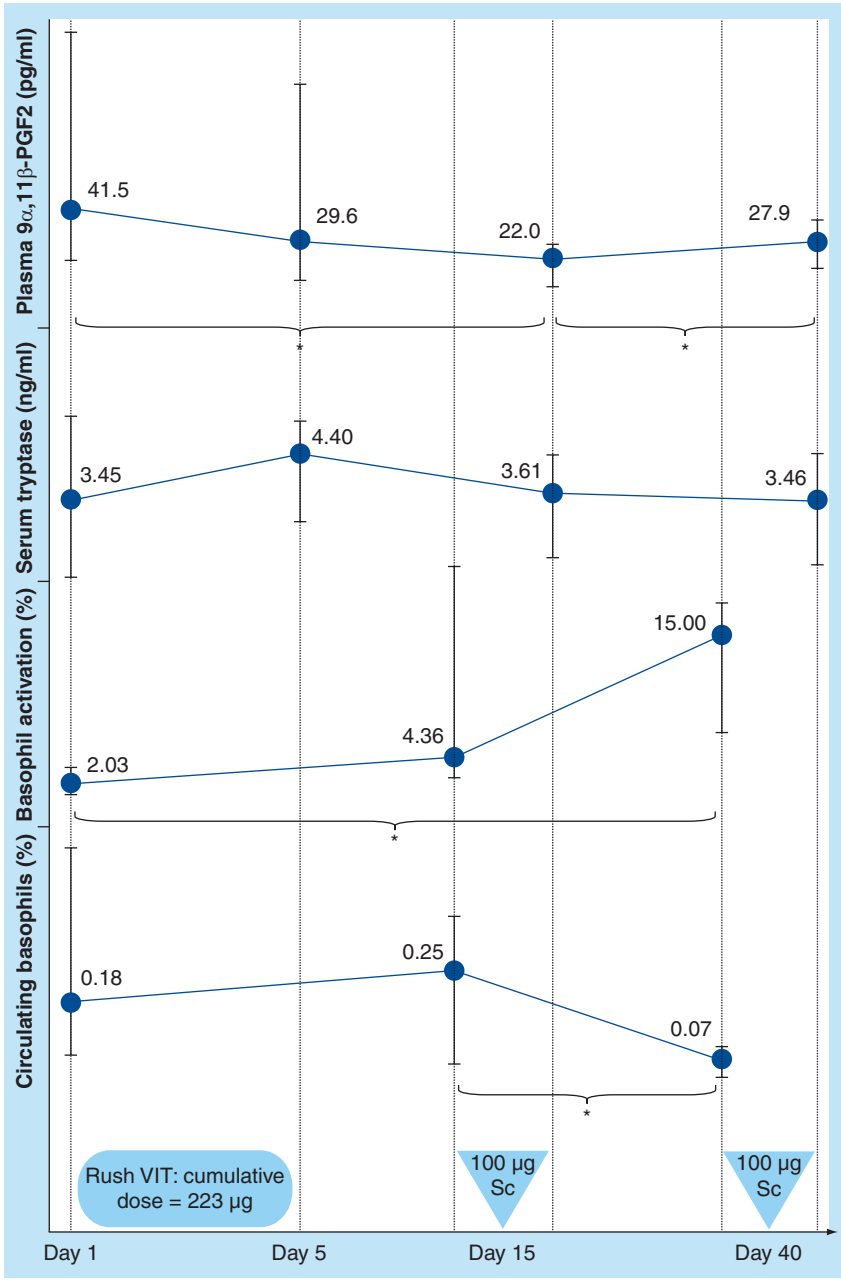

Figure 1. Schedule of taking blood samples and the results of relevant laboratory tests. Dots on the graph represent medians and the whisker lines represent the ranges of measured values.
*$p < 0.05$.
VIT: Venom immunotherapy.

This might be interpreted as an '*ex vivo*' provocation with a 2-week delay between allergen administration and outcome measurement. In our opinion, this increased stimulation of basophils, which is still visible 2 weeks after previous allergen administration, validates the decision about taking blood samples for BAT before administration of the new allergen. Our previous observations indicate that the increased baseline stimulation of basophils is no longer detectable after 6 weeks since the last allergy vaccination [Spiewak R *et al.*, Unpublished Data].

Conclusion

Our preliminary results suggest that serum levels of mast cell-derived 9α,11β-PGF2 decrease, while percentages of basophils expressing the activation marker CD63 increase during the course of the rush buildup phase and initial maintenance of bee venom immunotherapy in children allergic to BV.

Future perspective

This study was aimed at gaining knowledge about the behavior of selected activation markers of mast cells and basophils during rush immunotherapy with bee venom. These preliminary results provide information necessary for designing large studies assessing the usability of those markers in monitoring venom immunotherapy in children. Such future studies should involve larger groups of patients and the study period should include the whole course of immunotherapy (usually 3–5 years) and a post-treatment follow-up. Our results suggest that all markers analyzed in the present study deserve further attention, especially those demonstrating statistically significant changes under the limiting circumstances of a small study group. A potential practical application of these markers might be in the monitoring of patient safety in the course of immunotherapy. One example could be the establishment of 'safety limits' for the percentage of $CD63^+$ basophils during immunotherapy – a measurement above the limit would prompt for the subsequent allergen dose being reduced or postponed.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Mediators of mast cells and basophils provoke interest as potential markers of anaphylactic reactions that may be useful both in the diagnosis of venom allergy and monitoring venom immunotherapy.

Aim

- To study activation markers of mast cell (tryptase and 9α,11β-PGF2) and basophils (basophil count and CD63 expression) in honeybee venom allergic children during initial rush induction and subsequent 1-month maintenance immunotherapy.

Patients & methods

- Five pediatric patients with history of systemic reactions after bee sting, who were qualified for bee venom immunotherapy.
- The study period encompassed 40 days of immunotherapy, including a 5-day rush build-up phase with a cumulative dose of 223 µg bee venom allergen and two maintenance doses each of 100 µg.
- Changes in 9α,11β-PGF2 and tryptase levels were monitored to assess activity of mast cells.
- Peripheral blood basophil count, baseline basophil activation and response to bee venom allergen (basophil activation test) were monitored to assess basophil activity.

Results

- We have observed a decrease in mast cell activity, accompanied by an increase in basophil activity during the rush venom immunotherapy.
- In four patients, the basophil activation test using bee venom allergens remained positive throughout the whole study, while it was negative on all occasions in the fifth patient, in whom a *post hoc* analysis revealed certain differences to the remaining patients.

Conclusion

- In the initial phase of bee venom rush immunotherapy, mast cell and basophil activation markers seem to follow different trends: mast cell activation markers decrease, while numbers of activated basophils increase.
- The above results must be taken with caution due to the small size of the study group.

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