

ORIGINAL ARTICLE

Monitoring honeybee venom immunotherapy in children with the basophil activation test

Simona E. K. Žitnik¹, Tina Vesel¹, Tadej Avčin¹, Mira Šilar², Mitja Košnik² & Peter Korošec²

¹Department of Allergy, Rheumatology, and Clinical Immunology, Ljubljana University Children's Hospital, Ljubljana, Slovenia; ²University Clinic of Respiratory and Allergic Diseases, Golnik, Slovenia

To cite this article: Žitnik SEK, Vesel T, Avčin T, Šilar M, Košnik M, Korošec P. Monitoring honeybee venom immunotherapy in children with the basophil activation test. *Pediatr Allergy Immunol* 2011 Doi:10.1111/j.1399-3038.2011.01233.x

Keywords

children; honeybee venom immunotherapy; basophils; CD63.

Correspondence

Assistant Professor Peter Korošec,
University Clinic of Respiratory and Allergic
Diseases, Laboratory for Clinical
Immunology & Molecular Genetics, Golnik
36, 4204 Golnik, Slovenia
Tel.: +386 4 256 9432
Fax: +386 4 256 9143
E-mail: peter.korossec@klinika-golnik.si

Accepted for publication 4 October 2011

DOI:10.1111/j.1399-3038.2011.01233.x

Abstract

Background: New *in vitro* methods are essential for developing better follow-up criteria for venom immunotherapy (VIT).

Methods: Thirty-one children with a history of honeybee venom-induced systemic anaphylaxis were included in this prospective, single-blinded study. The basophil CD63 activation test (BAT) was assessed before starting VIT, at the end of the build-up phase (day 5), 6 months later, and after 2–4 yr of VIT.

Results: Basophil CD63 activation test allowed identification of the culprit insect in 74% of honeybee venom-allergic children. In comparison, IgE reactivity was single positive in only 52% of children. Five days after starting VIT, BAT was highly comparable to before VIT. However, after 6 months and further after 2–4 yr of VIT, a significant and approximately fourfold decrease was demonstrated in CD63 response at sub-maximal 0.1 µg/ml allergen concentration, which mainly represents cellular sensitivity. No such differences were found at a higher 1 µg/ml of allergen concentration. Person-to-person analyses showed that after 2–4 yr of VIT, a marked CD63 decrease was evident in 85% of children. In addition, elevated basophil sensitivity measured before VIT was associated with the appearance of side effects observed during the build-up phase of VIT.

Conclusion: Basophil CD63 allergen-specific sensitivity seems to be a promising tool for monitoring protective immune response in honeybee VIT.

Life-threatening anaphylactic reactions to Hymenoptera venom affect between 0.3% and 7.5% of the general population, and up to 75% of patients develop further systemic symptoms when re-stung (1, 2). In general, children have a better prognosis for severe systemic sting reactions, mortality, and the natural course of insect venom allergy, although the precise mechanisms for this are not known (3, 4). It has been well documented that specific venom immunotherapy (VIT) can lead to years of tolerance to insect stings. VIT is used in conjunction with positive skin and/or specific serum IgE tests in both children and adults with a history of severe systemic reactions such as respiratory and cardiovascular symptoms (5–7). Unfortunately, these standard diagnostic tests can neither predict the occurrence or severity of systemic reaction, nor can they predict the outcome of VIT. Recent advances in understanding basophil biology have led to advances in testing, such as flow cytometry

quantification of basophil activation by measurement of CD63 expression (8–14). This measurement may significantly improve diagnostic sensitivity in complex cases in which other testing methods have yielded inconclusive results (11). Moreover, several reports have suggested that changes in specific basophil CD63 responses might be related to adverse reaction or VIT outcome (10, 12, 13), although some authors were not able to demonstrate such correlations (8, 14). To evaluate the role of basophil testing in Hymenoptera venom allergies, we studied a group of 31 children with severe honeybee venom allergy. We measured and compared the results of CD63 testing prior to starting honeybee VIT, at the end of the build-up phase (5 days), 6 months later, and after 2–4 yr of VIT. We also investigated whether basophil CD63 activation test (BAT) results can predict adverse reactions during the build-up phase of VIT.

Material and methods

Study population

Thirty-one children (mean age 10.7 yr; range 4–18; 8 girls and 23 boys) with a clear history of systemic allergic reaction after honeybee sting (Grade I–II or higher according to Müller) (15) were included in this prospective, single-blinded study. None of the children had reported a systemic allergic reaction to wasp sting. Whole blood was collected from the patients after informed consent and approval of the Slovenian National Medical Ethics Committee was obtained. All of the children had positive (>0.35 kU/l) honeybee venom-specific IgE (median 22.8 kU/l; range 0.6 to >100) and 15 of them (48%) also had wasp venom-specific IgE (median 4.3 kU/l; range 0.5–24.5; Immulite, Siemens AG, Munich, Germany; Table 1). The median honeybee venom IgG4 was 1.9 mgA/l (range <0.01 to >30), and the median wasp venom IgG4 was 0.06 mgA/l (range <0.01 to 4.79; CAP-FEIA, Phadia, Uppsala, Sweden). The median total IgE was 318 kU/l (range 17.9–1907; CAP-FEIA). Skin prick and intradermal tests with honeybee and wasp venom were not performed. Basal serum tryptase levels were below 10 $\mu\text{g/l}$ (median 2.4; range 1–9.8) in all children. Testing occurred at least 2 wk after the sting reaction.

Honeybee venom immunotherapy

All 31 children started VIT (in a period of <1 yr after anaphylactic reaction) with honeybee venom (Venomenhal, HAL, Haarlem, the Netherlands) according to the position paper of the EAACI, reaching the maintenance dose of 100 μg of honeybee venom within 5 days using the rush protocol (15, 16). The maintenance dose was administered at intervals of 4 wk thereafter. The first BAT sample was taken just before starting VIT and the second sample was taken on the morning of the 5th day (the end of the build-up phase). The third and fourth samples were taken after 6 months and

2 (16 children) to 4 (10 children) years of VIT, just before the maintenance dose was administered. At the 6-month and 2- to 4-yr time point, we also collected a serum sample for specific IgE and IgG4 measurement. Five children were lost to follow-up after 2 (three children) to 4 (two children) years of VIT.

Basophil activation assay

The BAT was performed as previously described (10, 11). Briefly, heparinized whole blood was incubated in a basophil stimulation buffer with IL-3 (Buhlmann Laboratories, Basel, Switzerland) containing a final concentration of 0.1 or 1 $\mu\text{g/ml}$ of honeybee or wasp venom (3–5) (Hal Allergie), 0.55 $\mu\text{g/ml}$ of anti-Fc ϵ RI mAb (Buhlmann Laboratories), or 2 μM fMLP (N-Formyl-Met-Leu-Phe, Sigma, St. Louis, MO, USA) at 37°C for 15 min. Degranulation was stopped by chilling on ice, after which FITC-conjugated anti-CD63 mAb, PE-conjugated anti-CD123 mAb, and PerCP-conjugated anti-HLA-DR mAb (BD Biosciences, San Jose, CA, USA) were added and incubated for 20 min on ice. Finally, whole blood probes were lysed, washed, fixed, and analyzed within 2 h on a FACSCalibur flow cytometer (BD Biosciences). Data were acquired using an FL2 threshold that eliminated most CD123-negative cells, and at least 600 CD123-positive cells were acquired per sample. Basophils were identified as low side scatter, CD123-positive, and HLA-DR-negative cells, and the quantitative percentage determination of degranulated basophils was measured in FL1. The threshold value for diagnostically positive results was defined as 15% of CD63-positive basophils. This threshold was described previously (10, 11).

Statistical analysis

Data distribution was calculated using the D'Agostino & Pearson normality test. Depending on the distribution of the data, we used either an ANOVA or Kruskal–Wallis test, and Mann–Whitney tests. Data were expressed as median and range, if not otherwise stated. Correlation coefficients were calculated using the Pearson or Spearman test. For side effect analysis, a ratio between basophil CD63 expression after stimulation with honeybee venom at concentrations of 0.1 and 1 $\mu\text{g/ml}$ (0.1/1 index) was calculated, as described in detail previously (10). Probability values (p) <0.05 were accepted as significant.

Results

Honeybee venom immunotherapy

Of the 31 children included in the study, five experienced Grade I systemic reactions and two experienced Grade II systemic reactions during the build-up phase of VIT. After reducing the rate of honeybee venom dosage increase, all children reached the maintenance dose of 100 μg of honeybee venom without any further side effects.

Table 1 Diagnostic comparison of venom-specific IgE measurement and basophil activation test in 31 honeybee venom-allergic children. None of the children had a history of systemic allergic reaction to wasp sting

	Positive sIgE*	Positive BAT†
Honeybee venom	31/31	31/31
Wasp venom (additional wasp sensitization during honeybee VIT)‡	15/31 (25/31)	8/31 (14/31)
Sensitivity	100%	100%
Specificity	52%	74%

BAT, basophil CD63 activation test; VIT, venom immunotherapy.

* >0.35 kU/l.

†The threshold value for diagnostically positive venom CD63 results was defined as a positive reaction in 15% basophils at 1 $\mu\text{g/ml}$ of venom concentration (6, 7).

‡After 4 months ($N = 5$) or 2–4 yr ($N = 26$) of honeybee VIT.

Specific IgE and IgG4

Honeybee venom-specific IgE after 6 months of VIT (median 20.9 kU/l; range 1.7 to >100) were comparable to values before VIT. However, after 2–4 yr of VIT, these IgE values significantly decreased (median 4 kU/l; range <0.35 to 57, $p < 0.001$). Fifteen of 31 children had double-positive (honeybee and wasp) venom-specific IgE before starting VIT. Another six children became double-positive 6 months after introduction of VIT (novel wasp sensitization), and four more after 2–4 yr of VIT (Table 1). Serum concentration of honeybee venom-specific IgG4 significantly increased 6 months (median 17.1 mgA/l; range 0.7 to >30) and 2–4 yr after starting VIT (median 16.4 mgA/l; range 2.4 to >30, $p < 0.0001$). However, no increase was observed for wasp venom-specific IgG4 (median 0.09 and 0.13 mgA/l; range 0.01 to >30).

Basophil baseline, fMLP, and anti-FcεRI response

The baseline CD63 response was similarly low before VIT (median 3.4%; range 1.4–8.8), after 5 days (median 3.7%; range 0–7.3), after 6 months (median 3.5%; range 2.4–5.1), and after 2–4 yr of VIT (median 3.9%; range 2.4–5.1; Fig. 1). The response to fMLP was comparable to before VIT (med-

ian 45.5%; range 6.8–83.3), after 5 days (median 44.1%; range 17.5–85.9), after 6 months (median 50.4%; range 17.7–82.5), and after 2–4 yr of VIT (median 58.6%; range 9–80.4). The basophil response to anti-FcεRI mAbs was equally high before VIT (median 75.5%; range 14.1–99.2), after 5 days (median 79.3%; range 12.4–98.9), and after 6 months (median 63.4%; range 12–99.2); however, it became higher after 2–4 yr of VIT, but only in comparison with 6 months of VIT (median 95.6%; range 13–100; $p < 0.01$; Fig. 1).

Basophil honeybee venom response

All 31 children had a positive (by >15%) BAT response to honeybee venom before starting VIT at 1 µg/ml (median 77.8%; range 17.9–97.4). The response to 0.1 µg/ml of honeybee venom concentration (median 33%; range 5.3–91.5) was about one-half lower in approximately two-thirds of children. The CD63 response was nearly identical when tested 5 days after starting VIT (median 72.2%; range 11.3–99.5 for 1 µg/ml, and median 45.5%; range 0.5–96 for 0.1 µg/ml; Fig. 1). However, 6 months after VIT, the CD63 response decreased almost fourfold following stimulation with 0.1 µg/ml of honeybee venom (median 8.7%; range 2.3–81.5) and it stayed similarly low after 2–4 yr of VIT (median 10.8%; range 2.1–73.3; five children were lost to

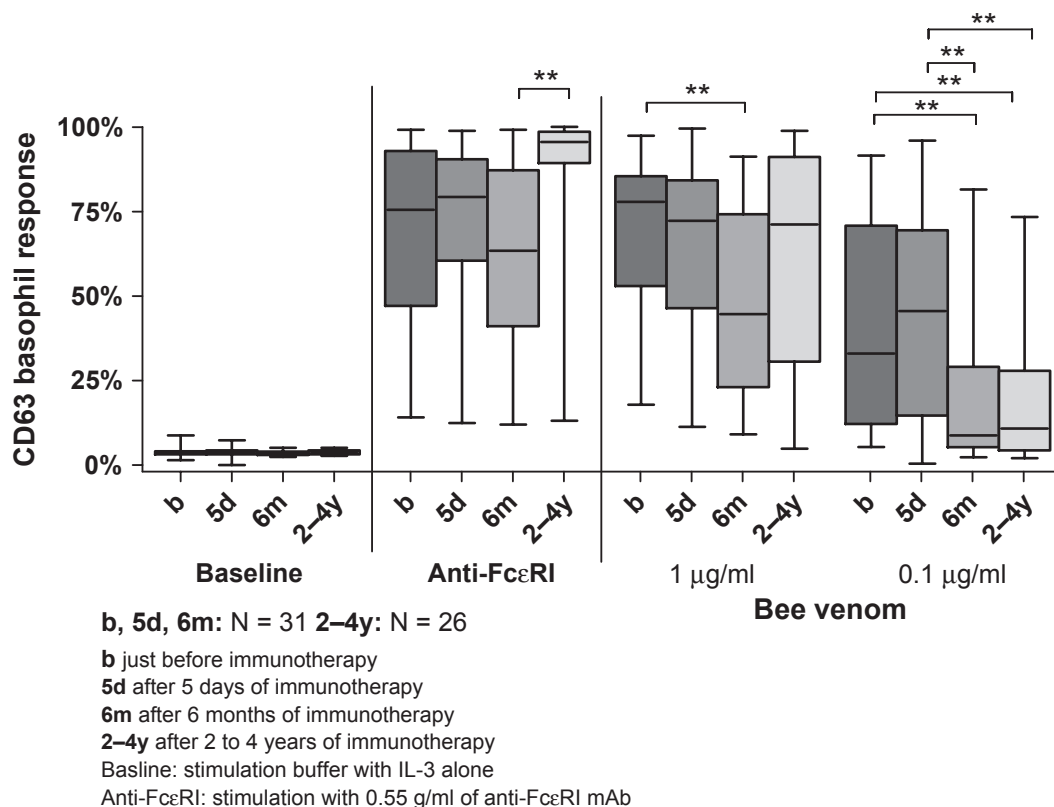


Figure 1 *In vitro* basophil CD63 response just before starting honeybee VIT, after 5 days, after 6 months (N = 31), and after 2–4 yr of honeybee VIT (N = 26). The box plot indicates the median (horizontal line), interquartile range (box), and minimum and maximum values (whiskers). * $p < 0.05$; ** $p < 0.01$.

follow-up at this point). This suppression of allergen-specific response was highly significant (Kruskal–Wallis test with Dunn's Multiple Comparison; Fig. 1). The basophil CD63 responses at 1 µg/ml of venom had transiently decreased after 6 months of VIT (median 44%; range 9.1–91.2), but it returned to previous values after 2–4 yr of VIT (median 71.2%; range 4.8–98.8). Person-to-person analyses showed that after 2–4 yr of honeybee VIT, of 26 children, 22 (85%) showed a significant decrease and four children (15%) showed an increase in basophil-specific response (Fig. 2a). There was no significant correlation between CD63 responses at 1 or 0.1 µg/ml of honeybee venom and concentration of IgG4 antibodies to honeybee before, after 6 months, or after 2–4 yr of VIT. Moreover, a subgroup of 22 children that showed a decrease in basophil response after 2–4 yr displayed no differences in IgG4 or IgE dynamic in comparison with a subgroup of four children that showed an increase in CD63 response (Fig. 2b,c).

Basophil wasp venom response

Twenty-three children had a negative (by <15%) and eight had a positive (by >15%) initial BAT response to wasp venom (median 7.9%; range 3.7–86.6 at 1 µg/ml and median

4.6%; range 1.1–88 at 1 µg/ml; Fig. 3). Seven of those negative children had positive wasp venom-specific IgE (Table 1). The CD63 response remained comparably low after 5 days and also after 6 months of honeybee VIT; however, it became higher (although not significantly) after 2–4 yr of VIT, preferably at a maximum 1 µg/ml of wasp venom concentration (median 23.8%; range 1.2–100), but not at sensitivity-related sub-maximal 0.1 µg/ml of allergen concentration. Consequently, six children additionally showed a positive CD 63 response (by >15% at 1 µg/ml) to wasp venom after 6 months or 2–4 yr of VIT.

Basophil 0.1/1 index and systemic side effects of VIT

The basophil 0.1/1 index was calculated as a ratio between the basophil CD63 response to honeybee venom at 0.1 and 1 µg/ml, as previously reported (10). We found that the 0.1/1 index was significantly higher in a subgroup of seven children that had experienced systemic side effects during the build-up phase (median 1.03; range 0.61–1.08) as compared to the 24 children without adverse systemic reactions (median 0.45; range 0.06–1.7; Fig. 4a). The 0.1/1 index was further evaluated by ROC curve analysis. The area under the ROC curve was 0.83 and $p < 0.01$. The optimal calculated threshold index was >0.69, with a

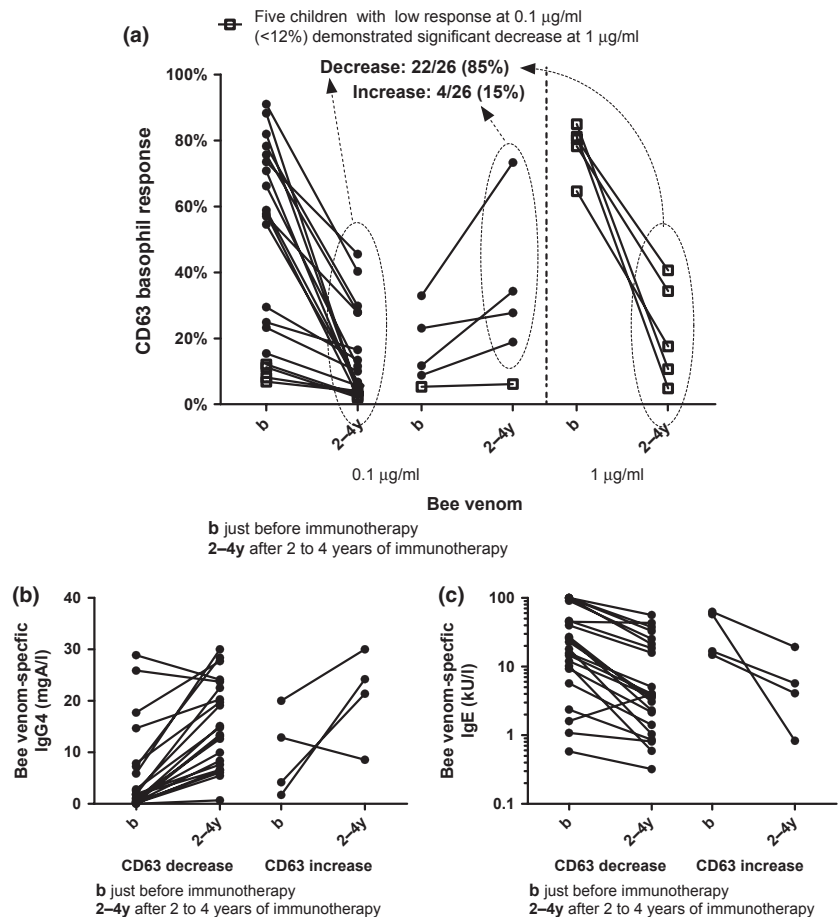


Figure 2 (a) Person-to-person analysis of basophil CD63 responses to honeybee venom before and after 2–4 yr of honeybee VIT. Twenty-two of 26 children showed a marked decrease in basophil response, whereas four children showed an increase. Those two subgroups demonstrated no differences according to the changes in bee venom-specific (b) IgG4 and (c) IgE antibodies.

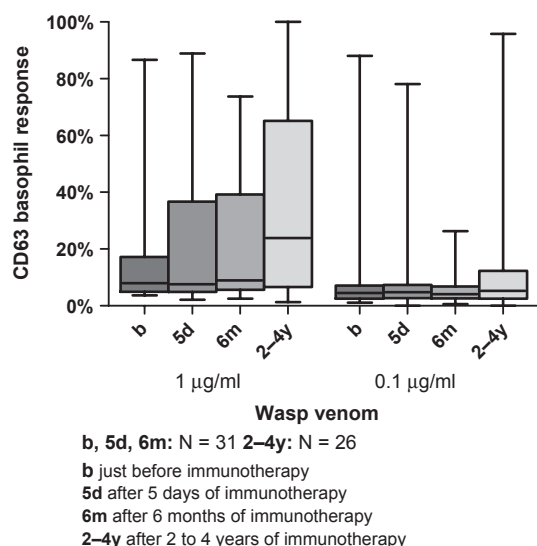


Figure 3 *In vitro* basophil CD63 responses to wasp venom before, after 5 days, after 6 months (N = 31), and after 2–4 yr of honeybee VIT (N = 26). The box plot indicates the median (horizontal line), interquartile range (box), and minimum and maximum values (whiskers).

true positive rate (sensitivity) of 86% and a false positive rate of 33% (giving a specificity of 67%). We also compared the 0.1/1 index during honeybee venom VIT and found a highly significant decrease in the sensitivity index after 6 months and after 2–4 yr of VIT ($p < 0.001$; ANOVA with Newman–Keuls multiple comparison test, Fig. 4b).

Discussion

This study showed that venom immunotherapy in children induces a significant and long-term decrease in allergen-

specific basophil sensitivity, which was evident after 6 months and after 2–4 yr of VIT. Furthermore, we also demonstrated the usefulness of basophil testing for identifying the culprit venom and showed that initially increased basophil allergen sensitivity is associated with adverse reactions during the build-up phase of VIT.

Basophils and mast cells are directly responsible for the clinical symptoms of IgE-mediated reactions by releasing histamine and other mediators. At the cellular level, the response of basophils corresponds to two independent variables: reactivity and sensitivity (17). Reactivity is the maximal response to allergen stimulation at the plateau of the dose response curve. In our study, reactivity is presented by the response at the 1 µg/ml of venom concentration and it was important for diagnostic confirmation of IgE sensitization, with a threshold set at more than 15% of CD63-positive basophils (10, 11). In addition to diagnostic confirmation, reactivity is very useful for defining the culprit venom in patients that exhibit simultaneous skin tests and/or IgE double-positivity (18). Double-positivity can be a result of the presence and serological recognition of cross-reactive carbohydrate epitopes, which lack allergenic activity and hence may give rise to false positive *in vitro* diagnostic results (19). Basophil testing overcomes those factors because it can distinguish between biologically active and inactive allergens. Consequently, our study showed that almost half of the IgE double-positive children (7 of 15) had positive BAT results for honeybee venom only. Similar observations were demonstrated by Ebo et al. (20) for diagnosing latex allergy. In this report, Ebo showed that BAT is a very specific tool that can determine the relevance of sensitization in relation to cross-reactive carbohydrate epitopes that cause positive latex IgE without apparent clinical significance.

Basophil sensitivity represents the threshold concentration of allergen that elicits a positive response (17). It can be evaluated by various approaches previously described. One of the first approaches, which was also used in this study, was to

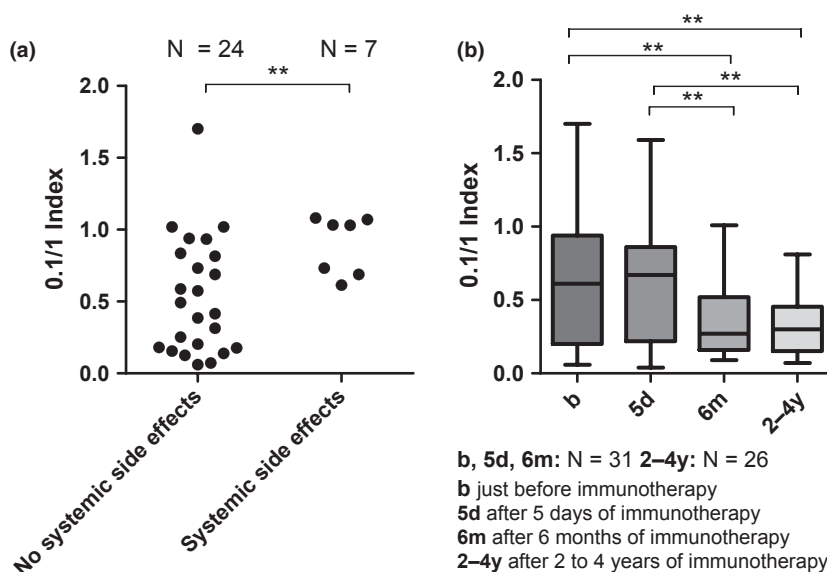


Figure 4 (a) Ratio between basophil CD63 response at 0.1 and 1 µg/ml of honeybee venom dilutions (0.1/1 index) determined just before honeybee VIT in children with systemic side effects of immunotherapy (N = 7) and in children without systemic adverse reactions (N = 24). (b) Basophil honeybee 0.1/1 index before, after 5 days, after 6 months (N = 31), and after 2–4 yr of honeybee VIT (N = 26). * $p < 0.05$; ** $p < 0.01$.

calculate the ratio (0.1/1 index) of basophil CD63 expression between a value on the plateau and a value on the steep part of the response curve, using two log concentrations (10). When using this approach, it is important that the median response at a step-log allergen concentration be about half of the response at the highest allergen concentration (10, 11, 13). This was also the case for the majority of our subjects because the median response to honeybee venom before VIT was 33% at 0.1 µg/ml and 77.8% at 1 µg/ml. Nevertheless, additional person-to-person analyses showed that this approach was limited to about one-fifth of the children. Namely, children that have an initially low response at 0.1 µg/ml, and were therefore out of the steep part of the response curve at this concentration, showed only minor changes at 0.1 µg/ml, but significant changes at 1 µg/ml, following 2–4 yr of VIT. Therefore, other more precise and sophisticated approaches, which are now more broadly available, should be used in further basophil sensitivity studies. One possibility is to construct a dose-response curve and calculate the allergen concentrations, giving 50% of maximum CD63 response; if this value is inverted and multiplied by 100, then it is referred to as 'CD-sens' (21–24). Another very robust and useful approach is to expand the CD63 response by at least 4 log allergen concentrations and compare the responses between different sub-maximal concentrations (24, 25). Skin tests are not suitable for evaluating allergen sensitivity because they cannot be measured precisely enough to permit exact quantitative evaluation. For allergen sensitivity measured by a skin test, a coefficient of variation (CV) of 100–200% was reported (21, 22). On the other hand, for allergen sensitivity measured by the CD63 basophil *in vitro* response, a CV of 5–15% was reported (21, 22).

Our study showed that honeybee venom-specific basophil sensitivity decreases significantly after 6 months and after 2–4 yr of honeybee VIT. This decrease was clearly evident in more than 80% of children. No long-term changes were observed at the level of basophil reactivity. In addition, no such changes were observed for the CD63 response to wasp venom. However, for wasp venom we demonstrated an increase at the level of the basophil reactivity and therefore additional CD63 positivity (>15%) in seven children. Nevertheless, wasp venom-specific basophil sensitivity (the response to sub-maximal venom concentration) did not change during honeybee VIT and it stayed similarly depleted to before VIT (median ≤ 5.3% at 0.1 µg/ml). This might suggest that additional wasp venom sensitization probably had no clinical relevance because it was evident only at the level of cellular allergen reactivity, but not at the level of allergen sensitivity. There were also no marked changes for anti-FcεRI stimulation, which acts via the IgE high-affinity receptor. Unfortunately, in this study we did not perform additional dose-dependent anti-IgE antibody stimulations, which might have allowed a more detailed assessment of the possible influence of VIT on IgE-mediated responsiveness.

Currently, we do not know whether the changes in basophil sensitivity are associated with induction of long-term tolerance after completing VIT. Nevertheless, during the

course of immunotherapy we carefully followed up a re-sting history and we found that 14 of 31 children experienced a honeybee field re-sting. However, none of them showed a systemic allergic reaction. We also monitor for novel wasp stings and re-stings. These were evident in five and three children, respectively. Of these five children, four became wasp venom-positive for CD63 testing. Again, none of them showed a systemic allergic reaction.

Similar observations concerning a decrease in basophil sensitivity were observed during pollen immunotherapy (23, 25) and after 6 months of oral peanut immunotherapy (26). Pollen immunotherapy models showed a significant association between the reduction in basophil allergen sensitivity and the rise of allergen-specific IgG antibodies (23, 25).

Nevertheless, we could not find any correlation between decreases in basophil sensitivity and an increase in specific IgG4 antibodies. Similar observations were demonstrated by Varga et al. (27), showing that, in contrast to pollen immunotherapy, IgG-dependent serum inhibitory activity might not be relevant for the clinical efficacy of wasp venom immunotherapy. On the other hand, Mikkelsen et al. (28) demonstrated that after 3–7 wk of VIT the involvement of the plasma component correlates with the decrease of basophil sensitivity. Currently, the precise mechanism for suppressing basophile sensitivity during VIT is unknown, as is whether it is a humoral or intracellular one, or maybe even a combination of both. Obviously, further studies are needed, including in relation to induction of long-term tolerance after completing a course of VIT.

We confirmed previous observations obtained using an adult population (6) that demonstrated that increased sensitivity on the basophil 0.1/1 index is significantly associated with systemic side effects observed during the build-up phase of VIT. For the group of children we studied, we also performed ROC curve analysis to determine the diagnostic usefulness of the 0.1/1 index. We found a high diagnostic sensitivity of 86%, but a relatively limited diagnostic specificity of 67%. In other words, some of the children passed the build-up phase without adverse reactions, thus displaying high allergen basophil sensitivity. On the other hand, among the children that experienced side effects, almost all showed high allergen sensitivity exceeding the threshold value of 0.69. In addition, we could not demonstrate any correlation between side effects and basal serum tryptase levels. A recent report suggests that increased basal tryptase may be associated with side effects in vespid VIT, but not with side effects in honeybee VIT (29).

In conclusion, our findings showed that basophil CD63 testing is a highly specific method for diagnostic evaluation of insect-sting allergies in children and that changes in basophil sensitivity might be a promising tool for monitoring the immune response initiated by venom immunotherapy.

Acknowledgment

This study was supported by the Slovenian Research Agency (Grants L3-0624 and P3-0360).

References

- Novembre E, Cianferoni A, Bernardini R, et al. Epidemiology of insect venom sensitivity in children and its correlation to clinical and atopic features. *Clin Exp Allergy* 1998; **28**: 834–8.
- Jennings A, Duggan E, Perry IJ, Hourihane JO. Epidemiology of allergic reactions to hymenoptera stings in Irish school children. *Pediatr Allergy Immunol* 2010; **21**: 1166–70.
- Graft DF, Schuberth KC. Hymenoptera allergy in children. *Pediatr Clin North Am* 1983; **30**: 873–86.
- Hompes S, Köhli A, Nemat K, et al. Provoking allergens and treatment of anaphylaxis in children and adolescents – data from the anaphylaxis registry of German-speaking countries. *Pediatr Allergy Immunol* 2011; **22**: 568–74.
- Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005; **60**: 1339–49.
- Moffitt JE, Golden DB, Reisman RE, et al. Stinging insect hypersensitivity: a practice parameter update. *J Allergy Clin Immunol* 2004; **114**: 869–86.
- Konstantinou GN, Manoussakis E, Douladiris N, et al. A 5-year venom immunotherapy protocol with 50 µg maintenance dose: safety and efficacy in school children. *Pediatr Allergy Immunol* 2011; **22**: 393–7.
- Erdmann SM, Sachs B, Kwiecien R, Moll-Slowsky S, Sauer I, Merk HF. The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004; **59**: 1102–9.
- Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy* 2004; **59**: 1110–7.
- Kosnik M, Silar M, Bajrovic N, Music E, Korošec P. High sensitivity of basophils predicts side-effects in venom immunotherapy. *Allergy* 2005; **60**: 1401–6.
- Korošec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. *Clin Exp Allergy* 2009; **39**: 1730–7.
- Ebo DG, Hagendorens MM, Schuerwegh AJ, et al. Flow-assisted quantification of *in vitro* activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. *Cytometry B Clin Cytom* 2007; **72**: 196–203.
- Peternelj A, Silar M, Erzen R, Kosnik M, Korošec P. Basophil sensitivity in patients not responding to venom immunotherapy. *Int Arch Allergy Immunol* 2008; **146**: 248–54.
- Eberlein-König B, Schmidt-Leidescher C, Rakoski J, Behrendt H, Ring J. *In vitro* basophil activation using CD63 expression in patients with honeybee and wasp venom allergy. *J Investig Allergol Clin Immunol* 2006; **16**: 5–10.
- Müller U, Mosbech H. EAACI position paper: immunotherapy with *Hymenoptera* venoms. *Allergy* 1993; **48**: S37–46.
- Bonifazi F, Jutel M, Biló MB, Birnbaum J, Müller U, EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy. Guidelines for clinical practice. *Allergy* 2005; **60**: 1459–70.
- MacGlashan DW Jr. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993; **91**: 605–15.
- Peternelj A, Silar M, Bajrovic N, et al. Diagnostic value of the basophil activation test in evaluating Hymenoptera venom sensitization. *Wien Klin Wochenschr* 2009; **121**: 344–8.
- Altmann F. The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007; **142**: 99–115.
- Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ. Validation of a two-color flow cytometric assay detecting *in vitro* basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy* 2002; **57**: 706–12.
- Johansson SGO, Nopp A, van Hage M, et al. Passive sensitization by blood transfusion. *Allergy* 2005; **60**: 1192–9.
- Nopp A, Johansson SG, Ankerst J, et al. Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006; **61**: 298–302.
- Nopp A, Cardell LO, Johansson SG, Oman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009; **64**: 811–4.
- Zidarn M, Košnik M, Šilar M, Grahek A, Korošec P. Rhinitis symptoms caused by grass pollen are associated with elevated basophil allergen sensitivity and a larger grass-specific immunoglobulin E fraction. *Clin Exp Allergy* 2011 Aug 22. [Epub ahead of print].
- Lalek N, Kosnik M, Silar M, Korošec P. Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin Exp Allergy* 2010; **40**: 1186–93.
- Jones SM, Pons L, Roberts JL, et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009; **124**: 292–300.
- Varga EM, Francis JN, Zach MS, Klunker S, Aberer W, Durham SR. Time course of serum inhibitory activity for facilitated allergen-IgE binding during honeybee venom immunotherapy in children. *Clin Exp Allergy* 2009; **39**: 1353–7.
- Mikkelsen S, Bibby BM, Dolberg MK, Dahl R, Hoffmann HJ. Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy. *Clin Mol Allergy* 2010; **8**: 2.
- Rüeff F, Przybilla B, Biló MB, et al. Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol* 2010; **126**: 105–11.