

Flow-Assisted Quantification of In Vitro Activated Basophils in the Diagnosis of Wasp Venom Allergy and Follow-up of Wasp Venom Immunotherapy

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Background: Correct identification of the culprit venom is a prerequisite for specific venom immunotherapy (VIT). Despite the efficacy of VIT, issues as how to monitor treatment and when to discontinue maintenance therapy remain to be established.

Methods: To evaluate diagnostic performances of the basophil activation test (BAT) in wasp venom allergy, 80 patients with a definite history of wasp venom anaphylaxis (systemic reactors) and 14 wasp-stung asymptomatic controls (stung controls) were enrolled. Venom-induced basophil activation was analyzed flow cytometrically by double-labeling with anti-IgE and anti-CD63. Results were compared to wasp IgE levels and results of a venom skin test (VST). To establish whether the BAT constitutes a candidate marker to monitor VIT, the BAT was repeated in 22 patients on the 5th day of a build-up course and after 6 months of maintenance VIT. Whether the BAT could contribute in the decision of discontinuing VIT was assessed in a cross-sectional analysis in 30 patients receiving treatment for 3 years.

Results: Comparison between systemic reactors and stung controls revealed a sensitivity of 86.4% and specificity of 100% for venom IgE, and sensitivity of 81.8% for VST, respectively. In contrast to stung controls, patients demonstrated dose-dependent venom-induced basophil activation. The BAT attained a sensitivity of 83.8% and specificity of 100%. At the end of the build-up course, no effect of VIT on the BAT was demonstrable. When the BAT was repeated after 6 months of treatment, submaximal stimulation of the cells demonstrated a significant decreased CD63 expression ($P < 0.04$). Patients having VIT for 3 years also demonstrated significantly lower venom-induced CD63 expression ($P < 0.001$). After 3 years, 60% of the patients had a negative BAT for submaximal stimulation of the cells whereas only 17.9% of the patients had negatigation of wasp IgE.

Conclusions: The BAT is a reliable instrument for the diagnosis of wasp venom anaphylaxis and might constitute an instrument to monitor wasp VIT. © 2006 International Society for Analytical Cytology

Key terms: flow cytometry; CD63; immunotherapy; hymenoptera venom

Hymenoptera venom allergy constitutes an important health problem and correct diagnosis is a prerequisite for effective and potentially life-saving management, i.e. specific venom immunotherapy (VIT). Currently, diagnosis of venom anaphylaxis generally relies upon an evocative history corroborated by positive venom skin tests (VST) and venom-specific IgE. Although each method can provide useful information, both have in se limitations (1–7). Sting challenge tests have been thoroughly described (8), but several practical and mainly ethical issues, as well as the observation that a single negative sting challenge does not definitely indicate absence of hypersensitivity certainly limits its application. In fact, as deliberate sting challenges carry a considerably risk for anaphylaxis, the technique is

not recommended as a diagnostic instrument in untreated patients (9–11). We have demonstrated venom-induced lymphocyte proliferation to have a sensitivity of 72% in wasp venom anaphylaxis (12). Therefore, supplementary

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Table 1
General Characteristics of the Patients

Parameter	Longitudinal group	Cross-sectional group
N	22	30
M/F	13/9	15/15
Age: median (range)	46.5 (17–73)	44 (15–77)*
Total IgE: median (range)	56 kU/L (5–306)	125.5 (24–2629)**
Wasp IgE: median (range)	4.72 kUa/L (<0.35–96.80)	6.90 kUa/L (<0.35 to >100)*
Percentage positive skin tests for wasp	81.8%	85.7%
Severity of reaction	All patients were selected if they presented with a compelling history of anaphylaxis with urticaria, angio-oedema, bronchospasm, hypotension, shock.	

*Not significant; ** $P = 0.03$.

in vitro tests to diagnose venom anaphylaxis are of tremendous interest.

Upon encounter of specific allergen that bridges FcεRI-bound IgE, basophils not only synthesize and secrete bioactive mediators but also up-regulate the expression of certain activation markers (e.g. CD63 and CD203c) that can be quantified by flow cytometry. The value of flow cytometry-assisted allergy diagnosis has been reported in allergies to pollen, house dust mite, foods, drugs, and *Hevea* latex (for review see Refs. 13 and 14). The technique also proved reliable to diagnose hymenoptera venom anaphylaxis (15–19), with a sensitivity and specificity for wasp venom between 85 and 92% and 80 and 83.3%, respectively (18,19). Nevertheless, as recently pointed out by the EAACI Interest Group on Insect Venom Hypersensitivity (7), additional larger and comprehensive studies remain necessary to confirm these results. Therefore, the primary aim of this study was to compare the diagnostic performance of the basophil activation test (BAT) in IgE-mediated wasp venom allergy with established tools such as skin tests and venom-specific IgE.

In spite of the high efficiency of VIT, an effective instrument to monitor successful VIT is still lacking, and an important unanswered question relates to the optimal duration of maintenance immunotherapy. Both, venom-specific IgE and VST fail to reflect induction of clinical tolerance and seem of little predictive value for future (deliberate) sting reactions VIT (20–26). As the technique closely resembles the in vivo pathway leading to symptoms, the second purpose of this study was to evaluate whether the BAT could constitute an in vitro and thus safe instrument to monitor successful VIT. Therefore in a subgroup of patients, the test was repeated at different predefined time points during treatment. Finally, a separate cross-sectional analysis was performed to evaluate the contribution of the BAT in the decision of stopping VIT safely.

METHODS

Patients and Controls

To compare the accuracy of the different diagnostic tools, 80 systemic reactors to wasp venom were included (47 men, 33 women; age 17–79 years). All (and if necessary their relatives) were carefully interviewed with a

standardized questionnaire. Patients were selected if they presented a compelling history of wasp venom anaphylaxis based on the type of reaction (e.g. urticaria, angio-oedema, rhinoconjunctivitis, bronchospasm, generalized anaphylaxis with hypotension, or shock) and entomologic identification of the stinging insect. Correct identification of the culprit was promoted by detailed description and showing a picture of the two predominant hymenoptera species (i.e. wasp and honeybee) in Belgium.

To address the time course of venom-specific IgE and in vitro basophil activation during early VIT, in 22 patients, the BAT was repeated on the 5th day of a semi-rush build-up hyposensitization course and after 6 months of maintenance immunotherapy. All patients were treated according to a standardized protocol (12). Each patient was initially administered a dose of 1 µg of wasp venom (Pharmalgen, ALK-Abellö, A/S, Denmark). The dose was progressively increased over 5 days. On the fifth day of this build-up regimen 80 and 100 µg were injected. A maintenance dose of 100 µg was administered at days 12, 26, 37, and once every 4 weeks during the first year, and subsequently every 6 weeks during at least 3 years.

To evaluate whether the BAT could contribute in the decision when VIT can be discontinued safely, a separate cross-sectional comparison on an additional 30 systemic reactors that had comparable wasp venom-induced allergic reactions and were receiving maintenance VIT for 3 years was carried out. It is noteworthy that 8 (26.7%) of them were resting by a wasp without systemic reaction. Table 1 summarizes the relevant characteristics of the patients in longitudinal follow-up and those analyzed cross-sectionally.

Fourteen healthy volunteers (8 men, 6 women) aged from 28 up to 70 years with a previous wasp sting without symptoms served as stung controls.

Serum IgE

Specific IgE for wasp (*Vespula vulgaris*) venom was quantified with a solid-phase technique according to the manufacturer's instructions (Immuno-CAP FEIA, Phadia AB, Brussels, Belgium).

Venom Skin Tests

All systemic reactors had a VST using an intradermal endpoint titration method (10^{-5} – 10^{-1} $\mu\text{g/mL}$ solutions of wasp venom Pharmed, ALK-Abellö, A/S, Denmark). VST were considered positive when the wheal and flare reaction exceeded a diameter of 5 mm.

Basophil Activation Test

The technique of the basophil activation test (BAT) has already been described elsewhere (27). Within 3 h after sampling (endotoxin free heparinized tubes, Vacuette, Greiner Labortechnik GmbH, Kremsmünster, Austria), aliquots of 100 μL whole blood were preincubated for 10 min at 37°C with stimulation buffer containing interleukin (IL)-3 (2 ng/mL, PeproTech, Inc Rocky Hill, NJ). Pre-activated blood samples were stimulated for 20 min at 37°C in a water bath with 100 μL commercial wasp venom (Pharmed, ALK-Abellö, A/S, Denmark; serial dilution: 0.01, 0.1, 1, and 10 $\mu\text{g/mL}$), 100 μL (10 $\mu\text{g/mL}$) anti-IgE (clone G7-18; mouse IgG2a; Pharmingen, BD Biosciences, Erembodegem, Belgium) as a positive control, or 100 μL washing solution to measure spontaneous CD63 expression (negative control). The optimal stimulation time and temperature were determined in preliminary experiments. To quantify activated basophils, cells were stained with 20 μL monoclonal biotinylated anti-human IgE (clone GE-1; mouse IgG2b; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 10 μL monoclonal phycoerythrin (PE)-conjugated anti-human CD63 (clone H5C6; mouse IgG1; Pharmingen, BD Biosciences, Erembodegem, Belgium), or PE-conjugated irrelevant-control antibody of identical isotype (Pharmingen) during 20 min in the dark, on ice. After washing, 20 μL of Streptavidin AlexaFluor 488 (Invitrogen, Paisley, UK) was added for 15 min at room temperature. Red blood cells were lysed and white blood cells were fixed (FACS Lysing solution, BD) during 10 min at room temperature. After centrifugation (5 min, 250g, 4°C) cells were resuspended in 200 μL of washing solution. Flow cytometric analysis of basophil activation was performed on a FACScan flow cytometer (BD Immunocytometry Systems). IgE-staining and side scatter were employed to gate on at least 500 basophils that expressed high density of surface IgE. Subsequently, within this gate the percentage of activated basophils, i.e. coexpressing CD63, was measured. For this purpose, a marker was set on the 99th percentile value obtained with an irrelevant isotype-control antibody. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression (negative control) from the value obtained with allergen stimulation.

Statistical Analysis

All results were expressed as median (range). The Kruskal-Wallis, Mann-Whitney *U*-test, Friedman, Wilcoxon, and Spearman's rank correlation test were applied where appropriate. Differences were considered as significant at a *P* value less than 0.05. Two graph-receiver operating

characteristics curve analysis was performed to calculate the optimal threshold value that corresponds to the best sensitivity and specificity (28,29). All statistic calculations were performed using SPSS version 12.

RESULTS

Venom-Specific IgE

None of the stung controls had a wasp venom-specific IgE equaling or exceeding 0.35 kUa/L. In systemic reactors, before therapy, median (range) of wasp venom IgE was 4.72 kUa/L (<0.35–96.80). According to the 0.35 kUa/L threshold value, wasp venom IgE showed a sensitivity of 86.4% and specificity of 100%. After 6 months of VIT wasp venom IgE was 3.05 kUa/L (<0.35–84.30).

In the 30 patients treated for at least 3 years, wasp venom specific IgE before therapy was 6.90 kUa/L (<0.35 to >100) and significantly declined to 1.88 kUa/L (<0.35–26.20) (*P* < 0.001). Negativation of wasp venom-specific IgE, however, was restricted to 5/28 (17.9%) of the patients who had positive wasp venom IgE before therapy.

Venom Skin Test

The wasp VST before VIT was positive in of 81.8% of the patients.

Basophil Activation Test

Dose-finding. The preliminary dose-finding experiments are summarized in Figure 1. Stung controls (*n* = 14) and systemic reactors to wasp venom (*n* = 15) demonstrated comparable spontaneous and anti-IgE induced CD63 expression. Actually, spontaneous CD63 expression was 3% (1–11) in stung controls and 3% (0–16) in systemic reactors, whereas anti-IgE-induced CD63 expression relative to spontaneous expression was 57% (27–79) in stung controls and 47 (16–66) in systemic reactors. In stung controls wasp venom-induced basophilic CD63 expression remained merely unaltered and was comparable to spontaneous CD63 expression. Actually, percentage of wasp venom-induced CD63 positive basophils above spontaneous expression was 0% (0–3), 1% (0–4), 1% (0–26), and 1% (0–26) for a venom stimulation concentration of 0.01, 0.1, 1, and 10 $\mu\text{g/mL}$, respectively. In contrast, systemic reactors showed a dose-dependent up-regulated wasp venom-induced basophilic CD63 expression with a median (range) of CD63 positive basophils above spontaneous expression of 12% (0–85), 45% (9–80), 60% (23–80), and 52% (39–78) for a stimulation concentration of 0.01, 0.1, 1, and 10 $\mu\text{g/mL}$. Although stimulation with 0.01 $\mu\text{g/mL}$ elicited a significantly lower basophilic activation as compared to incubation of the cells with 0.1, 1, and 10 $\mu\text{g/mL}$ (*P* ≤ 0.002), two-graph ROC analysis demonstrated all four stimulation concentrations to be highly discriminative between patients and stung controls (Fig. 2).

Sensitivity and specificity. Given the potentially life-threatening course of wasp venom allergy, false-negative

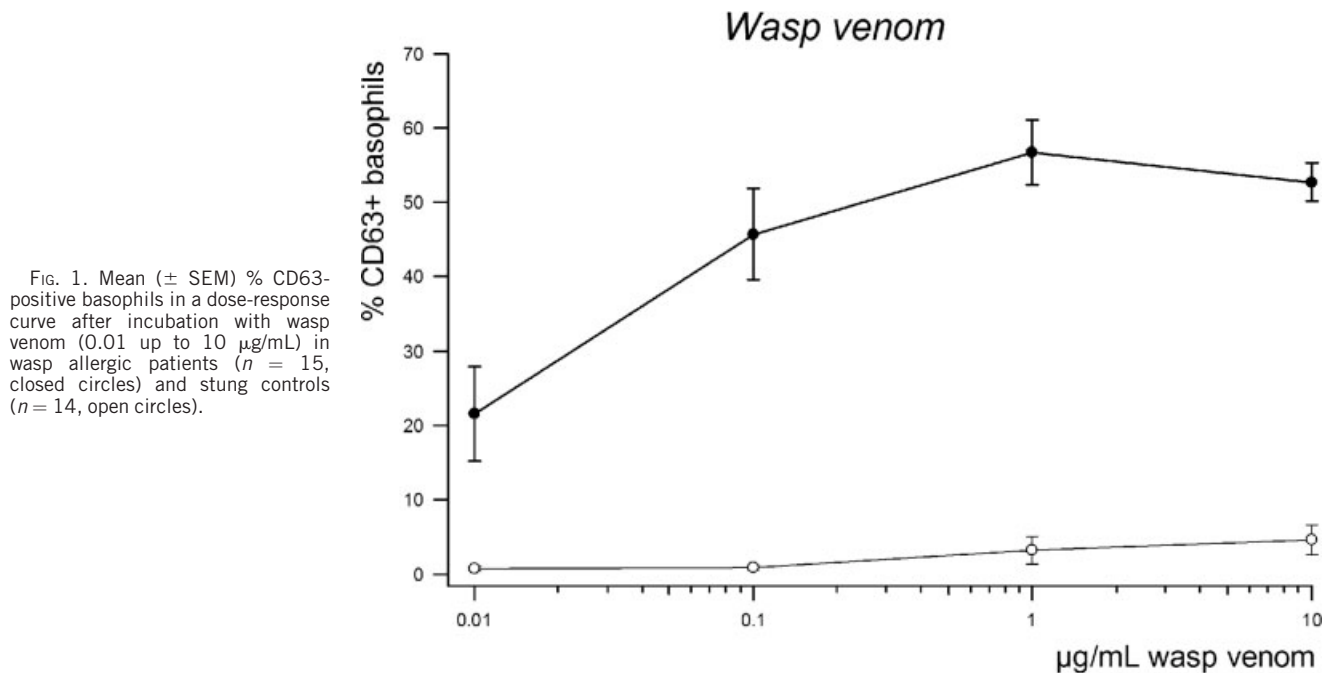


FIG. 1. Mean ($\pm$ SEM) % CD63-positive basophils in a dose-response curve after incubation with wasp venom (0.01 up to 10 $\mu\text{g/mL}$) in wasp allergic patients ($n = 15$, closed circles) and stung controls ($n = 14$, open circles).

test results need to be minimized. Consequently, further evaluation of the diagnostic performance of the BAT was performed with a stimulation concentration that proved absolutely discriminative, i.e., 10 $\mu\text{g/mL}$. In an expanded analysis with 80 patients, 68 responded to positive control

stimulation with anti-IgE and the BAT reached a sensitivity of 98.5% and specificity of 100%. Taking into account the 12 nonresponders to positive control stimulation with anti-IgE who also demonstrated negative allergen stimulation, the overall sensitivity of the BAT decreased to 83.8%.

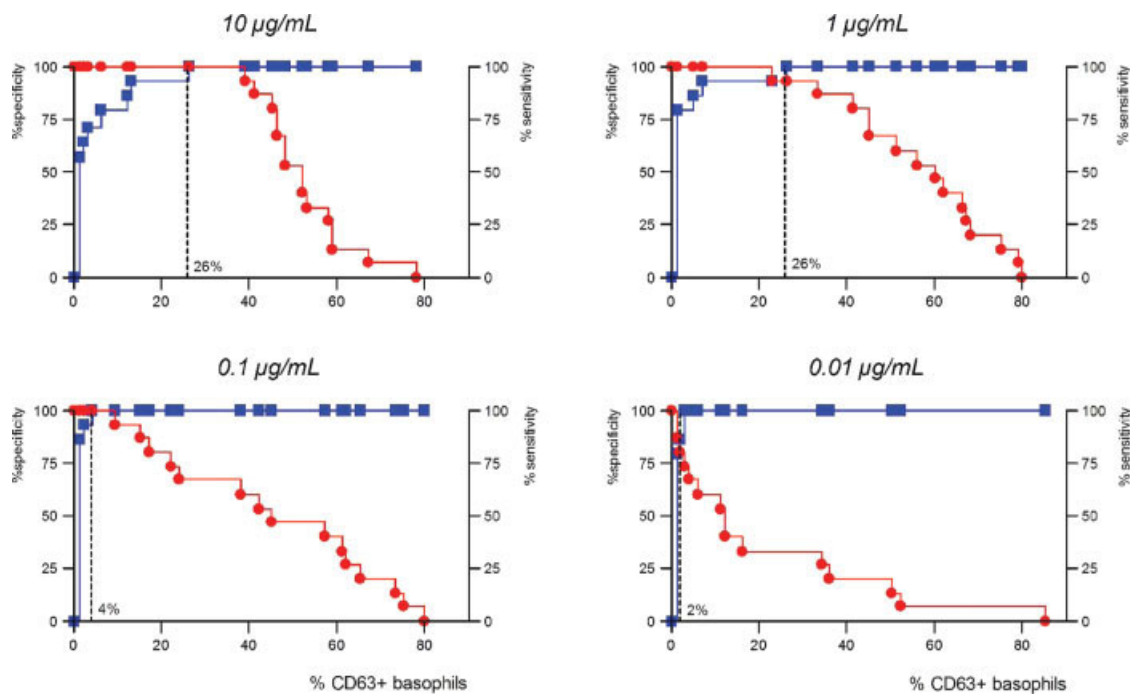


FIG. 2. Two-graph ROC curve for wasp venom-induced CD63 expression generated between stung controls ($n = 14$) and wasp venom allergic patients ($n = 15$). Threshold values of venom-induced CD63 positive basophils (dotted lines) were 26, 26, 4, and 2% above spontaneous expression for a stimulation concentration of 10, 1, 0.1, and 0.01 $\mu\text{g/mL}$, respectively. Circles represent sensitivity and squares specificity, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

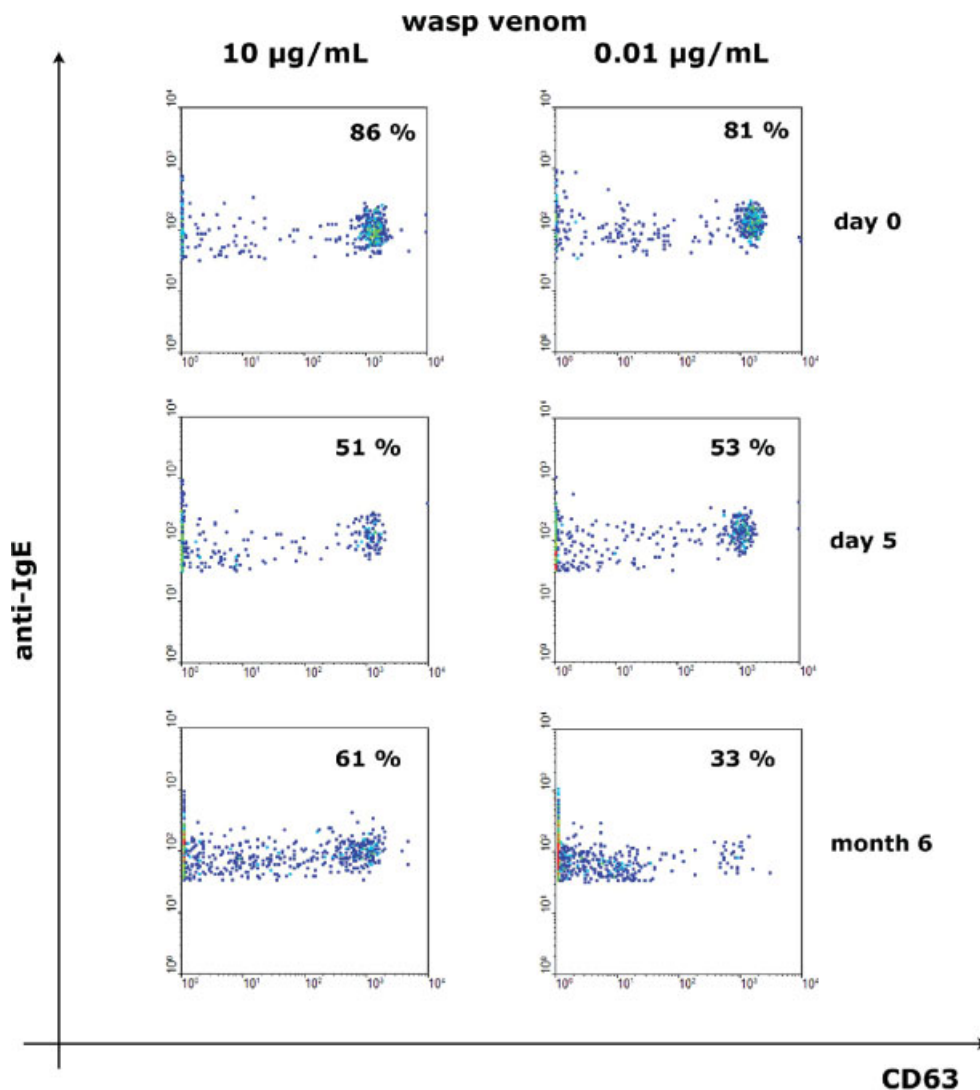


FIG. 3. Basophil activation before (day 0), after a semirush hyposensitization course (day 5) and finally after 6 months of maintenance VIT (month 6). Results expressed in % CD63-positive basophils. The left panel represents stimulation with 10 µg/mL and right panel 0.01 µg/mL. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Follow-up of immunotherapy. Figure 3 shows a representative example of basophil responsiveness before, after the 5 day semirush VIT course and finally after 6 months of maintenance VIT.

Figure 4 (upper and mid panel) summarizes the individual time course of the BAT during VIT for maximum stimulation of the cells with 10 and 0.1 µg/mL, whereas the lower panel shows the results for submaximal stimulation with 0.01 µg/mL ($n = 22$). From these figures it emerges that a high-dose 5-day semirush hyposensitization course had no significant effect on the basophil responsiveness as assessed by the venom-induced up-regulation of CD63 expression, irrespective the stimulation concentration. Before treatment, incubation of the basophils with 0.01, 0.1, and 10 µg/mL of wasp venom resulted in a median (range) % of CD63-positive basophils of 29% (0–85), 68% (5–90), and 49% (30–68), respectively. After the semirush hyposensitization course corresponding median (range) % of CD63-positive basophils were 48% (1–87), 63% (2–94), and 42% (7–69), respectively. When the BAT in these

patients was repeated after 6 months of maintenance VIT, median (range) % of venom-induced CD63 was 14% (0–47), 51% (0–90), and 59% (0–82) for a stimulation concentration of 0.01, 0.1, and 10 µg/mL. As compared to pretreatment values, submaximal cell stimulation with wasp venom 0.01 µg/mL demonstrated a significant inhibited CD63 expression ($P < 0.04$). In contrast, stimulation at a 10-fold higher dose of 0.1 µg/mL remained unaltered, suggesting a 10-fold decrease in basophil responsiveness. Complete negatvation of the BAT was restricted to 5/22 patients (22.7%). During early maintenance VIT spontaneous and anti-IgE-induced CD63 expression remained unaltered (data not shown).

Figure 4 also summarizes the individual percentages of CD63-positive basophils from the cross-sectional comparison with an additional 30 patients who were receiving maintenance VIT for 3 years. Median (range) % of anti-IgE induced CD63 expression in this group treated for 3 years was 49% (2–90) and did not differ from pretreatment values of the group studied longitudinally (47% (19–66)).

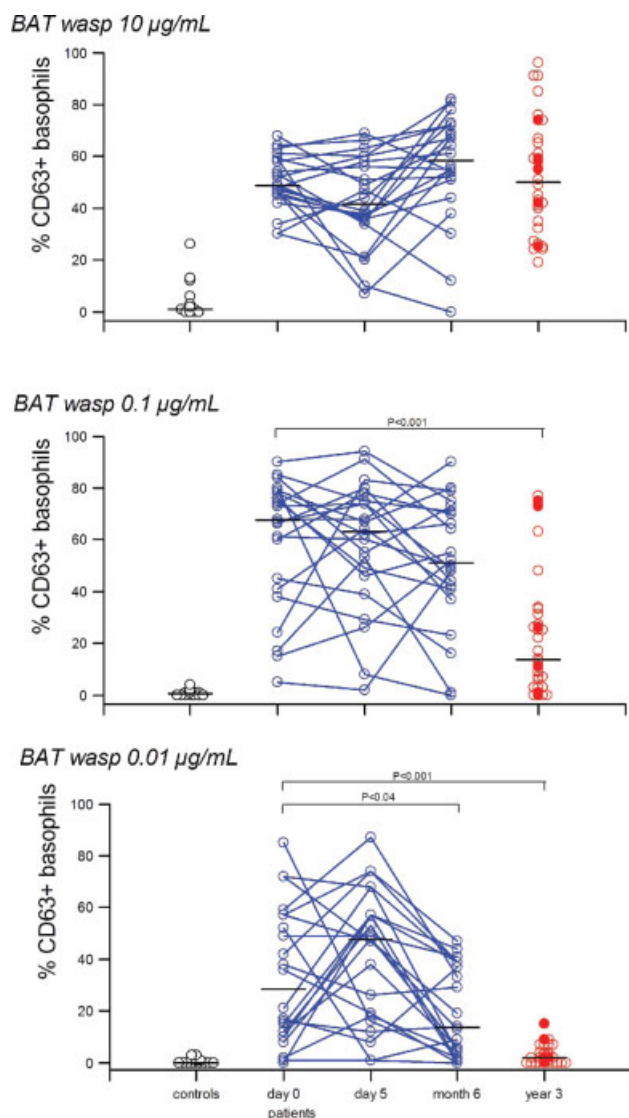


Fig. 4. Percentages of CD63-positive basophils in stung controls ($n = 14$, left) and wasp venom allergic patients ($n = 22$, mid) before treatment (day 0), after semirush hyposensitization (day 5) and after 6 months of maintenance therapy (month 6). Percentages of CD63 positive basophils of a separate cross-sectional analysis in patients treated for 3 years ($n = 30$, right). Closed circles represent the 8 patients who tolerated a field sting. The bold lines denote the medians. Upper, mid and lower panel represent the data for a stimulation concentration of 10, 0.1, and 0.01 $\mu\text{g/mL}$, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Median (range) % of venom-induced CD63 expression in this group was 2% (0–15), 14% (0–83), and 50% (19–96), for a stimulation concentration of 0.01, 0.1, and 10 $\mu\text{g/mL}$. As compared to pretreatment values of the systemic reactors, significant lower CD63 expression was observed for submaximal stimulation with 0.01 $\mu\text{g/mL}$ and also stimulation with 0.1 $\mu\text{g/mL}$ ($P < 0.001$). In contrast, stimulation of the cells with 10 $\mu\text{g/mL}$ remained unaltered, suggesting a further decrease in basophil responsiveness. Moreover, after 3 years of VIT, 18/30 (60%) of the patients demonstrated a negative BAT for a stimulation concentra-

tion with 0.01 $\mu\text{g/mL}$ (% of venom-induced CD63-positive basophils $\leq 2\%$). For stimulation with 10 and 0.1 $\mu\text{g/mL}$ the number of negative basophil activation responses was 5 and 8 out of 30, respectively.

DISCUSSION

The only effective and potentially life-saving treatment of wasp venom anaphylaxis is specific VIT. Currently diagnosis of wasp venom anaphylaxis rests upon an evocative history along with the demonstration of wasp venom-specific IgE antibodies in the skin or serum (for a recent review of the EAACI Interest Group on Insect Venom Hypersensitivity on the diagnosis of venom allergy the reader is referred elsewhere (7)). Our data confirm that VST and quantification of venom-specific IgE can be considered as quite performant diagnostic tools for wasp venom anaphylaxis. However, as already addressed in the introductory paragraph, none of the tests demonstrated absolute diagnostic reliability and even when performed in a complementary way did not identify all patients at risk for subsequent sting anaphylaxis.

Our dose-finding experiments demonstrate that wasp venom-induced basophilic activation, assessed flow cytometrically through the coexpression of IgE and the activation marker CD63, is not restricted to a single concentration of the allergen but rather covers different venom stimulation concentrations with a kind of plateau of maximal cell stimulation. These findings are in line with prior observations (15–19). Most importantly, the observation that the BAT discriminates between patients and controls for different allergen concentrations spanning several log scales allows determination of an optimal diagnostic stimulation concentration for further validation of the technique. Given the potentially life-threatening course of wasp venom anaphylaxis, evaluation of the diagnostic performance of the BAT was performed with a venom stimulation concentration (10 $\mu\text{g/mL}$) that proved most discriminative between patients and controls. According to a two-graph ROC-generated threshold of 26% above spontaneous CD63 expression, the BAT confirmed to be a reliable diagnostic instrument in wasp venom allergy and demonstrated an overall sensitivity of 83.8 and 100% specificity. Obviously, these figures include the patients who demonstrated nonresponsiveness to positive control stimulation with anti-IgE and for whom, in the absence of venom-induced CD63 expression, the BAT cannot be considered as a diagnostic tool. Often, those individuals whose results should be considered as “false-negatives” in specificity calculation are not reported in the literature. Obviously, this is not correct.

Conversely, the BAT allowed correct identification of the culprit venom in two patients with a compelling history of wasp-venom anaphylaxis that presented with urticaria, angio-edema, and respiratory symptoms, but who demonstrated a negative skin test and venom-specific IgE result. Similar findings on the BAT to constitute a potential diagnostic tool in patients with negative IgE and skin test results were recently observed by Kosnik et al. (30).

Despite the high efficiency of VIT, the mechanisms and biomarkers associated with clinical efficacy remain elusive (for review see Ref. 31). Studies monitoring conversion of venom-specific IgE and skin test responses have repeatedly demonstrated these tests to be of poor predictive value and to fail to reflect induction of clinical tolerance (20–26). In our cross-sectional analysis, only 18% of the patients demonstrated negative wasp venom IgE after 3 years of treatment. This is in line with the data of van Halteren et al. (22) who found IgE negatization in approximately one-tenth of their patients. On the other hand, anaphylaxis due to sting challenges has been reported in patients with venom-specific IgE or skin test conversion (21,23–26). Therefore, current practice is to offer VIT for a finite period of 3–5 years, irrespective the persistence of a positive skin test or IgE result (32–35).

Alternatively, a variety of studies have demonstrated in vitro desensitization of basophils during IgE-mediated stimulation of the cells. From these data, different mechanisms that might lead to loss of patient's responsiveness during semirush immunotherapy have been postulated (36–40). As in vitro stimulation of basophils closely resembles the in vivo pathway finally leading to symptoms, we speculated that suppression of venom-induced CD63 expression during treatment might be an in vitro correlate of clinical tolerance and be useful to monitor successful VIT. Therefore, the BAT was repeated at the end of a 5-day build-up course and after 6 months of maintenance treatment, at both time-points clinical protection has already been demonstrated by a well-tolerated sting challenge (18,41,42) and mediator release by basophils to some extent to be inhibited (43,44). At the end of 5 days of semirush immunotherapy there was no significant change in basophilic expression of CD63, yet the patients tolerated successive escalating exposure to venom without difficulty. A possible explanation for this apparent paradox could be expression of CD63 not necessarily to reflect final behavior of the cell, i.e. mediator release, but merely to represent a distinct point in the signaling pathway or a different functional endpoint (45). In other words, the semirush course inhibits degranulation but not fusion of the intracellular granules with the outer basophil membrane.

Alternatively, after 6 months of maintenance treatment a decrease in venom-induced basophilic activation was observed. However, as compared to pretreatment values, inhibition of basophil activation was restricted to the submaximal stimulation experiments (0.01 µg/mL venom) and was not demonstrable when the cells were stimulated maximally (0.1 and 10 µg/mL), suggesting 6 months of immunotherapy to induce an up to 10-fold decrease of basophil responsiveness rather than a complete inhibition of the cells. This observation parallels the findings of Mothes et al. (46) who demonstrated inhibition of allergen-induced release of histamine by therapy-induced blocking IgG antibodies to be restricted to those experiments in which the basophils were stimulated submaximally. Moreover, our findings might explain the failure of Erdmann et al. (18) to demonstrate suppression of basophilic activation after 6 months of VIT. Revision of the dose-finding curves from this study reveals that the time-course of the BAT during VIT was assessed with a stimulation concentration resulting in maximal cell response.

philic activation after 6 months of VIT. Revision of the dose-finding curves from this study reveals that the time-course of the BAT during VIT was assessed with a stimulation concentration resulting in maximal cell response.

To evaluate whether the BAT could guide the decision when to discontinue VIT safely, a separate cross-sectional comparison with 30 patients (8 being re-stung without reaction) treated for 3 years was performed. At this time-point, as compared to pretreatment values of systemic reactors, significant inhibition of basophil activation was demonstrable for stimulation with 0.01 and also for 0.1 µg/mL venom. Although one could argue longitudinal analysis over 3 years to provide more robust data, we believe our cross-sectional investigation in matched patients to be highly suggestive for basophil responsiveness further to decrease with time, with more and more of the patients becoming negative during prolonged maintenance therapy. This frequency of reduced and absent basophilic responsiveness is consistent with the trend to diminished skin test sensitivity demonstrated by others (24). In this study these authors demonstrated VIT administered for more than 5 years to induce an at least 10-fold decline in skin test sensitivity in over 95% and complete negatization of skin test responses in approximately 60–70% of the patients, respectively.

Taken together, from our data it emerges flow cytometry constitute a reliable tool to diagnose wasp venom anaphylaxis. Furthermore, we confirmed the technique to provide the physician with an instrument to take the sting out of difficult cases with inconsistent IgE and skin test results that hinder correct identification of the culprit (19,30,47).

Finally, for the first time, it is demonstrated that the technique might offer an in vitro tool to monitor successful VIT. However, it should be acknowledged that responsiveness in our assay might not really represent a basophil property, but rather mirror less IgE that is cross-linked on the cell membrane because of decreased specific IgE or induction of blocking IgG antibodies.

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