

Diagnosis of stinging insect allergy: utility of cellular in-vitro tests

Kathrin Scherer^a, Andreas J. Bircher^a and Ingmar A.F.M. Heijnen^b

^aAllergy Unit, Department of Dermatology, University Hospital Basel and ^bImmunology Laboratory, Department of Rheumatology, University of Basel, Felix Platter Hospital, Burgfelderstrasse 101, CH-4012 Basel, Switzerland
Tel: +41 61 326 4250; fax: +41 61 326 4252;
e-mail: ingmar.heijnen@fps-basel.ch

Correspondence to Dr Ingmar A.F.M. Heijnen, Immunology Laboratory, Department of Rheumatology, University of Basel, Felix Platter Hospital, Burgfelderstrasse 101, CH-4012 Basel, Switzerland
Tel: +41 61 326 4250; fax: +41 61 326 4252;
e-mail: ingmar.heijnen@fps-basel.ch

Current Opinion in Allergy and Clinical Immunology 2009, 9:000–000

Purpose of review

Diagnosis of stinging insect allergy is based on a detailed history, venom skin tests, and detection of venom-specific IgE. As an additional diagnostic tool, basophil responsiveness to venom allergens has been shown to be helpful in selected patients. This review summarizes the current diagnostic procedures for stinging insect allergy and discusses the latest developments in cellular in-vitro tests.

Recent findings

Cellular assays have been evaluated in patients with Hymenoptera venom allergy. The diagnostic performance of the cellular mediator release test is similar to that of the flow cytometric basophil activation test (BAT), but the BAT has been the most intensively studied. BAT offers the possibility to assess basophil reactivity to allergens in their natural environment and to simultaneously analyze surface marker expression and intracellular signaling. It has been demonstrated that BAT represents a valuable additional diagnostic tool in selected patients when used in combination with other well established tests. A major limitation is the current lack of unified, standardized protocols. Flow cytometry offers huge possibilities to enhance knowledge of basophil functions.

Summary

The BAT may be used as an additional test to confirm the diagnosis of stinging insect allergy in selected patients, provided that it is performed by an experienced laboratory using a validated assay. Test results have to be interpreted by clinicians familiar with the methodological aspects. The utility of the BAT to confirm allergy diagnosis and to predict the risk of subsequent systemic reactions may be improved by combined analysis of multiple surface markers and intracellular signaling pathways.

Keywords

basophil activation test, flow cytometry, hymenoptera, mediator release test, stinging insect allergy

Curr Opin Allergy Clin Immunol 9:000–000
© 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

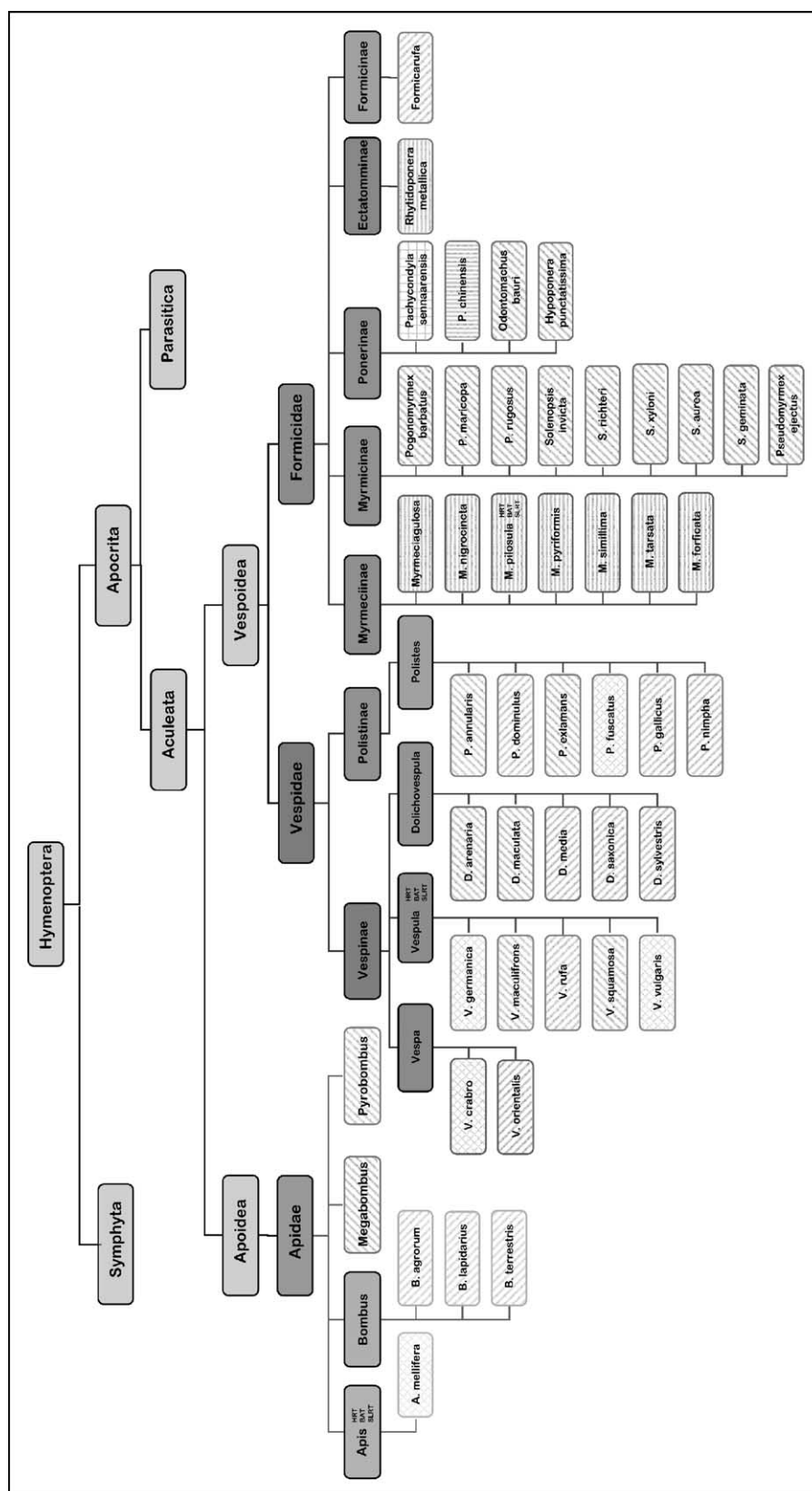
Introduction

Stings from insects are a very common event and cause severe systemic hypersensitivity reactions in 2–3.5% of the general population [1]. In the order of Hymenoptera, besides honey bees (*Apis*) and bumblebees (*Bombus*) of the Apidae family, various members of the Vespidae family, most notably yellow jackets (*Vespula*) and hornets (*Vespa*) of the Vespinae subfamily and some members of the Polistinae subfamily, a diverse array of ant species (Formicidae family) belonging to different subfamilies and many genera have the capacity to induce systemic hypersensitivity reactions in humans [2,3] (Fig. 1). In addition, some members of other classes and orders, such as pigeon ticks (*Argas* reflexus, Arachnidae-class, Acari-order/subclass), mosquitoes (Diptera order, Nematocera suborder) and

others are mentioned as elicitors of anaphylactic reactions [4,5].

Individuals who have experienced a systemic reaction following an insect sting are subsequently recommended to undergo a diagnostic procedure to assess the underlying pathomechanism (allergic versus nonallergic) and to identify the species of the offending insect [6]. Because diagnosis of allergy forms the basis for treatment, diagnostic tests should be sensitive and specific. Ideally, a diagnostic test should also be able to adequately predict clinical reactivity to a future sting. In recent years, numerous studies have demonstrated that the investigation of cellular responses to allergen exposure *in vitro* may help improve our diagnostic armamentarium. Here, we summarize the current state of diagnostic procedures

Figure 1 Taxonomy of the members of the Hymenoptera order causing allergic reactions in humans, their geographic distribution and medical importance



Data from <http://www.telefonica.net/web2/insect/> adapted with permission from [2,3].

for stinging insect allergy and review the latest developments of cellular in-vitro tests.

Present status of diagnostic testing in stinging insect allergy

The diagnosis of insect sting allergy requires a history of a prior systemic reaction confirmed with accurate diagnostic tests.

History

Besides taking a detailed medical history in general, the situation in which the allergic reaction occurred, concomitant intake of drugs, and other possibly aggravating factors (e.g. alcohol, physical or emotional stress, climatic factors, and inflammatory disease) are of special importance. To identify the insect, it is helpful to know the time and circumstances of the reaction with respect to season, urban or rural location, and proximity to foods. In addition, it should be clarified whether the sting remained in place and which insect the patient suspects [7]. To distinguish dangerous immediate-type reactions from toxic or delayed-type reactions, the time course in which symptoms appeared and the exact morphology (e.g. localized swelling, flush, weals, angioedema, pain, pruritus) are important.

In-vivo diagnostic tests

There are a number of in-vivo diagnostic testing methods that have historically been the preferred diagnostic confirmatory test in cases of insect sting allergy.

Skin tests

Skin prick tests and intradermal tests (IDTs) with standardized whole venom extracts at least 2 weeks after the reaction to a sting are the mainstay of diagnostics in suspected venom allergy [1]. IDT are performed with progressive dilutions up to 1 µg/ml of insect venom extract. A number of studies have recently demonstrated high sensitivities (>90%) and specificities (>90%) of IDT [8,9,10^{*}], albeit that substantially lower sensitivities (70–90%) have also been reported [11]. The reproducibility of venom IDT using a standardized technique in patients with systemic reactions to bee and wasp has recently been seriously questioned by Graif *et al.* [12], in contrast to the generally accepted view [12,13].

Sting challenge tests

As in-vivo tests with purified insect venom preparations are not reliable [14], and well tolerated immunotherapy with Hymenoptera venom does not necessarily indicate tolerance of a field-sting, intentional sting challenges with live Hymenoptera have been used to diagnose allergy and to evaluate the efficacy of immunotherapy. However, tolerance to a single sting challenge does not definitely rule out venom hypersensitivity. The prognos-

tic reliability of a tolerated sting challenge with respect to a later field sting is only 85–95% [15]. Apparently, additional factors related to the insect (e.g. age) and the patient have to be considered when interpreting test results [16]. In venom allergic patients, a single sting may induce potentially life-threatening anaphylaxis. Furthermore, a single exposure to venom can sensitize or act as a booster [17]. Thus, the deliberate administration of an insect sting is not recommended as a diagnostic test. Sting challenge with live Hymenoptera in an experienced intensive care setting is generally accepted as safe enough to evaluate the efficacy of immunotherapy [18], but is only offered at a few specialized centers.

In-vitro diagnostic tests

Although the in-vivo skin test is generally an accurate assessment for the majority of individuals who experience systemic insect sting reactions, additional in-vitro assays are used as confirmatory tests.

Specific IgE

It is current practice to measure venom-specific IgE in serum for the assessment of stinging insect allergy [6]. Large improvements in reagents and instrumentation of quantitative IgE assays have been made in the last decade and testing is now performed in many clinical laboratories on closed and fully automated analyzers that guarantee high levels of analytical standardization. It should, however, be noted that specific IgE test results obtained by using different commercial test systems may still not be equivalent [19]. The sensitivity of specific IgE serum tests in patients with systemic insect sting reactions appear to be somewhat lower than that of IDT, but also for this test sensitivity values of higher than 90% have repeatedly been reported [8,9,10^{*}]. It is generally accepted that the specific IgE levels do not predict severity of a possible future sting reaction [20]. However, using a modern quantitative standardized assay, a trend between the level of specific IgE and the severity of a sting challenge was observed, although this correlation was not significant [21]. Interestingly, patients with severe sting reactions showed significantly lower levels of total IgE [21]. These interesting findings have to be confirmed in future studies.

Specific IgG

The presence of venom-specific IgG (subclasses) in the serum indicates previous allergen exposure, that is, history of a sting, but does not correlate with the presence or absence of an allergic sting reaction [1]. Specific IgG may be of relevance as elicitors of sting-induced serum sickness-like disease or other unusual reactions [18]. Neither the presence nor the level of specific IgG is predictive for the likelihood or the severity of a future reaction or the protective effect of immunotherapy [18,22,23].

Cellular assays

The potential role of cellular in-vitro assays as routine procedure in the diagnosis of Hymenoptera venom allergy is currently the subject of intensive discussions [24–27]. The latest position papers of the European and American professional societies (EAACI and AAAAI/ACAAI, respectively) on this subject classify the cellular assays as additional tests that are being evaluated and that may demonstrate sensitization [1,6]. Some groups, however, consider one of the cellular assays, namely the flow cytometric basophil activation test, as a validated and reliable tool for the diagnosis of Hymenoptera venom allergy [25,27].

Basophil-based cellular tests in stinging insect allergy

Although allergen-induced activation of lymphocytes has been used in previous studies [28], the cellular assays performed today for diagnosis rely on the human basophil.

Background and current status

Functional cellular in-vitro assays based on the measurement of cellular reactivity after direct stimulation with allergen theoretically represent ideal diagnostic tests. These tests aim to measure if and – depending on the method used – by which mechanism key cells of the allergic response react to an allergen challenge *ex vivo*. In fact, the use of such a ‘miniature in-vitro anaphylaxis system consisting of the human basophil, blood, and antigen’ had already been reported for patients with bee sting allergy in 1961 [29], several years before the discovery of IgE [30]. Since then, numerous attempts have been made to develop improved cellular assays. Tests based on human basophils all have an initial stimulation step with allergen in common, but differ in the methodology to detect a response.

Basophil degranulation test

The original basophil degranulation test follows the morphological changes of allergen-stimulated basophils by light microscopy [29]. It is obvious, however, that the subjective microscopic evaluation represents a critical drawback of this test and although modified staining techniques with improved reproducibility have been reported for bee and wasp venom allergic patients [31], this cumbersome test never became part of routine practice.

Histamine and sulfidoleukotriene release tests

Another strategy that has been pursued relies on the analysis of mediators released by the activated cells. Cross-linking of IgE on basophils causes the release of both histamine and sulfidoleukotrienes, especially after pretreatment with cytokines such as IL-3 [32]. The measurement of these two mediators in the cellular supernatant by radio-immunoassays or enzyme-immunoassays

has traditionally been used to assess the validity of this diagnostic test in Hymenoptera venom allergy [33,34]. In general, the release of sulfidoleukotrienes has been found more sensitive than that of histamine [34,35]. Using a standardized commercially available sulfidoleukotriene release assay (CAST-ELISA) in a large group of individuals with a positive history, sensitivities were 88.5 and 94% for wasp and bee venom, respectively [10[•]]. Although the release of histamine and sulfidoleukotrienes often correlate, discordant results were noted [34,35], indicating that these mediators may be released via different pathways.

Although the potential diagnostic utility has thus been shown, basophil mediator release tests have not been widely used in routine diagnostic practice. This may be explained by the practical limitations inherent to all functional cellular tests, which can be identified in the preanalytical (e.g. sample storage), the analytical (e.g. priming conditions with IL-3, allergen concentration), and the postanalytical (e.g. positivity criteria) phase.

Basophil activation test

A major step forward in the development of functional basophil assays was made by the introduction of the analysis of basophil immunophenotype (i.e. expression of cell surface markers) by flow cytometry [36]. Upon in-vitro stimulation with allergen, basophils may rapidly upregulate specific cell surface molecules, of which CD63 and CD203c have been used in the diagnosis of stinging insect allergy. These allergen-induced changes of basophil immunophenotype have been used to analyze basophil responsiveness to Hymenoptera venom. It has been documented in over 10 controlled studies that this technique, the so-called basophil activation test (BAT), is sensitive (77–100%) and specific (70–100%) for Hymenoptera venom allergy, yielding results comparable to venom skin test and specific IgE results (summarized in [10[•]]). Moreover, it has recently been shown that the value of the BAT becomes more apparent when used in combination with other diagnostic tests. Ebo *et al.* [37] reported that in about half the patients with venom anaphylaxis, diagnosis is not unequivocal due to discrepant or negative results of skin test and specific IgE. The BAT helped to identify the culprit insect in the majority of these cases. Likewise, the combined use of skin test, specific IgE and cellular assays (i.e. BAT and CAST-ELISA) resulted in a positive predictive value of 100% for both bee and wasp venoms, if all tests were positive, and a negative predictive value of 100%, if at least one test was positive [10[•]]. These data basically confirm the proposed indications for BAT as suggested by Kleine-Tebbe *et al.* [24]: history of IgE-mediated allergy with important therapeutic implication (i.e., venom immunotherapy) combined with negative skin test and negative specific IgE, or discordance between skin test and specific IgE.

It remains to be established whether the BAT is useful in the follow-up of venom immunotherapy. Several papers have addressed this issue, but the data are not consistent. Two studies, one with wasp and one with ant venom allergic patients, concluded that the BAT failed to predict the outcome of a sting challenge after completed immunotherapy [28,38]. In contrast, other studies have shown lower frequencies of CD63 expressing basophils after immunotherapy, when the cells were submaximally stimulated [39,40], and this was associated with treatment success [39]. As these groups used different methodological conditions, it is obvious that further evaluation with standardized methods is necessary.

Basophil activation test: methodological aspects and perspectives

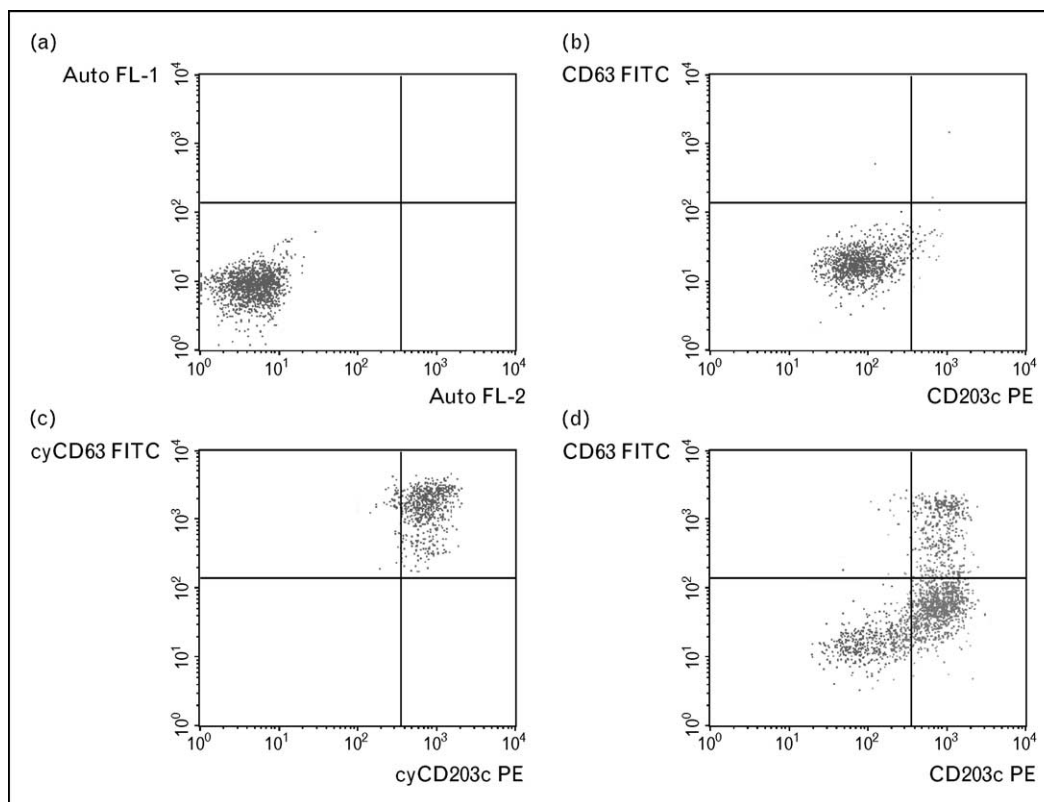
In-vitro measurements of allergen-induced basophil reactivity represent functional bioassays that are subject to more variables than quantifying specific IgE. These variables may be interrelated, such as the composition of the IgE repertoire [41[•]] and the intrinsic cellular sensitivity and reactivity of the basophils, and determine the performance and final outcome of basophil-based tests, regardless of the test readout used, that is, mediator release or surface marker expression [24]. Furthermore, a number of additional factors influencing the test result are introduced by the analytical protocol after blood sample collection. Methodological and technical aspects of sample preservation and storage, basophil preparation and stimulation, and detection methods for basophil reactivity may have an impact on test results and have been a matter of ongoing debate [24,25,42]. The comprehensive documentation of technical factors influencing BAT test results has only recently begun [43[•]]. Although the basophil mediator release assays and the basophil activation marker tests both 'suffer' from the above-mentioned variables, the latter lends itself better to routine diagnostic use for several reasons.

First, flow cytometry has an extraordinary sensitivity and is ideally suited for the measurement of leukocyte subsets in whole blood without the need for prior enrichment. This is important because in-vitro manipulation of basophils may induce priming or activation and thus influence test outcome [24]. In addition, assessment of basophil reactivity in conditions that mimic the basophil's natural environment as close as possible may have the advantage to integrate effects of regulatory factors that are normally present in the blood, but are lost when analyzing purified cells. This has recently been illustrated by a study using the basophil activation test for evaluating basophil responses in children with milk allergy. It could be demonstrated that basophils from individuals with milk allergy, but who are able to tolerate heat-denatured milk, directly stimulated in a whole blood

assay are less reactive to milk allergen than basophils from those who are reactive to heat-denatured milk. Interestingly, the suppressed basophil reactivity of the tolerant individuals was mediated in part because of a serum inhibitory factor [44[•]]. By the same rationale, it may be argued that the basophil activation test should be performed without the addition of extra priming factors such as IL-3, which is often done just for historical reasons. Although the two most recently reported studies on BAT and Hymenoptera venom immunotherapy documented a decrease in basophil responsiveness after therapy when using a whole blood assay with IL-3 preincubation, the decrease was only significant at submaximal stimulation conditions [39,40]. Given that IL-3 upregulates the protein tyrosine kinase Syk, which is associated with the ability of IgE-unresponsive basophils to regain responsiveness [45], it does not seem too far-fetched to propose that further BAT evaluation studies should be performed without IL-3 preincubation.

Second, flow cytometry enables us to examine basophils at the single-cell level in a multiparameter approach. This allows not only identification of the basophil in a heterogeneous leukocyte population but also simultaneous characterization. Most of the BAT protocols used today, including the ones that are commercially available, use one or two immunophenotypic markers to identify basophils (e.g. anti-IgE or, alternatively, CD123 and HLA-DR) and subsequently rely on only one marker (CD63 or CD203c) to determine basophils' activity. However, as may be appreciated from numerous other applications of multiparameter flow cytometry in the clinical laboratory, resolution and information dramatically increase when more markers are combined [46[•]]. This may be well exemplified when CD63 and CD203c are analyzed in combination. Resting basophils moderately express CD203c and only marginally express CD63 on the cell surface, whereas high levels of both markers are present inside all basophils (Fig. 2) [36]. In response to specific allergen, anti-IgE, or other stimulants, basophils show a typical and consistent activation pattern. First, CD203c is rapidly upregulated on the cell membrane, which may be accompanied by a dim upregulation of CD63. Subsequently, basophils may (or may not) continue to strongly upregulate CD63 expression, without an additional increase in CD203c. Up to three subpopulations of basophils may thus be detected after activation when both CD63 and CD203c are analyzed (Fig. 2). Interestingly, upregulation of CD203c by an activated basophil not only precedes but also appears to be a 'conditio sine qua non' for strong CD63 upregulation on the same cell. Although this activation pattern has been recognized by several groups (I. Heijnen *et al.*, unpublished data, and [47–49]), it has received little attention. It is, however, apparent that, although the pattern itself seems conserved, the relative distribution

Figure 2 Bivariate dot plot histograms illustrating the typical results of immunophenotypic analysis of basophils in whole blood from a patient allergic to bee venom

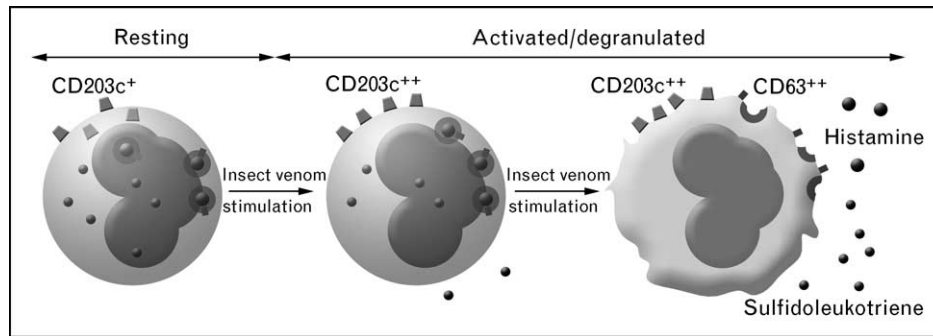


In comparison with baseline autofluorescence control (a), unstimulated resting basophils readily express moderate levels of CD203c and hardly detectable CD63 on the cell surface (b). High levels of CD63 and CD203c are present inside all basophils, as detected by cytoplasmic (cy) staining (c). After in-vitro stimulation for 15 min with 0.1 μ g/ml bee venom, different basophil subpopulations may be recognized: residual resting CD203c^{pos} CD63^{borderline} cells (d, lower left quadrant), CD203c^{bright} CD63^{dim} basophils (d, lower right quadrant), and CD203c^{bright} CD63^{bright} basophils (d, upper right quadrant).

of the different subpopulations depends on the stimulant, time of activation, and inherent cell and sample characteristics (e.g. IgE repertoire). The exact association of the identified subpopulations with mediator secretory processes is presently unclear. For instance, there have been convincing reports of a strong correlation of CD63 upregulation, anaphylactic degranulation (AND), and histamine release [35,36,48]. On the contrary, histamine may also be secreted via small vesicles in the absence of entire granule exocytosis, a process termed piecemeal degranulation (PMD) [50]. It is this alternative pathway that is suggested to be associated with upregulation of CD203c [51]. A unifying hypothesis would be that PMD is paralleled not only by a clear translocation of CD203c from intracellular stores to the plasma membrane, but also by a low level increase in membrane CD63 derived from intracellular granules accompanying mediator release. The low levels of CD63 on these cells may be detectable by sensitive flow cytometry (Fig. 2d, lower right quadrant). Depending on a number of factors, the basophil may then continue to AND, resulting in fusion between granules with the membrane, and, hence, rapid and

strong further increase of membrane CD63 expression (Figs 2 and 3). It is interesting to note that an association between CD63 mobilization and PMD has also been implicated in eosinophil activation [52]. Although the clinical association and diagnostic utility of multiparameter analysis of basophils by flow cytometry remain to be fully established, it is clear from the above discussion that the use of one single activation marker may cause confusion about what is meant by activation. In addition, in light of the above finding, it does not make much sense to debate which marker is the 'best' for measuring basophil activation [53]. These markers stain different but overlapping basophil subpopulations. Only the combined use of multiple markers may optimally identify those basophils that are clinically and diagnostically important. In this context, it is interesting to note that besides surface membrane expression, simultaneous assessment of intracellular signaling is feasible, as has recently been evaluated in birch pollen allergic patients [54*].

Finally, flow cytometry is the preferred measuring method for basophil-based tests because it is amendable

Figure 3 A model for basophil activation

In resting basophils, the majority of CD63 is associated with intracellular granules, whereas CD203c is partly present on the cell membrane and partly in intracellular stores (left cell). As a first stage of basophil activation (middle cell), CD203c translocates to the cell surface; CD63 may become dimly expressed on the surface as a result of vesicle transport [piecemeal degranulation (PMD)]. Subsequently, as a result of fusion between CD63-containing granules and cell membrane [anaphylactic degranulation (AND)], CD63 is expressed with high density without a concomitant further upregulation of CD203c (right cell). PMD and AND are accompanied by the release of mediators such as histamine and sulfidoleukotrienes.

to standardization, which is a prerequisite for introduction in the routine clinical laboratory. The benefits of standardizing flow cytometric methods have been illustrated previously for numerous different assays, including functional bioassays assessing cellular responses after antigen stimulation [55]. Unfortunately, to the best of our knowledge, multicenter studies evaluating unified BAT procedures have not been published yet. It is, therefore, not known whether the reported protocols used by the different laboratories allow meaningful comparisons between the different studies. The recent establishment of EuroBAT (a working group under the EAACI Interest Group on Allergy Diagnosis) holds promise for the development of standardized assays in the future.

Conclusion

Basophil responsiveness to Hymenoptera venom allergens can be detected *in vitro* by measuring the release of mediators or assessing basophil activation marker expression (BAT). BAT represents a valuable additional diagnostic tool in selected patients when used in combination with other well established tests. However, different BAT protocols are currently in use and because it is not known whether these protocols allow meaningful comparisons between different laboratories, multicenter standardization efforts are urgently needed.

In the future, diagnostic utility of the BAT to confirm clinical diagnosis and predict risk of subsequent systemic reactions to insect stings may be improved by use of multiparameter flow cytometry for combined analysis of surface marker expression patterns and intracellular signaling pathways. Until then, the BAT can be used to 'take the sting out of difficult cases' [37], provided that the test has been performed by an experienced laboratory using a validated assay, and that results have

been interpreted by a clinician familiar with the methodological aspects.

Acknowledgements

The authors thank Mrs Suzanne Link and Mr Dieter Hofer for their valuable assistance in preparing the figures.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Bilo BM, Rueff F, Mosbech H, *et al.* Diagnosis of Hymenoptera venom allergy. *Allergy* 2005; 60:1339–1349.
- 2 Klotz JH, deShazo RD, Pinna JL, *et al.* Adverse reactions to ants other than imported fire ants. *Ann Allergy Asthma Immunol* 2005; 95:418–425.
- 3 Fernandez J. Distribution of vespid species in Europe. *Curr Opin Allergy Clin Immunol* 2004; 4:319–324.
- 4 Sabbah A, Sainte-Laudy J, Hassoun S, *et al.* Allergy to mosquitoes and use of new immunobiological parameters. *Allerg Immunol (Paris)* 1997; 29:126–128.
- 5 Rolla G, Nebiolo F, Marsico P, *et al.* Allergy to pigeon tick (*Argas reflexus*): demonstration of specific IgE-binding components. *Int Arch Allergy Immunol* 2004; 135:293–295.
- 6 Bernstein IL, Li JT, Bernstein DI, *et al.* Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol* 2008; 100 (3 Suppl 3):S1–S148.
- 7 Perez Pimiento AJ, Vasquez Bautista AA, Prieto Lastra L, *et al.* Hypersensitivity to Vespula and Polistes: can we tell the primary sensitization from the clinical history? *Allergol Immunopathol (Madr)* 2007; 35:225–227.
- 8 Sturm GJ, Bohm E, Trummer M, *et al.* The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy* 2004; 59:1110–1117.
- 9 Eberlein-Konig B, Schmidt-Leidescher C, Rakoski J, *et al.* In vitro basophil activation using CD63 expression in patients with bee and wasp venom allergy. *J Investig Allergol Clin Immunol* 2006; 16:5–10.
- 10 Scherer K, Weber JM, Jermann TM, *et al.* Cellular in vitro assays in the diagnosis of Hymenoptera venom allergy. *Int Arch Allergy Immunol* 2008; 146:122–132.

This study investigates in detail the usefulness of a sulfidoleukotriene-release test and of flow cytometry-based assessment of basophil CD63 expression in the diagnosis of Hymenoptera venom allergy in comparison to other approved tests with respect to establishing the culprit insect.

- 11 Golden DB, Kagey-Sobotka A, Norman PS, *et al.* Insect sting allergy with negative venom skin test responses. *J Allergy Clin Immunol* 2001; 107:897–901.
- 12 Graif Y, Confino-Cohen R, Goldberg A. Reproducibility of skin testing and serum venom specific IgE in Hymenoptera venom allergy. *Ann Allergy Asthma Immunol* 2006; 96:24–29.
- 13 Reisman RE. Diagnostic venom skin tests and venom specific IgE assays: do we need to worry? *Ann Allergy Asthma Immunol* 2006; 96:5–6.
- 14 Bousquet J, Menardo JL, Aznar R, *et al.* Clinical and immunologic survey in beekeepers in relation to their sensitization. *J Allergy Clin Immunol* 1984; 73:332–340.
- 15 Van Halteren HK, van der Linden PW, Burgers SA, Bartelink AK. Hymenoptera sting challenge of 348 patients: relation to subsequent field stings. *J Allergy Clin Immunol* 1996; 97:1058–1063.
- 16 Rueff F, Przybilla B, Muller U, Mosbech H. The sting challenge test in Hymenoptera venom allergy. Position paper of the Subcommittee on Insect Venom Allergy of the European Academy of Allergology and Clinical Immunology. *Allergy* 1996; 51:216–225.
- 17 Spoerl D, Scherer K, Bach S, Bircher AJ. Sensitisation to wasp venom by one accidental injection in a patient desensitized for bee venom allergy. *Swiss Med Wkly* 2008; 138 (Suppl 163):60S.
- 18 Przybilla B, Rueff F, Fuchs T, *et al.* Insect venom allergy. *Allergo J* 2004; 13:186–190.
- 19 Hamilton RG. Diagnostic methods for insect sting allergy. *Curr Opin Allergy Clin Immunol* 2004; 4:297–306.
- 20 Van der Linden PW, Hack CE, Struyvenberg A, van der Zwan JK. Insect-sting challenge in 324 subjects with a previous anaphylactic reaction: current criteria for insect-venom hypersensitivity do not predict the occurrence and the severity of anaphylaxis. *J Allergy Clin Immunol* 1994; 94:151–159.
- 21 Sturm GJ, Heinemann A, Schuster C, *et al.* Influence of total IgE levels on the severity of sting reactions in Hymenoptera venom allergy. *Allergy* 2007; 62:884–889.
- 22 Wilson AB, Deighton J, Lachmann PJ, Ewan PW. A comparative study of IgG subclass antibodies in patients allergic to wasp or bee venom. *Allergy* 1994; 49:272–280.
- 23 Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol* 2004; 4:313–318.
- 24 Kleine-Tebbe J, Erdmann S, Knol EF, *et al.* Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int Arch Allergy Immunol* 2006; 141:79–90.
- 25 De Weck AL, Sanz ML, Gamboa PM, *et al.* Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008; 146:177–189.
- 26 Dubois AE, van der Heide S. Basophil-activation tests in Hymenoptera allergy. *Curr Opin Allergy Clin Immunol* 2007; 7:346–349.
- 27 Ebo DG, Bridts CH, Hagendorens MM, *et al.* The basophil activation test in the diagnosis and follow-up of hymenoptera venom allergy: an alternative point of view. *J Investig Allergol Clin Immunol* 2008; 18:493–494.
- 28 Brown SG, Haas MA, Black JA, *et al.* In vitro testing to diagnose venom allergy and monitor immunotherapy: a placebo-controlled, crossover trial. *Clin Exp Allergy* 2004; 34:792–800.
- 29 Shelley WB, Juhlin L. A new test for detecting anaphylactic sensitivity: the basophil reaction. *Nature* 1961; 191:1056–1058.
- 30 Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reaginic antibody. IV: Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 1966; 97:75–85.
- 31 Mumcuoglu Y, Wortmann F. Modified basophil degranulation test in diagnosis of bee and wasp sting allergies. *Allergy* 1980; 35:335–340.
- 32 Demoly P, Lebel B, Arnoux B. Allergen-induced mediator release tests. *Allergy* 2003; 58:553–558.
- 33 Yocum MW, Johnstone DE, Condemni JJ. Leukocyte histamine release in Hymenoptera-allergic patients: correlation with skin test reactivity and changes following hyposensitization therapy. *J Allergy Clin Immunol* 1973; 52:265–277.
- 34 Maly FE, Marti-Wyss S, Blumer S, *et al.* Mononuclear blood cell sulfidoleukotriene generation in the presence of interleukin-3 and whole blood histamine release in honey bee and yellow jacket venom allergy. *J Investig Allergol Clin Immunol* 1997; 7:217–224.
- 35 Sainte-Laudy J, Sabbah A, Drouet M, *et al.* Diagnosis of venom allergy by flow cytometry: correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. *Clin Exp Allergy* 2000; 30:1166–1171.
- 36 Knol EF, Mul FP, Jansen H, *et al.* Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; 88:328–338.
- 37 Ebo DG, Hagendorens MM, Bridts CH, *et al.* Hymenoptera venom allergy: taking the sting out of difficult cases. *J Investig Allergol Clin Immunol* 2007; 17:357–360.
- 38 Erdmann SM, Sachs B, Kwiczen R, *et al.* The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004; 59:1102–1109.
- 39 Peternelj A, Silar M, Erzen R, *et al.* Basophil sensitivity in patients not responding to venom immunotherapy. *Int Arch Allergy Immunol* 2008; 146:248–254.
- 40 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* 2007; 72:196–203.
- 41 Christensen LH, Holm J, Lund G, *et al.* Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *J Allergy Clin Immunol* 2008; 122:298–304.
- The authors investigated how distinct properties of the IgE repertoire, such as total and allergen-specific IgE antibody concentration, IgE affinity, and IgE clonality affect effector cell degranulation.
- 42 De Weck AL, Sanz ML, Gamboa PM, *et al.* Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. II: Technical issues. *J Investig Allergol Clin Immunol* 2008; 18:143–155.
- 43 Sturm GJ, Kranzelbinder B, Sturm EM, *et al.* The basophil activation test in the diagnosis of allergy: technical issues and critical factors. *Allergy* 2009. [Epub ahead of print]
- This study thoroughly analyzes technical and other factors that influence the results of the basophil activation test.
- 44 Wanich N, Nowak-Węgrzyn A, Sampson HA, *et al.* Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. *J Allergy Clin Immunol* 2009; 123:789–794.
- The authors demonstrate the benefit of analyzing basophils in a whole blood setting by showing that basophils from milk allergic patients, who tolerate extensively heated milk, are less reactive to milk allergen when tested in a whole blood assay than those cells that have been enriched, in part because of a serum inhibitory factor.
- 45 Kopley CL. Antigen-induced reduction in mast cell and basophil functional responses due to reduced Syk protein levels. *Int Arch Allergy Immunol* 2005; 138:29–39.
- 46 Chattopadhyay PK, Hogerkerp CM, Roederer M. A chromatic explosion: the development and future of multiparameter flow cytometry. *Immunology* 2008; 125:441–449.
- This review highlights the benefits of modern multiparameter flow cytometry and discusses new clinical applications of this technology.
- 47 Buhring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004; 133:317–329.
- 48 Hennersdorf F, Florian S, Jakob A, *et al.* Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* 2005; 15:325–335.
- 49 Chirumbolo S, Vella A, Ortolani R, *et al.* Differential response of human basophil activation markers: a multiparameter flow cytometry approach. *Clin Mol Allergy* 2008; 6:12.
- 50 Dvorak AM, MacGlashan DW, Morgan ES, Lichtenstein LM. Vesicular transport of histamine in stimulated human basophils. *Blood* 1996; 88:4090–4101.
- 51 Shreffler WG. Evaluation of basophil activation in food allergy: present and future applications. *Curr Opin Allergy Clin Immunol* 2006; 6:226–233.
- 52 Mahmudi-Azer S, Downey GP, Moqbel R. Translocation of the tetraspanin CD63 in association with human eosinophil mediator release. *Blood* 2002; 99:4039–4047.
- 53 Eberlein-Konig B, Varga R, Mempel M, *et al.* Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006; 61:1084–1085.
- 54 Aerts NE, Dombrecht EJ, Bridts CH, *et al.* Simultaneous flow cytometric detection of basophil activation marker CD63 and intracellular phosphorylated p38 mitogen-activated protein kinase in birch pollen allergy. *Cytometry B* 2008; 76:8–17.
- This study demonstrates that antigen-induced p38 MAPK phosphorylation in human basophils essentially contributes to CD63 upregulation and is a sensitive and specific intracellular marker for allergy diagnosis.
- 55 Maecker HT, Rinfret A, D'Souza P, *et al.* Standardization of cytokine flow cytometry assays. *BMC Immunol* 2005; 6:13.