Diagnostic Tests Based on Human Basophils: More Potentials and Perspectives than Pitfalls

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Key Words
Basophil activation • Drug allergy • Flow cytometry • Food allergy • Insect venoms • β-Lactams • Latex • Myorelaxants • NSAID

Abstract
For the diagnosis of allergy, cellular basophil activation tests (BAT), e.g. histamine or sulfidoleukotriene release tests, have long been introduced, but the expression of basophil activation markers such as CD63 and CD203c detected by flow cytometry has attracted more recent attention. A recent opinion paper in this Journal has stressed not only the potential but also the possible pitfalls of flow-cytometric BAT. We have applied clinical validation of various BAT in various ways for several years, and our experience shows that these new technologies have more potentials and perspectives than pitfalls. A comprehensive review of clinically validated studies on allergy to aeroallergens, insect venoms, food allergens and drugs, e.g. myorelaxants, β-lactams, pyrazolones and non-steroidal anti-inflammatory drugs, as well as chronic urticaria shows clearly that even with different protocols, reproducible and meaningful results can be obtained. Although the available technologies may still be optimized and better standardized, there are no serious reasons to deprive allergic patients of clinically indicated BAT, which can be performed reliably by any laboratory with allergy and flow-cytometric capacity and expertise.

Introduction

In a recent issue of this Journal an opinion paper was devoted to diagnostic tests in allergy based on human basophils [1]. Although reviewing first many publications about histamine release as an outcome of basophil activation, the main discussion and criticisms of this paper focus on the flow-cytometric basophil activation test (BAT), which has recently attracted increased attention.

The overall picture is strongly influenced by the experience gained with anti-IgE- and allergen-induced histamine release. In a number of ways, expression of basophil activation markers, particularly when applied to diagnostic clinical evaluation of basophil activation, obeys different rules, and a number of pitfalls are simply counteracted by our long clinical experience with such tests. The above-mentioned opinion paper [1] also nourishes the general impression that the diagnostic use of flow-cytometric BAT is plagued with many unresolved ques-
tions and that it should be reserved for the time being to basophil-experienced laboratories.

In our opinion, some points need to be readdressed to complete the discussion. First, the clinical issues are discussed in a comprehensive review. More technical issues and controversies are addressed in a following paper [2]. Readers specifically interested in flow-cytometric BAT are also referred to some recent reviews [3–10].

Mechanisms of Basophil Activation

The mechanisms of IgE-receptor-mediated activation and the subsequent transmission of signals leading to the release of various mediators such as histamine, LTC₄ (sLT) and lymphokines, e.g. interleukin (IL)-4 and IL-13, have been well described [1] (fig. 1). However, the expression mechanisms of various membrane proteins, which are the basis of diagnostic BAT, as well as the various mechanisms of non-IgE-mediated activation have not yet been differentiated, leading to the impression that the same quantitative and qualitative rules may apply to the various outcomes of basophil activation (e.g. CD63/CD203c expression, histamine release, LTC₄ and lymphokine formation). Indeed, the authors [1] state that ‘the parameters comprehensively studied during histamine release form the basis of the test outcome, independently of the final test readout. Promoting cellular tests for the evaluation of IgE-mediated sensitization has the caveat of introducing a number of interrelated variables’. As a matter of fact, and as discussed in greater detail elsewhere [2], the various test readouts do not strictly follow the rules established for histamine release: a number of differences in their activation cascades and regulation have been well documented [11–19].

These differences probably explain why in clinical practice, although often correlated, the various outcomes may occur independently from each other in some individuals (fig. 1).

For example, in a number of individuals and according to the mode of activation, expression of CD63, LTC₄ production and histamine release may be entirely dissociated for the same allergen concentration (fig. 2). In addition, for in vitro IgE-mediated reactions, the expression of CD63 is much more sensitive to the external Ca²⁺ concentration than LTC₄ production, and this relative Ca²⁺ sensitivity varies from one individual to another. This has led in some circumstances to the paradoxical finding of a control negative BAT but positive sLT (CAST assay) [Sanz, unpubl. data]. Even the expression of two different

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**Fig. 1.** Dissociation of basophil activation outcomes in some individuals. Despite overall correlation between skin prick tests, sLT (CAST), histamine release and basophil activation (CD63; % = % of activated cells), individual patients may show total dissociation between these outcomes. Data for peach-allergic patients and rPrp p 3 allergen [data from ref. 71]. nPru p 3: correlation between BAT and CAST (a; r = 0.39); BAT and the histamine release test (b; r = 0.66); BAT and the skin prick test (SPT; c; r = −0.35), and CAST and the histamine release test (d; r = 0.49).
membrane markers, CD63 and CD203c, obeys different rules and regulations [20, 21].

One must therefore be careful when extrapolating general conclusions drawn from experience gained with histamine release to other BAT, a point more extensively discussed elsewhere [2].

**Clinical Issues in the Diagnostic Use of BAT**

In addition to the large number of anecdotal studies at the beginning of the BAT era in the 1990s [22–24], during the past 5 years, a large number of clinically validated studies have been published in peer-reviewed and highly ranked journals that focus on clinical allergy. Most of these publications emerged from European groups. Clinical studies encompassing at least 15–20 confirmed allergic patients were considered validated, demonstrated by compelling history and/or provocation tests as well as other positive skin tests and/or allergen-specific IgE for example. In addition, at least 10, non-allergic controls negative to skin tests and other allergy diagnostic tests with no history of aeroallergen sensitization should be included. For some allergens, e.g. food, enrolment of atopic patients is required, i.e. patients with IgE to aeroallergens but negative to challenge with the food allergen tested. Many of these studies have been reviewed recently [8–10]. Herewith, tables 1–7 summarize the results of validated clinical studies performed with various allergens such as inhalant allergens [25–33] (table 1), hymenoptera venoms [34–56] (table 2), latex [57–63] (table 3), food [64–80] (table 4) and drugs [81–125] (tables 5–7). The techniques applied are also indicated. In our opinion, these studies demonstrate the diagnostic utility of BAT for a number of clinical indications.

Since some specific questions or criticisms have been raised [1] about some of these studies, we feel it is appropriate to address them in more detail.

**Clinical Indications of BAT**

**Hymenoptera Allergy**

The utility of BAT in hymenoptera venom hypersensitivity [34–56] has been confirmed hitherto by many more than the two studies quoted by Kleine-Tebbe et al.
The main benefit of BAT in that indication is that it may confirm clinical history in the absence of positive skin tests or positive allergen-specific IgE. In addition, by using BAT, the insect responsible for clinical sensitivity may be detected leading to a more specific indication for immunotherapy [43, 45]. There is no reason to cast doubt on the results, as possibly due to aggregated platelets [1], since specific investigations on that point [54, 126] have been negative. In the largest series reported so far [44, 45], correlation of BAT with skin tests ranged from 0.71 to 0.82. In a recent large series of 118 patients, a definite diagnosis of venom anaphylaxis [history + unequivocal single positive specific IgE (sIgE) and skin test] was observed in only about 55% of the patients. In 40%, diagnosis is hindered by equivocal or discrepant test results (mostly double-positive sIgE results for both venoms). In 7 patients (5%), there was no proof of sensitization (sIgE and skin test negative). Considering only BAT responders (86% of all patients), BAT confirmed the final diagnosis in almost two thirds of the patients with equivocal test results and, most importantly, in all 7 patients with negative sIgE and skin test results [48]. This paper [48] has documented that BAT may also be used to monitor venom immunotherapy, although

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Authors, year</th>
<th>Allergens</th>
<th>Patients n</th>
<th>Controls n</th>
<th>Sens., %</th>
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<tr>
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<td>Lolium perenne</td>
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<td>29</td>
<td>Hauswirth et al., 2002</td>
<td>Bet v1, 2 Phil p 1, 3, 5</td>
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</table>

Baso = BASOTEST; BD = FastImmune Becton-Dickinson; HC = healthy controls; II = isolated leukocytes; NA = not available; Ref. = reference; Sens. = sensitivity; Spec. = specificity; SPT = skin prick test; Wb = whole blood. For listed studies, see the reference list. In addition, several other studies have been presented and published, sometimes in abstract form [28, 30–33].

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Authors, year</th>
<th>Allergens</th>
<th>Patients n</th>
<th>Controls n</th>
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<th>Spec., %</th>
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<td>hymenoptera</td>
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<td>36</td>
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<td>wasp</td>
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<td>38</td>
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<td>hymenoptera</td>
<td>AS 28 HC 8 91 90 Wb 63 –</td>
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<td>39, 40</td>
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<td>AS 50 HC 20 92 80 Wb 63 –</td>
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<tr>
<td>43</td>
<td>Eberlein-König et al., 2004</td>
<td>hymenoptera</td>
<td>AS 14 HC 5 78 100 Wb 63 +</td>
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<td>44, 45</td>
<td>Scherer et al., 2005, submitted</td>
<td>wasp</td>
<td>AS 150 HC 40 93 85 IL 63 +</td>
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<td>46</td>
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<td>wasp, bee</td>
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<td>wasp, bee</td>
<td>AS 43 HC 25 97 89 Wb 203c –</td>
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<tr>
<td>48</td>
<td>Ebo et al., 2007</td>
<td>wasp</td>
<td>AS 80 HC 14 86 100 Wb 63 +</td>
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</table>

AS = Anaphylactic shock; HC = healthy controls; II = isolated leukocytes; n = number of patients; Ref. = reference; Sens. = sensitivity; Spec. = specificity; SPT = skin prick test; Wb = whole blood. [For additional studies on insect venoms, see ref. 49–56.]
### Table 3. Clinically validated studies for latex allergens

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Authors, year</th>
<th>Allergens</th>
<th>Patients</th>
<th>n</th>
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<td>57</td>
<td>Ebo et al., 2002</td>
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<td>Hx+ ST+ sIgE+</td>
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<td>HC, STsIgE</td>
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<td>+</td>
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<td>HC, STsIgE+</td>
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<td>58</td>
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<td>latex</td>
<td>Hx+ ST+</td>
<td>43</td>
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<td>93</td>
<td>100</td>
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<td>+</td>
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<tr>
<td>59</td>
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<td>203c</td>
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AC = Atopic controls; HC = healthy controls; Hx = history; Il = isolated leukocytes; NA = not available; Prov. = provocation; Sens. = sensitivity; sIgE = specific IgE; Spec. = specificity; ST = skin test; Wb = whole blood.

### Table 4. Clinically validated studies for food allergens

<table>
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<tr>
<th>Ref.</th>
<th>Authors, year</th>
<th>Allergens</th>
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<th>Controls</th>
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<td>100</td>
<td>Il</td>
<td>63</td>
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In addition to the listed studies, a number of reports on individual food-related allergens have been published [71–79]. AS = Anaphylactic shock. For further information, see legend to table 3.

### Table 5. Clinically validated studies for myorelaxants

<table>
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<th>Ref.</th>
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<th>Controls</th>
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<td>Wb</td>
<td>63</td>
<td>–</td>
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<td>82</td>
<td>Abuaf et al., 1999</td>
<td>myorelaxants</td>
<td>AS, ST+</td>
<td>41</td>
<td>HC Prov.–</td>
<td>21</td>
<td>64</td>
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<td>AS, ST+</td>
<td>39</td>
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<td>17</td>
<td>54</td>
<td>100</td>
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<td>myorelaxants</td>
<td>AS, ST±</td>
<td>21</td>
<td>HC</td>
<td>10</td>
<td>79</td>
<td>100</td>
<td>Wb</td>
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<td>Kvedariene et al., 2006</td>
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<td>AS, ST?</td>
<td>47</td>
<td>AC 40, HC 5</td>
<td>45</td>
<td>36 (86°)</td>
<td>93</td>
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<td>rocuronium</td>
<td>AS, ST+</td>
<td>14</td>
<td>HC ST– Prov.–</td>
<td>8</td>
<td>91</td>
<td>100</td>
<td>Wb</td>
<td>63</td>
<td>+</td>
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AS = Anaphylactic shock. For further information, see legend to table 3.

* In patients investigated within 3 years after the clinical reaction.
When BAT are performed in whole blood, the question remains whether the decrease in reactivity observed after immunotherapy is due to competing venom-specific serum IgG antibodies, as shown for histamine release [127], or whether a true decrease in cellular reactivity also occurs in parallel.

**Latex Allergy**

In several validated studies [57–63] (table 3), BAT was shown to demonstrate a sensitivity and specificity for CD63 expression of >90%, with one exception in which sensitivity was reported to be 75% for CD203c and 50% for the CD63 assay [60]. BAT is the test which reflects the most closely clinical provocation. In particular, it enables to sort out the clinically ‘false-positive’ cases, namely those with negative latex challenge but positive latex-specific IgE determinations due to cross-reactive carbohydrate determinants [57]. With other allergens, IgE to cross-reactive carbohydrate determinants seems to have little functional activity [128], too, and BAT should permit to sort out such cases.

**Food Allergy**

Food allergy will probably become one of the areas of allergy diagnosis in which BAT will give most useful services [64–80]. Although double-blind, placebo-controlled food challenge (DBPCFC) is constantly advocated as the gold standard, it is recognized that it is not flawless and that it is in practice less often used than publicly advocated. In numerous instances, it is not practicable or even not ethical (e.g. history of anaphylactic shock). The clinical picture of the oral allergy syndrome (OAS) after single-blind provocation is so obvious that the requirement for DBPCFC in that instance is questionable. There are several validated studies in the literature (table 4), some including recombinant food allergens [66, 69–71]. In birch-allergic patients with or without OAS stratified by DBPCFC, cellular tests, e.g. CAST [73] and BAT [68], have been found to be better predictors of provocation outcome than quantitative food-specific IgE. BAT may help to discriminate between relevant and irrelevant IgE to apple [68] and it has also shown its diagnostic usefulness in allergies to various foods [74–80].
**Chronic 'Idiopathic' Urticaria**

Numerous cases of chronic ‘idiopathic’ urticaria seem due to autoimmune phenomena, in particular to autoantibodies directed against IgE or its receptors (IgE-RI), leading to immediate skin test reactivity against autologous serum but also to basophil hyperreactivity. An increasing number of studies has confirmed the usefulness of BAT with donor basophils in the diagnosis and pathogenetic elucidation of chronic urticaria [129–138].

**Drug Hypersensitivity**

It is probably in the field of immediate-type allergic reactions to drugs that BAT has attracted the most interest in recent years, due to the fact that the performance of drug-specific IgE tests has been rather poor, especially in other drugs than β-lactam antibiotics, and since they are not available for many drugs. BAT can also detect immediate-type drug hypersensitivities due to non-IgE mechanisms.

**Myorelaxants** [81–87]. There are currently five validated studies (table 5) showing the utility of BAT in the diagnosis of immediate-type reactions to myorelaxants, with a sensitivity of 54–91% for CD63 expression and 36% for CD203c expression [84]. One may argue that sensitivity is too low to be really useful in routine diagnosis [83], but one must bear in mind that it is at least equal if not superior to that of skin tests and drug-specific IgE. On the other hand, BAT is highly specific. Accordingly, similar to many other tests in drug allergy, a positive test in an individual case is probably meaningful, while a negative test does not permit to exclude clinical drug allergy. Sensitivity apparently increases up to 80% when BAT is performed shortly after the clinical reaction [85]. BAT has proven to be complementary to skin tests in the evaluation of cross-reactivity between myorelaxants [86].

**β-Lactams** [88–96]. In quoting only one [88] of the several validated studies on BAT in the diagnosis of β-lactam allergy and in pretending that CD63 BAT was only performed in patients with positive skin tests to β-lactams, Kleine-Tebbe et al. [1] have presented an incomplete view of the literature. If the percentage of positive BAT is only about 50% of skin-test-positive cases [88–90], it is also positive in about 40% of the patients with entirely negative skin tests and a positive clinical history of an immediate-type systemic reaction confirmed by provocation [92], who constitute about 25% of all β-lactam-allergic patients. A lower sensitivity has sometimes been found for BAT in whole blood, but this is neither a consistent finding (table 6) nor one obtained by direct comparisons [124]. In studies comparing sensitivity and specificity of BAT with those of sIgE to β-lactam in the same patients [87–89, 91, 92], sensitivity of BAT determined by basophil activation was apparently superior (5–10% or more). Sensitivity of β-lactam sIgE varied between 24 and 74% [86, 92, 93, 95] depending on the group of patients selected and the time elapsed since the clinical reaction. Recently, these results have been confirmed in a large multicentric study implicating 10 European groups, 181 β-lactam-allergic patients and 80 controls [95]. In that study, it was also shown that the combination with CAST increases sensitivity to 62%. In terms of diagnostic performance, the combined use of skin tests, sIgE and BAT increases sensitivity to 85% and the addition of CAST to 95%.

**Pyrazolones.** In IgE-mediated allergies to single analgesics, e.g. pyrazolones, metamizol has attracted particular interest [99]. These patients differ from those with the NSAID hypersensitivity syndrome: they react specifically to a single drug or to a group of drugs of similar immunological structure. In 26 patients reacting to metamizol, BAT was found positive in 42%. BAT was also highly specific (100%). In combination with skin tests, sensitivity increased to 69% and combined with CAST to 77%.

**NSAID** [100–107]. Since several authors have stated that blood leukocytes from patients with the NSAID hypersensitivity syndrome may produce sLTs, as detected by CAST [108], the dogma that no in vitro tests may be associated with that condition has been crumbling. The first studies on BAT and NSAID by Sanz et al. [100] and Gamboa et al. [101] have not been properly quoted and interpreted [1]. If among 60 patients, only 18 have been effectively challenged, the remainder (42 patients) had a documented history of at least two or more immediate reactions to at least two different NSAID. This is a rather strict clinical inclusion criterion. Furthermore, in an ensuing multicentric study [102] on two similar populations who have been challenged or not (but with at least two clinical events) and analyzed separately, the percentage of positive BAT was identical (about 75%). It has also been questioned [1] why a BAT test to some NSAID could be considered positive when the culprit drug itself would be negative. One should realize that the mechanism of BAT in the NSAID hypersensitivity syndrome has nothing to do with an immunological reaction and that the classical notions of specificity for culprit drug and cross-reactivity do not apply. In fact, BAT reactions in NSAID hypersensitivity are closely related to the pharmacological COX-1-inhibiting activity of NSAID and are strictly dose dependent [100–105]. The most likely and main mechanism of
basophil activation is the pharmacological abolition of prostaglandin E2 synthesis, a natural inhibitor of basophil activation, partly responsible for homeostasis [103–105]. In vitro, diclofenac and naproxen have a much stronger pharmacological activity than aspirin and are more likely to cause a BAT reaction in vitro, even if aspirin had been responsible for the clinical reaction in vivo. These findings [103, 105] have recently been confirmed in a large multicentric study including 12 European groups, 150 NSAID-hypersensitive patients and 163 controls [102]. A major new finding is that some NSAID-tolerant individuals also react to higher concentrations of NSAID in BAT: the clinical NSAID hypersensitivity syndrome appears to be reflected in a shift of the BAT dose-response curve to NSAID [101–105]. It has also been shown that an index based on BAT reactions to two concentrations of aspirin, diclofenac and naproxen (ADN index) enables to distinguish NSAID-hypersensitive from NSAID-tolerant patients with 80% accuracy [102]. Provocation indeed remains the ultimate proof needed for validation, but by now the BAT and the ADN index seem to be a practical option in many cases, e.g. when provocation cannot be done. There is some evidence that the mode of cell preparation (e.g. whole blood, buffy coat or isolated plasma leukocytes) may play some role in BAT reactivity to NSAID [103, 105] (table 7).

In some cases of anaphylactic shock to aspirin, an IgE-related mechanism has been suggested as the cause of a positive BAT [106]. It has also recently been claimed that diclofenac causes microscopic basophil degranulation but no CD63 expression [107]. Since these BAT-negative results have been obtained with drug concentrations 8–30 times lower than those found required for a positive BAT [97, 99], these results should not be considered relevant.

Others

Individual cases of immediate-type allergy to a number of other drugs or materials used in diagnostic or therapeutic procedures have been reported in recent years: iodinated povidone [109], cyclosporine [110], heparin [111, 112], chlorhexidine [113, 114], hydroxyethyl starch [115], patent blue [116, 117], omeprazole [118], hyaluronidase [119], *Viscum album* [120], Gelofusine [121, 122], dexamethasamine [123] or bovine serum albumin [124]. The main interest of BAT in such cases is to confirm the diagnosis, particularly when skin testing and/or determination of drug-specific IgE are not available.

In view of all these facts, the conclusion that the role of BAT in the diagnosis of drug hypersensitivity still remains to be elucidated [1], which has been drawn in some position papers [1, 139–141], no longer represents the state of the art.

**Indications, Performance and Interpretation of BAT**

BAT are not primary diagnostic measures; they are essentially complementary to skin tests and allergen-specific IgE determinations, particularly when these cannot be performed or have given equivocal/doubtful results in comparison with clinical history. They are particularly indicated in insect venom allergy, food allergy, latex allergy and the major immediate-type drug allergies. BAT appear to be useful for the study of cross-reactivity among various protein allergens and drugs and for monitoring immunotherapy with some allergens and particularly with anti-IgE antibodies [15, 125].

The proper performance of the test requires special attention to details, particularly regarding the storage of the blood samples, the preparation and quality of the reagents used and the flow-cytometric gating processes established. Most important is the quality and standardization of the allergens used, which must be submitted to special quality control measures for use in flow cytometry. If these flow-cytometric quality control measures are not met by commercially available allergens, the laboratory will have to perform them by itself. Matter of fact, experience with allergens is probably more important for quality BAT than experience with basophil handling, which is easily acquired by any flow-cytometric laboratory. Some of these technical details are discussed in the accompanying paper [2] and in various reviews [5, 8, 9].

In order to establish appropriate cutoff points for each allergen, it is necessary to have proper negative and positive controls as well as receiver-operating characteristic curves (ROC) establishing optimal sensitivity versus specificity [142]. In fact, the recommendation to determine cutoffs by this method [8] has been followed by the majority of groups having published BAT clinical studies for a number of years.

In the experience of the Pamplona group, for example, the following cutoff points offer the highest specificity and sensitivity values determined by ROC [4, 10]: for inhalant allergens >15%; food allergens >15%; latex >10%, hymenoptera venoms >10%; β-lactam antibiotics >5% and stimulation index (SI) >2; metamizol >5% and SI >5; aspirin and NSAIDs >5% and SI >2. For most other groups [8, 9], cutoff points have been very similar but usually not adding an SI as additional requirement. For
some special issues, e.g. NSAID, special criteria (e.g. the ADN index) [102] have to be applied.

It has been stated [1] that the quantitative degree of basophil activation, measured by the amount of histamine or sLT released or the quantitative expression of activation markers in BAT, has presumably no clinical diagnostic value. This statement needs some qualification. Some authors have indeed found a correlation between the degree of clinical sensitivity and the percentage of activated basophils [50], but other authors did not confirm this observation. In a recent paper [54], it has been claimed that patients at risk of anaphylaxis upon insect sting challenge may show higher BAT baseline levels, but this study used different technologies and mode of evaluation than most BAT studies reported hitherto. For muscle relaxants, a correlation between the degree of skin sensitivity and CD63 expression has been reported [82]. The same has been the case for the amount of sLT released (CAST) and nasal allergen provocation [143]. In hymenoptera venom allergy, the degree of BAT reactivity has been reported to be predictive of side effects during venom immunotherapy [50, 51], but this is not agreed upon by all [52]. The occurrence of high CAST and/or BAT levels to β-lactams has been correlated to systemic anaphylaxis [89]. Finally, it has been demonstrated that sLT levels (CAST) [73] and BAT [68] are highly predictive of DBP-CFC and of OAS in food allergy.

Conclusions

There should be little doubt for those following the literature that such tests are rapidly becoming an important addition to our allergy diagnostic capacities. However, the number of European groups possessing varied and sustained practical BAT experience in routine diagnosis is still rather limited. Furthermore, some defiance has been entertained for years by position papers often authored by scientists not yet using the method in their own laboratory and always requiring still ‘more evaluation’ [139, 140].

The most recent opinion paper [1] on this matter, to which we wish to contribute herewith with our BAT clinical experience, may give the impression of an ‘extraordinary variability of BAT responses’, of an avalanche of pitfalls and of a requirement to possess many skills before engaging in clinical BAT diagnosis, ‘which should be restricted to selected cases and to experienced laboratories’. It would be a pity if due to these warnings many patients would still be deprived of the benefits of BAT and flow-assisted allergy diagnosis.

In fact, the common positive scientific and clinical experiences with BAT gathered over a number of years are more valuable than the few technical issues still open to discussion, which are dealt with in detail elsewhere [2]. A special effort is under way to standardize procedures and evaluation at the European level [141]. In addition to a number of validated and published ‘home protocols’, we dispose at present of at least three widely clinically validated and commercially available BAT technologies (e.g. BASOTEST, Orpegen, Heidelberg, Germany; Fastimmune, Becton Dickinson, Mountain View, Calif., USA, and Flow Cast, Bühlmann Laboratories, Allschwil, Switzerland) [8, 10]. These may be considered as first-generation BAT. Modifications have been proposed (Allergenicity, Beckmann-Coulter, Marseille, France) [9] and additional second-generation tests still await an equivalent clinical validation. However, there are no rational or scientific reasons to temporize providing BAT services for proper clinical indications, in our opinion, for a clinical allergy group wishing to offer patients the benefit of optimal allergy diagnosis.

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