Current perspectives

Principles, potential, and limitations of *ex vivo* basophil activation by flow cytometry in allergology: A narrative review

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The major challenge of allergy diagnosis lies in the development of accessible and reliable diagnostics allowing correct prediction of the clinical outcome following exposure to the offending allergen(s) and cross-reactive structures. Since the late nineties, evidence has accumulated that flow-assisted analysis and quantification of *ex vivo*–activated basophils (according to the basophil activation test [BAT]) might meet this requirement for different IgE-dependent allergies and particular forms of autoimmune urticaria. Other so-called nondiagnostic applications of the BAT involve therapeutic monitoring, follow-up of natural histories, and identification of allergenic recognition sites. However, it has also become clear that appropriate use of the BAT necessitates knowledge about degranulation metrics and guidance to guarantee correct execution and interpretation of the results. Here, we have reviewed the most relevant applications and limitations of the BAT. Some personal statements and views about its perspectives are made. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: *Allergy, anaphylaxis, basophil, basophil activation, CD63, CD203c, flow cytometry, histamine, HistaFlow, IgE, mast cell, mast cell activation*

Abbreviations used

AIT:	Allergen specific immunotherapy
BAT:	Basophil activation test
Can s:	*Cannabis sativa*
CCD:	Cross-reactive carbohydrate determinant
CRD:	Component resolved diagnosis
DPT:	Drug provocation test
FCM:	Flow cytometry
HVA:	Hymenoptera venom allergy
IDHR:	Immediate drug hypersensitivity reaction
MC:	Mast cell
MRGPRX2:	Mas-related G protein–coupled receptor X2
sIgE:	Specific IgE
STAT5:	Signal transducer and activator of transcription 5
ST:	Skin test
TG-ROC:	2-Graph receiver operating characteristic
VIT:	Venom immunotherapy

Degranulation of basophils and mast cells (MCs) can be triggered by various processes (Fig 1[1-3]). IgE-dependent degranulation involves synthesis of allergen-specific IgE (sIgE) antibodies by plasma cells. These sIgE antibodies bind to their high-affinity receptor (FcεRI) present on the surface membrane of MCs and basophils to form sIgE-FcεRI complexes. Encounter of a specific allergen that cross-links such sIgE-FcεRI complexes can induce degranulation with release of preformed mediators and *de novo* synthesis of inflammatory mediators. However, IgE-mediated activation is not only achieved by traditional allergens but can also occur by means of lectins with a binding specificity that matches the glycosylation of IgE and/or the FcεRI, or by means of other molecules such as superallergens (protein Fv, HIV gp120) or *Schistosoma mansoni* IPSE/alpha-1.[4,5] IgE-independent activation results from occupation of various receptors, such as C3aR and C5aR (receptors for the anaphylatoxins C3a and C5a) or Mas-related G protein–coupled receptor 2 (MRGPRX2) on MCs by drugs, as observed in some immediate drug hypersensitivity reactions (IDHRs).[6,7]

Generally, diagnosis of sIgE-mediated allergies starts with history taking together with skin tests (STs) and/or quantification of sIgE level (including component resolved diagnosis [CRD]). However, as none of these tests is absolutely predictive, many have focused on *ex vivo* basophil activation tests (BATs) to close the gaps in their diagnostic instrumentation and to avoid sometimes potentially dangerous provocations. In BATs, functionality of the cells can be explored via quantification of released mediators and phenotyping of intracellular and/or surface changes by flow cytometry (FCM). This review focuses on the main applications and limitations of FCM-based BATs and provides some guidance to guarantee correct execution and interpretation of these tests. It appears that although BATs are more than a diagnostic aid, further standardization and harmonization are required before their entrance into mainstream use. Most of the points to consider relate to the infrastructure and expertise of the laboratory, choice of readout, allergen preparation, metrics of basophil

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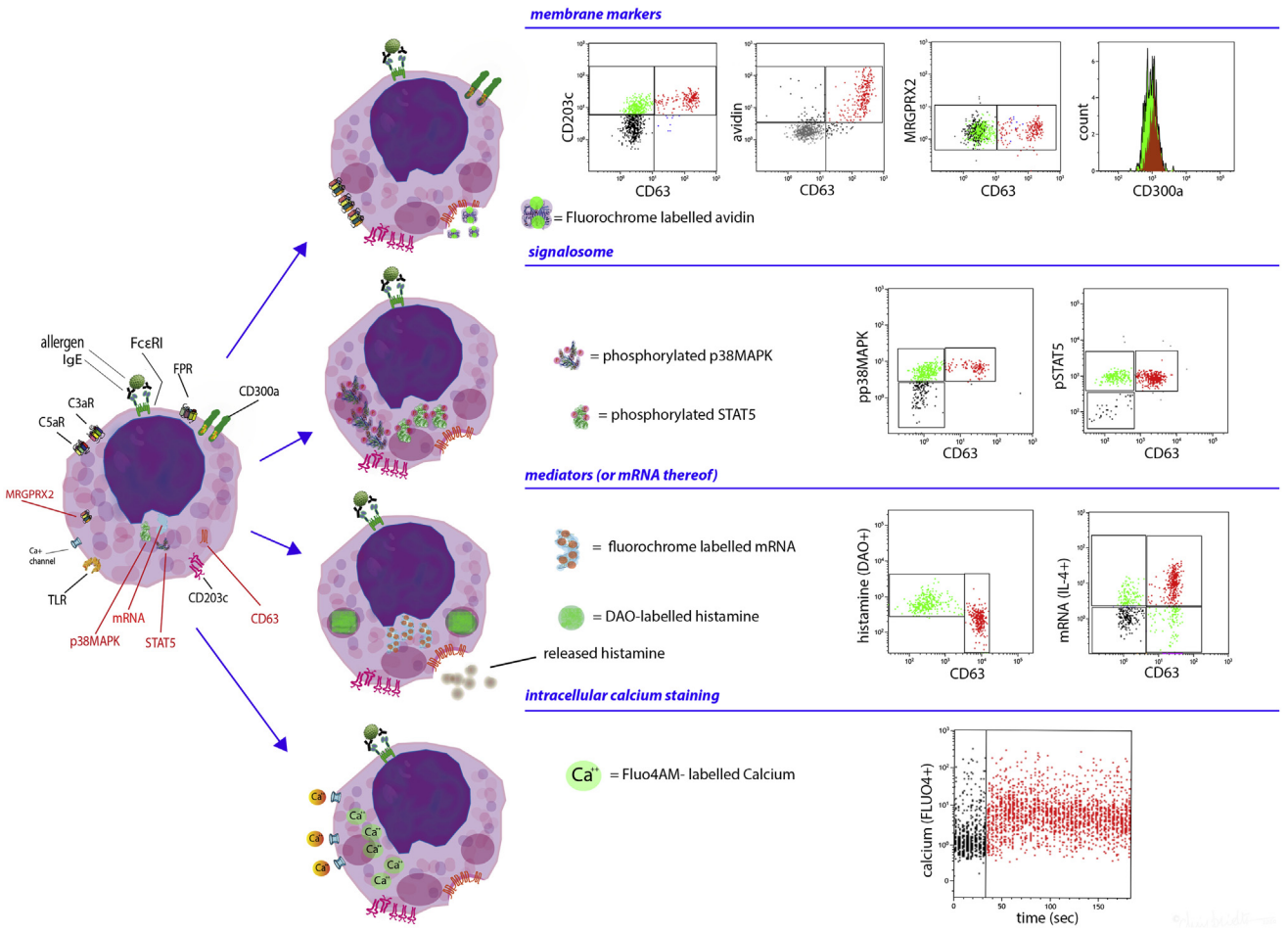

FIG 1. Basophil identification and activation/degranulation markers. Basophils express various molecules that can be used in isolation or in combination to identify the cells and measure their activation/degranulation status by flow cytometry in a technique designated as the BAT. Basophil activation/degranulation can occur via IgE/Fcεl-dependent and IgE/FcεRI-independent pathways. Individual cell activation/degranulation can be measured by FCM on 4 levels: (1) via appearance or upregulation of surface markers (such as CD203c, CD63, CD300a, MRGPRX2, and avidin binding of membrane-associated exteriorized anionic proteoglycans released from granules); (2) via phosphorylation of signaling molecules (such as p38 mitogen-activated protein kinase [p38MAPK] and STAT5); (3) via changes in mediator content (such as decrease of histamine or increase of trapped cytokines or their mRNA); and (4) via increased intracellular calcium staining. Intracellular molecules are denoted in pink. For more details on the identification and activation/degranulation markers see the text and Table E1 (available in this article's Online Repository at www.jacionline.org). For technical details, see Ebo et al,[1] Bridts et al,[2] and Elst et al.[3] *DAO,* Diamino oxidase; *pp38MAPK,* phosphorylated p38 mitogen-activated protein kinase.

activation, responder status of the cells, and correct positioning of the BAT in the diagnostic algorithm. It is needless to stress that state-of-the-art equipment and expertise are required for correct execution of commercial and/or homemade BATs and that interlaboratory comparison of results should benefit from harmonization and standardization of both instruments and protocols. Users of BATs must realize that data from different laboratories are not always readily interchangeable and that local validation procedures are mandatory.

THE BAT VIA FCM: PRINCIPLES AND TECHNICAL ASPECTS

Basophil identification and activation/degranulation markers

The foundations of modern FCM-based BATs date from 1991, with the first description of the lysosomal-associated membrane protein CD63 as being a degranulation marker of basophils.[8] At present, different protocols allowing detection of surface marker alterations, intracellular changes, and exteriorization of granular content have been developed.[1-3] As shown in Figs 1 and 2 and Table E1 (in this article's Online Repository at www.jacionline.org), traditional FCM-based BATs use whole blood and rely on cellular identification and quantification of activation and/or degranulation markers on the surface membrane. These changes are detectable and quantifiable on an individual-cell level by using specific fluorescent-labeled mAbs. Actually, in most studies basophils have been identified by scatter characteristics, reflecting cellular size and granularity combined with staining of surface markers such as IgE/CD203c, CCR3/CD3, or CD123/HLA-DR. Subsequently, after activation, the appearance or upregulation of specific markers, such as lysosomal CD63 or the lineage-specific ectoenzyme CD203c, is measured, with degranulating basophils being defined as $CD203c^{++}CD63^{+}$ cells. On the basis

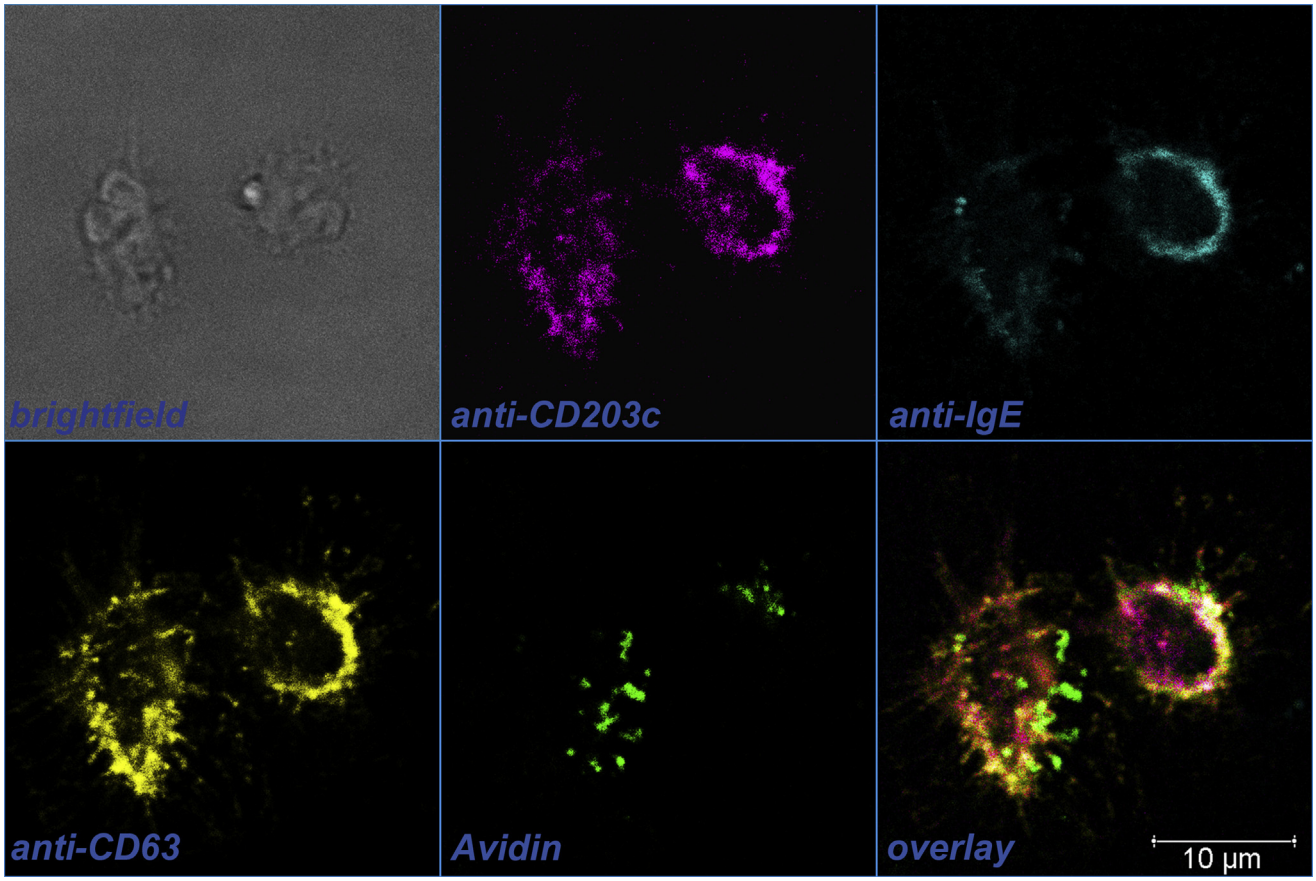

FIG 2. Confocal microscopy image. Confocal microscopy image of anti-IgE–stimulated basophils stained with anti-CD203c–APC, anti–surface IgE-AF405, antilysosomal-associated protein CD63-PE, and avidin-AF488 (binding membrane–associated exteriorized anionic proteoglycans released from granules), as well as composite view.

of divergent time kinetics, responses to secretagogues and/or inhibitory compounds, signalosome, and absence or presence of mediator release, a CD63 and CD203c compartment has been identified. The CD203c-compartment is characterized by a rapid and significant upregulation of CD11b, CD13, CD164, and CD203c.[9-11] In the CD63 compartment, maximum upregulation of CD63 and CD107a is slower and reflects anaphylactic degranulation.[11] For a comprehensive description of these activation marker profiles, their different relationships to piecemeal and anaphylactic degranulation, and their signal transduction processes, the reader is referred elsewhere in the *Journal of Allergy and Clinical Immunology*.[12] Whether the CD63- and/or CD203c-based microfluidic immunoaffinity BAT technique[13] and mass cytometry (CyTOF)[14] can keep their promise remains to be established. Other surface markers that can be used to quantify *ex vivo* basophil activation and degranulation are the inhibitory receptors CD300a and CD200R and the activating receptor CD300c.[15-17] In addition, basophilic activation can also be analyzed by studying the phosphorylation status of signaling molecules such as p38 mitogen-activated protein kinase or signal transducer and activator of transcription 5 (STAT5).[18,19] Degranulation of basophils can also be explored by measuring the exteriorization of granule matrix and decrease in intracellular histamine content. Briefly, anionic proteoglycans from exteriorized basophil granule matrix are stained by cationic fluorescent avidin probes,[1,20,21] whereas the quantification of the intracellular histamine relies on a histaminase affinity technique in which diamino oxidase is coupled to a fluorochrome.[22] Newly synthesized cytokines such as IL-4 and IL-13, or their mRNA, can be trapped and measured intracellularly.[23] Finally, basophil activation can also be measured by imaging of intracellular Ca^{++}.[24]

Whole blood versus separated cells and effects of IL-3

Most applied BAT methods use whole blood and study cells in their "natural" environment. However, there are various strategies to enrich and/or purify the cells.[25] If the analyses are performed in whole blood, one should verify the possibility for *in vivo* stimulation (eg, by exposure to IL-3). Such exposure is not associated with an upregulation of CD63, but it might be demonstrable by an upregulated expression of CD69, CD203c, p38MAK, and STAT5.[1,18,19,26,27] Note that in whole blood settings the outcome of the test can be influenced by blocking antibodies (through CD32)[28] or stoichiometrically via interference with the IgE-allergen interaction.[29] These blocking antibodies can be deleted by washing leukocytes or using purified basophils.[25] Although modern purification protocols are designed to avoid cell loss and activation (eg, by using low temperatures, $Ca2^{+}$/$Mg2^{+}$-free buffers [with added EDTA], and using negative

immunomagnetic selection), cell loss and background activation can occur.[30,31] Alternatively, basophils can be primed intentionally with IL-3 to optimize analytical sensitivity (ie, responsiveness to allergen[1,32,33] and autoreactive antibodies[34]).

Passive sensitization experiments

Although protocols have been developed to permit BATs up to 24 hours after sampling,[14,35] a major weakness of the test remains the necessity of viable basophils, as sensitivity of the test decreases over time.[33,36] To circumvent this limitation, several groups have adopted BATs with passive sensitization of stripped donor basophils.[37,38] This involves stripping of bound sIgE antibodies from their surface FcεRI receptors with the aid of acidic buffers, incubating these stripped cells with patient's serum (containing sIgE antibodies to the allergen being investigated), and finally challenging the passively sensitized basophils with the allergen at the first stage of the BAT. The donor cells for sensitization should be from a healthy subject whose basophils are known to be good responders. Both unstripped and stripped donor basophils should be included in the controls. This procedure, apart from being laborious and involving a number of extra steps, carries the risk of nonspecific stimulation or damage to basophils and is difficult to standardize. The results so far indicate rather varying performance. For example, in the study by Moneret-Vautrin et al,[39] a food-specific IgE level result between of 3.50 and 35 kUA/L was required for effective passive sensitization. In the study by Mueller-Wirth et al,[40] passive sensitization was already demonstrable at drug-specific IgE titers of 1.0 KUA/L. Whether, cell lines (eg, rat basophil leukemia or LAD2 MCs) or donor MCs constitute valuable alternatives to circumvent the limitations of BATs remains to be established, but the preliminary results seem promising.[41-44] In the comparative study by Larsen et al,[37] the BAT was shown to have better performance than histamine release testing and passive histamine release testing.

Selection of the optimal allergen and dose (degranulation metrics)

Other crucial elements to keep in mind are the correct selection of source material, preparation and storage of the allergen extract, and metrics of the allergen dose-response curves.[12] For many applications, BATs use crude allergen extracts.[45-47] However, the inherent variability and instability of natural allergens complicate selection of the best source material and optimal extraction procedure (for a review, see Larsen and Dreborg[48]). For example, thermal processing can influence the capacity of peanuts to trigger basophils. This impact seems highly divergent between patients and unpredictable by SDS-PAGE or IgE binding.[49] Or, as shown in Fig E1 (in this article's Online Repository at www.jacionline.org), in contrast to sesame oil, a whole sesame seed extract might not trigger basophil degranulation in a patient who has experienced sesame oil anaphylaxis. Fortunately, it is not all doom and gloom. On several occasions, BATs have been shown to benefit diagnosis in difficult patients demonstrating clinically irrelevant sIgE results because of sensitization to cross-reactive carbohydrate determinants (CCDs).[50,51] For a summary of allergen concentrations, see Hoffmann et al.[52] Note that these concentrations are only indicative and might not apply to laboratories using different equipment and protocols. It is also important to remember that there can be a significant difference between the stock concentration and final concentration in the aliquot. For drugs and related compounds, it is recommended that concentrations be expressed on a molar base.

Basophil responses are characterized by a broad variability necessitating use of different stimulation concentrations enabling construction of dose-finding curves (Fig 3).[53] These curves encompass different metrics, including basophil sensitivity, median effective concentration, and basophil reactivity, which differ according to the stimulation conditions and applied readout. For an explanation and implications of the metrics of dose-response curves, the reader is referred elsewhere.[12,54-57] Clearly, clinical validation of BATs cannot be considered appropriate when it failed to carefully establish allergen-specific dose responses. Ideally, these dose responses show a sigmoidal shape with a plateau or bell shape. However, because of the complexity of most allergens and relative affinity of different epitope-paratope interactions, dose-response curves can show unpredictable complex courses that can vary among allergens and subjects. In diagnostic settings, in which only a limited number of allergen concentrations are used, there is a chance of producing false-negative results. Nevertheless, in our experience, it is not rare to find 1 or 2 optimal specific stimulation concentrations, even for drugs that can induce false-negative results because of cytotoxicity. Other explanations for false-negative results are a nonresponder's cell status (as is observed in about 5% to 15% of the patients), poor storage conditions for the blood sample (analyses are best performed within 4 hours[33]), use of cytotoxic (concentrations of) allergens, degraded allergens,[58] (pharmacologic) interference with surface receptors,[59] inhibition by cross-reactive compounds,[60] and blunted responses because of a preactivated status of the cell.[30] Therefore, BATs should always include a negative control setting to assess spontaneous expression of the readout, a positive control to verify responsiveness of the cell on cross-linking of surface sIgE-FcεRI complexes. *N*-formylmethionyl-leucyl-phenylalanine, which acts independently from IgE-FcεRI can be of benefit to include such a positive control to verify cell viability. In nonresponders who do not react to positive control and allergen, negative results should be considered uninterpretable. If cells do not respond to a positive control but do respond to an allergen, the test can be considered positive provided there is no nonspecific stimulation in at least 3 to 5 (exposed) control individuals. Nonresponsiveness is attributed to disturbances in the signalosome of the FcεRI pathway, particularly failure to express the downstream tyrosine kinase Syk. Although IL-3 can restore nonreleaser status, this approach is of little help in traditional BATs, as it takes days for conversion.[61] It should be kept in mind that IL-3 can blunt responses measured via upregulation of expression of CD69 and CD203c and phosphorylation of p38 mitogen-activated protein kinase and STAT5.

Determination of the decision threshold (cutoff limit)

Validation of a diagnostic cannot be considered appropriate when it failed to establish a decision threshold differentiating between positive reactions and responses in control individuals. In such studies one should establish optimal allergen-specific decision thresholds and abandon predefined arbitrarily chosen cutoff limits for determination of sensitivity, specificity, predictive values, and performance of the test. Normally, the cutoff of *in vitro* tests is defined by the mean of blank tests plus or minus 3.3

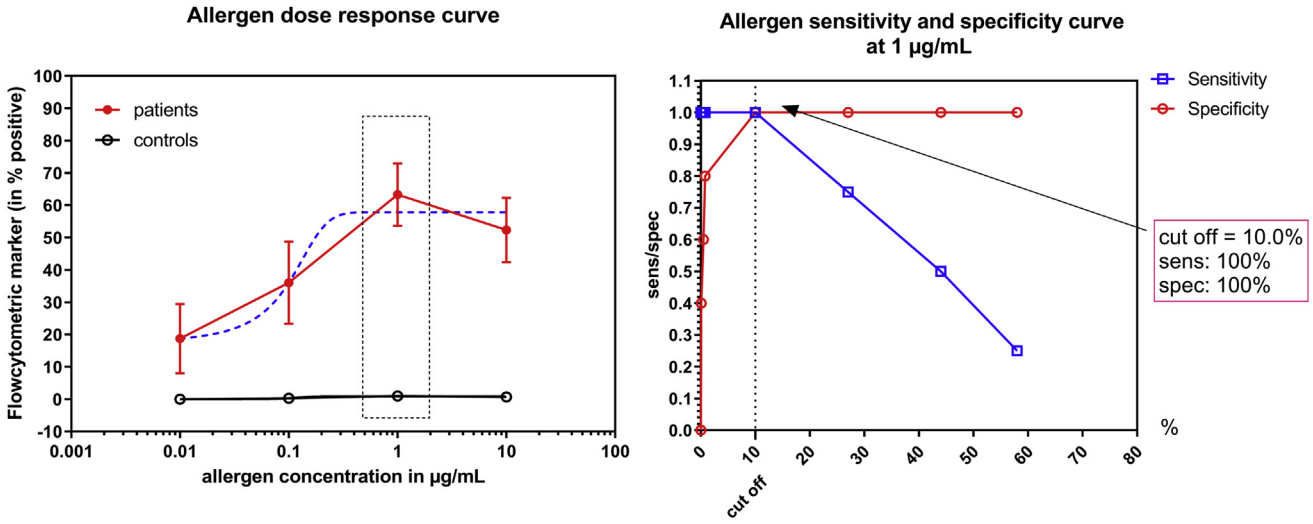

FIG 3. Allergen dose-response curve and TG-ROC analyses. Dose-response curve for *ex vivo* basophil degranulation by recombinant Can s 3 (rCan s 3), the nonspecific lipid transfer protein from *Cannabis sativa*, in 9 patients sensitized to Can s 3 (*solid symbols*). In contrast, in 7 control individuals, cells are unresponsive to Can s 3 (*open symbols*). Results are obtained from individuals responsive to positive control stimulation and expressed as a percentage of $CD63^+$ basophils; the dashed rectangle denotes 1 μg/mL as being the most discriminative stimulation concentration (*left*). Two-graph receiver operating characteristics curve combining sensitivity (*squares*) and specificity (*circles*) for the BAT rCan s 3 (1 μg/mL) (*right*). For a comparative validation of BAT rCan s 3 in cannabis allergy not restricted to Can s 3–sensitized patients, see Decuyper et al.[53]

SDs. However, for BATs this definition is rarely applied. As shown in Fig 3, an alternative method to calculate optimal decision thresholds is analysis of 2-graph receiver-operating characteristic (TG-ROC) curves. In TG-ROC curves, the test sensitivity and specificity are plotted against the threshold limit, assuming the latter to be an independent variable. For rare conditions, it might be difficult to include a minimum number of patients to construct TG-ROC curves. In such cases, comparison of the results in the patient(s) with control experiments in a minimum of 3 to 5 (exposed) control individuals is proposed. The importance of cutoffs in BATs is stressed by Dreborg, who argues the use of poorly documented decision thresholds.[62]

CLINICAL APPLICATIONS

Over 25 years, the BAT has evolved into a useful test in the evaluation of patients with inhalant, food, Hymenoptera venom, *Hevea* latex, immediate drug allergy, and some forms of chronic urticaria/angioedema. However, its place within the diagnostic algorithms is highly variable and often still poorly determined. Actually, the position of the BAT is determined by its ease of use in achieving a correct execution and interpretation, as well as by the availability of 1 or more safe, more accessible, and better-performing alternatives. For example, as drugs are manufactured according to the principles of Good Manufacturing Practice and only a few alternative easily accessible *in vitro* tests are available, it is likely that BATs deserve a more predominant place in the diagnostic algorithm for IDHRs than for other IgE-mediated allergies. For inhalant, food, Hymenoptera venom, and *Hevea* latex allergy, extract preparation and interpretation of results might be less obvious and the merit of the test must be seen in a more global context of other available diagnostics, including CRD.

Inhalant allergy

As shown in Table E2 (in this article's Online Repository at www.jacionline.org), the utility of the BAT has been explored in allergy to house dust mite, pollen and cat by using natural extracts and purified and/or recombinant components. Although the overall performance of BATs in inhalant allergy is good, the technique is rarely beneficial. Diagnosis of inhalant allergies can readily be established by other means such as STs and measurement of sIgE, including CRD. However, in cases of "entopy" (ie, cases of local allergic rhinitis with positive nasal provocation tests and negative skin prick tests and sIgE), BATs have allowed diagnosis in 8 of 16 patients.[63] Correlations between basophil sensitivity and nasal/bronchial provocation tests as well as asthma severity and efficacy of omalizumab treatment have been described.[54,64] BATs have also been used to monitor allergen-specific immunotherapy (AIT) for house dust mite, birch, timothy grass, *Lolium perenne*, mugwort, *Parietaria*, Japanese cedar, and cypress.[65-69] In these studies, reduced basophil sensitivity occurs early during AIT and is likely due to interference of blocking IgG antibodies.

Food allergy

The performance of the BAT in food allergy has already been studied extensively and reviewed. From these reports, the BAT has emerged as a potential diagnostic for primary and secondary food allergies, including the tick-borne alpha-gal syndrome.[70-72] However, it is premature to claim that BATs are "food challenges in a test tube."[71] The utility of BATs is allergen dependent and

requires validation for different allergens and phenotypes (eg, oral allergy syndrome vs anaphylaxis). However, such a validation might be challenging not only for reasons already addressed in this review but also because of distinct age- and geography-related sensitization patterns.[73,74] Finally, it should be kept in mind that test performance can depend on the control group. For example, in exploring a BAT's performance in the context of cross-reactivity syndromes, a comparison between patients and healthy control individuals is likely to result in overestimation of its specificity.[51,74,75] In other words, a comparison between true patients and healthy control individuals might not be representative for the general clinic population, in which one might need to differentiate between clinically relevant and irrelevant sensitization, rather than to dichotomize between patients and controls. Table E3 (in this article's Online Repository at www.jacionline.org) summarizes the sensitivity and specificity from BATs in food allergy. Predictive values are summarized in Table E9 (in this article's Online Repository at www.jacionline.org). BATs can be useful to discriminate between clinically relevant and irrelevant sIgE level test results or when no alternative *in vitro* tests are available[56,75-77] or for reducing the need for challenges in difficult cases in which the patient experienced severe anaphylaxis.[71,78] Alternatively, as already mentioned, one should keep in mind the possibility of clinically irrelevant BAT results induced by dietary lectins.[4,5]

With respect to BATs using components, it is noteworthy that a single component rarely covers the entire sensitization profile,[79,80] and that outcomes have been reported to be age and/or population dependent.[74] BATs have also been used to monitor AIT and allergen-specific oral immunotherapy for peanut,[57,81-83] sesame,[84] cow's milk,[85-87] and egg.[88] In line with the findings for aeroallergens, reduced basophil allergen sensitivity during AIT and oral immunotherapy with food is likely due to interference of blocking sIgG antibodies.[81-84,87,88] Acosta et al[89] recently provided an immunologic explanation as to why birch-associated apple allergy cannot be effectively treated by administration of the sensitizing pollen allergen (sublingual immunotherapy with recombinant *Betula verrucosa* 1, the major allergen from birch *Betula verrucosa*]). They found that treatment with recombinant *Betula verrucosa* 1 promotes specific IgG antibodies that cross-react with recombinant *Malus domesticus* 1 from apple but lack sufficient affinity to inhibit BATs with apple allergens and induce cross-protection. In contrast, treatment with the apple allergen induced a food allergen-specific *de novo* antibody response characterized by IgG1 antibodies with IgE-blocking bioactivity and specific for epitopes exclusive of the apple allergen. Some have claimed that BATs can also predict clinical severity and prognosis of food allergy[55,90-92] and food challenge responses,[56,92-94] predict thresholds of reactivity[55,95] to help determine when food can safely be (re)introduced,[96] and distinguish degrees of tolerance.[97] However, not all of the studies seem promising.[73,98] Therefore, and because of the possibility of reporting bias, additional comprehensive studies are required for confirmation. Ideally, these studies should compare the BAT with STs and/or CRD, as these might provide similar information but more easily. Recently, a passive BAT was used to demonstrate that mammalian glycolipid can activate allergic effector cells via surface-bound sIgE in alpha-gal allergy[38] and identify unique epitopes of certain peanut components.[99] The BAT has also been used to monitor the effect of omalizumab in food allergy.[100]

HVA

The utility of the BAT in wasp and honeybee venom allergy has been largely explored (see Table E4 in this article's Online Repository at www.jacionline.org). The BAT can benefit diagnosis of Hymenoptera venom allergy (HVA), especially in difficult cases that yield equivocal or negative sIgE and/or ST results. About 4% to 6% of patients with HVA demonstrate negative sIgE and ST results. In some of these cases, BATs can be useful to identify the culprit insect and guide treatment.[101,102] In addition, BATs can also help to take the sting out of difficult cases presenting with double-positive sIgE results resulting from sensitization to α-1,3-fucose–containing CCDs present on various Hymenoptera venom proteins.[101,103,104] However, the utility of the BAT in HVA needs to be reviewed in the global context of other diagnostics. With the venue of CRD using nonglycosylated recombinant proteins, the BAT likely lost ground; however, the technique remains useful in patients with negative ST and sIgE investigation results. It appears that the BAT is not predictive for severity of sting reactions.[105,106] With respect to venom immunotherapy (VIT), it has been shown to be a treatment to decrease basophil sensitivity but not reactivity.[105,107-111] The effects of VIT on basophils include early basopenia and a decrease in intracellular histamine content during maintenance treatment.[110] Some studies suggest that basophil sensitivity is predictive for side effects during the buildup phase of VIT.[108,112] However, this has not been the experience of others.[113] In patients with mastocytosis, the BAT adds little, if anything at all, to the diagnosis in cases with negative sIgE and ST results.[114,115]

Hevea latex allergy

As shown in Table E5 (in this article's Online Repository at www.jacionline.org), the first descriptions of the BAT in the diagnosis of allergy to latex from *Hevea brasiliensis* dates back from almost 2 decades ago.[51] The technique predominantly proved to be helpful to discriminate between clinically relevant and irrelevant sIgE results, the latter resulting mainly from sensitization to CCD and profilin.[116] However, as in HVA, with the venue of CRD using nonglycosylated components, the utility of the test lost ground. Alternatively, the BAT can help when other tests are unavailable (eg, sensitization to *Hevea brasiliensis* 12, the nonspecific lipid transfer protein from *Hevea*).[117]

Can s

Allergy to *Cannabis sativa* (Can s) has become a significant health problem and can be associated with complex cross-reactivity syndromes involving many vegetables, fruits, and latex.[53,118] Diagnosis of cannabis allergy can be challenging, mainly because of the moderate specificity of sIgE, ST results, or BAT results obtained by using natural extracts.[119] In such difficult cases, a BAT with Can s 3 (the nonspecific lipid protein of *C sativa*) can benefit correct diagnosis, especially as traditional sIgE testing is still not available.[119] The utility of the BAT with Can s 4 (the oxygen-evolving enhancer protein 2 [OEEP2]), in Can s 3–negative patients remains to be established.[120] Note that Can s 3 covers approximately two-thirds of the *Cannabis* IgE reactivity profile.[53,119]

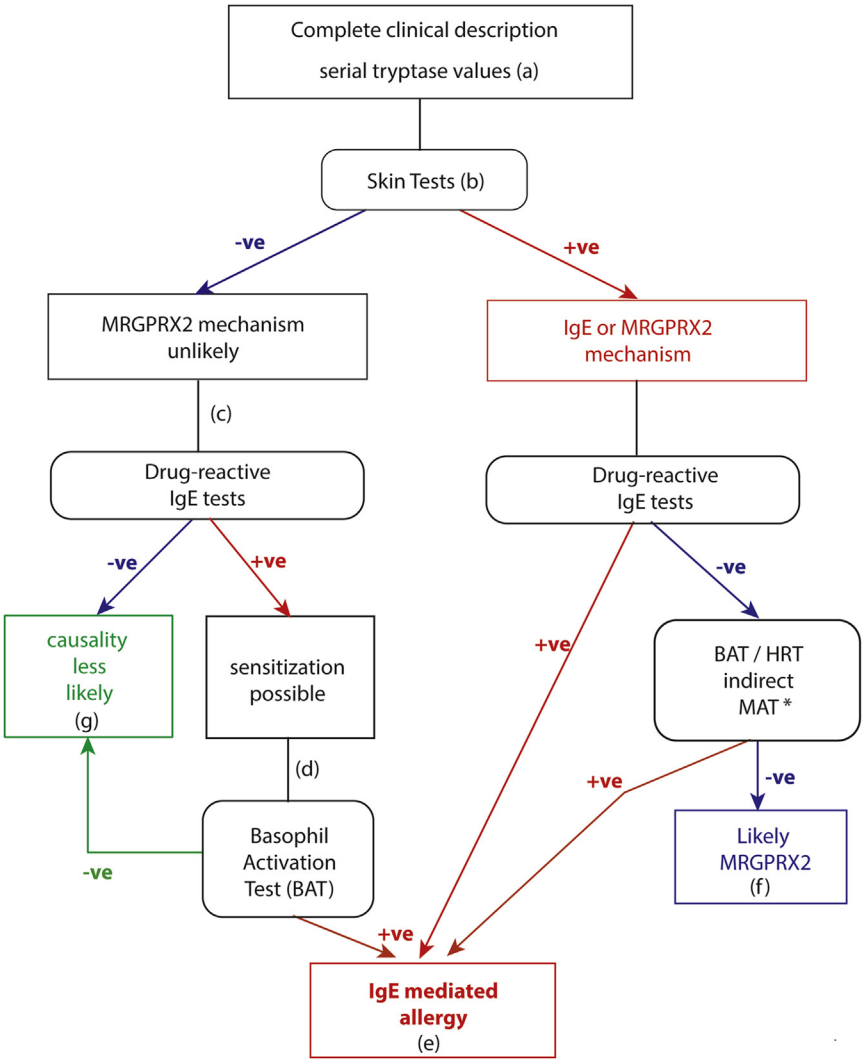

FIG 4. Resolving MRGPRX2- and IgE-binding properties of drugs: guidance. **A,** Referring physicians should provide complete information and tryptase values. **B,** Allergologic work-up of immediate drug hypersensitivity generally starts with STs. **C,** Negative ST results do not preclude sensitization. **D,** Diagnosis of drug allergy rarely can rely on a positive sIgE result in isolation. **E,** Cases with a negative ST result have been reported in IgE-mediated drug allergy. An IgE-dependent reaction might be confirmed in a passive MC activation test (*indirect MAT). **F,** In patients with an evocative history and positive ST result but incongruent negative combined sIgE, BAT, and indirect MAT result, the results may indicate an MRGPRX2-dependent reaction. **G,** In cases with negative investigation results, drug challenges might be indicated. Note that other mechanisms of IgE/FcεRI-independent MC activation by drugs can occur. *sens,* Sensitivity; *spec,* specificity.

Drug hypersensitivity

Although drug provocation tests (DPTs) are considered the criterion standard, a DPT might be difficult because of ethical and practical considerations. Consequently, confirmation of IDHRs generally relies on STs and quantification of sIgE. Unfortunately, diagnosis of IDHRs is not always straightforward, mainly because of uncertainties associated with STs and unavailability of drug-sIgE assays. As shown in Tables E6 to E8 (in this article's Online Repository at www.jacionline.org), many have explored BATs as a confirmatory diagnostic in IDHRs. It has emerged that the performance of BATs in IDHRs varies considerably according to the investigated drug (class), decision threshold,[62,121] and time elapsed between the index reaction and testing.[122] Alternatively, application of *ex vivo* basophil experiments in IDHRs might extend beyond diagnosis.[123] Different groups have claimed utility of the BAT in diagnosing IDHRs to neuromuscular blocking agents, β-lactam antibiotics, carboplatin, opiates, iodinated contrast media, and biologics.[124-126] For some of these drug(s) (classes), however, the evidence is limited[127] or controversial.[58,121,128,129] In our experience, the BAT adds little to the diagnosis of (amino)penicillin hypersensitivity (unpublished data). In addition, the technique could deepen our insights in immune IgE/FcεRI and nonimmune mechanisms of IDHRs,[60,127,129,130] unveil cross-reactivity between structurally related substances,[127,131,132] benefit identification of antibody recognition sites,[130] and monitor effects of rapid desensitisation strategies.[133,134] For some drugs such as opiates, the BAT is likely to constitute the only diagnostic.[127,135] Fig E2 (in this article's Online Repository at

www.jacionline.org) shows an example of a BAT to identify opiates in a patient who experienced anaphylaxis from pholcodine but tolerated a codeine and morphine DPT.

It is evident that the BAT adds to diagnosis only in the case of IDHRs that involve basophil degranulation. BATs cannot document IDHRs resulting from enzymatic inhibition of cyclooxygenase, as happens in nonselective hypersensitivity to nonsteroidal anti-inflammatory drugs and angioedema in response to angiotensin-converting enzyme inhibitors. Therefore, we do not advocate use of the BAT in this context. Alternatively, for selective nonsteroidal anti-inflammatory drug reactors, the BAT might benefit diagnosis and help to determine the presence or absence of a parent drug or metabolite reactive IgE antibodies.[136]

For diagnostic purposes, the fact that positive basophil responses cannot discriminate between sIgE-FcεRI cross-linking and alternative mechanisms is not a drawback. However, evidence of usefulness of BAT in IDHRs from IgE/FcεRI-independent pathways is limited and should be interpreted carefully. The reporting that basophils constitutively express the MRGPRX2 receptor[30] contradicts earlier observations,[3,137] and it is difficult to align with the observation that various MRGPRX2 agonists do not trigger basophil degranulation in control individuals.[121,128,129] Fig E3 (in this article's Online Repository at www.jacionline.org) shows plots of MRGPRX2 by MCs and basophils.[3] Fig 4 shows an algorithm indicating the place of BAT in the diagnostic work-up of IDHR. Particular attention is paid to the BAT's place in discriminating between sIgE-FcεRI– and MRGPRX2-mediated reactions.

Urticaria and angioedema

Acute and chronic histaminergic urticarias and angioedemas rest on MC and basophil degranulation via diverse innate and adaptive immune responses, including autoimmune processes.[138,139] At present, in chronic spontaneous urticaria, 2 groups of MC-degranulating signals have been identified (ie, IgE autoantibodies to autoallergens and IgG autoantibodies that target FcεRI or IgE-FcεRI complexes present on the MC surface). The presence of such anti-IgE or anti-FcεRI antibodies can be assessed functionally by using patients' sera in an autologous serum ST and/or to passively sensitize donor basophils in the autoimmune BAT (see Fig E4 in this article's Online Repository at www.jacionline.org).[140-142]

CONCLUSION AND FUTURE DIRECTIONS

The BAT provides the physician and clinical laboratory with a promising diagnostic approach, especially in difficult cases in which traditional tests are unavailable or yield uncertain results. However, before the BAT can enter into mainstream application, additional larger scale studies are required to confirm and critically verify some observations. Ideally, such studies should assess how the BAT relates to other diagnostics. Further automation of data analyses and bioinformatic tools should advance standardization and quality assurance and thus accelerate transition to the clinics.[143] Some points to consider and requirements to advance mainstream use of BAT are shown in Table E10 (in this article's Online Repository at www.jacionline.org). Although the nondiagnostic applications of the BAT are still in their infancy, with increasing use, the technique is expected to become an attractive and valuable asset to study various domains of basophil activation and degranulation biology. Evidence is emerging that the BAT might help to deepen our understandings in mechanistic endotypes of IDHRs, benefit identification of antibody recognition sites, expand our understanding of desensitization and tolerance induction strategies, and predict natural disease courses and prognosis.

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