

The basophil activation test in the diagnosis of immediate drug hypersensitivity

Expert Rev. Clin. Immunol. 7(3), 349–355 (2011)

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Hypersensitivity reactions to drugs account for 15% of all adverse drug reactions and represent an important health problem with significant morbidity and mortality. This article describes the current applications and perspectives of the basophil activation test by flow cytometry in the diagnosis of immediate-type drug allergy, with particular focus on its diagnostic performance in allergy to neuromuscular blocking agents, antibiotics and NSAIDs and on future applications.

KEYWORDS: basophil activation test • CD203c • CD63 • drug allergy • flow cytometry • specific IgE

Hypersensitivity reactions to drugs account for 15% [1] of all adverse drug reactions and represent an important health problem, causing major morbidity and associated mortality [2].

Although drug provocation tests (DPTs) are regarded as the ‘gold standard’ to establish or exclude the presence of hypersensitivity to a certain drug, DPTs are hampered by obvious ethical and practical issues and are therefore not suitable for routine clinical practice [3]. As a consequence, physicians have traditionally relied upon skin tests, quantification of specific IgE antibodies and basophil release tests to confirm their clinical suspicions. However, neither skin tests or quantification of specific IgE antibodies (if available) demonstrate an absolute predictive value and there is certainly room for novel diagnostic instruments. This article summarizes the current literature on the flow-assisted basophil activation test (BAT) in the field of drug allergy, focusing on its diagnostic performance.

Mechanism

IgE-mediated immediate-type adverse drug reactions are initiated by the introduction of drugs (or their metabolites), which are processed by antigen-presenting cells with subsequent activation and polarization of naive T-helper cells to a Th2 phenotype. These Th2 cells are involved in the switching of IgE class in B cells and in further maturation to IgE-secreting plasma cells. Secreted IgE sensitizes mast cells and basophils by binding to FcεRI. Subsequent exposure to the culprit drug (or its metabolites) leads, by

bridging/cross-linking of FcεRI receptors, to activation of these cells with consequent release of various mediators such as histamine, leukotrienes, prostaglandins and cytokines. These mediators are responsible for the pathologic reactions of an immediate hypersensitivity reaction, ranging from cutaneous symptoms (e.g., urticaria or angioedema) and respiratory symptoms (e.g., asthma) to anaphylaxis and anaphylactic shock [4].

Principle of the assay

Flow cytometry is a useful tool for the analysis of different cellular types and can be used to identify different cell populations, even basophils, that present <1% of the total number of leukocytes in the peripheral blood.

The principle of the BAT is described extensively elsewhere [5,6]. Briefly, the BAT relies on flow cytometric identification and quantification of alterations of specific activation markers on the surface-membrane or inside the basophils. These changes can be detected and quantified on a single-cell basis using specific monoclonal antibodies coupled to particular fluorochromes.

Practically, basophils are identified by specific markers such as CCR3⁺/CD3⁺, CD123⁺/HLA-DR⁺ and IgE⁺/CD203c⁺. Of these markers only CD203c is lineage specific.

In a second step, the upregulation of specific activation markers is quantified. CD63 and CD203c are the most commonly used cell markers (TABLE 1 & FIGURE 1). Furthermore, activation of basophils can also be measured by means

of intracellular molecules. For example, by measurement of p38 MAPK phosphorylation [7] or CD300a [8]. However, whether these markers are applicable for allergy diagnosis by BAT remains to be established.

Current applications

Currently, the BAT has multiple applications. The technique has proven to be sensitive and specific for the diagnosis of several IgE-mediated allergies including inhalant allergens, food allergies, hymenoptera venom allergy and natural rubber latex allergy [9–11]. In the context of diagnosis of drug allergy, BAT has been validated for neuromuscular blocking agents (NMBAs) [10,12–16], β -lactam antibiotics and clavulanic acid [17–21], iodinated contrast media [22,23], the antiseptic chlorhexidine [24,25], gelatine-based plasma expanders and the infusion-excipient carboxymethylcellulose [11,26]. An important observation from these studies is the fact that chemicals, such as drugs, elicit less pronounced *in vitro* basophil activation, compared with protein allergens such as inhalant and food allergens. As this may lead to diagnostic error, it underscores the paramount importance of abandoning arbitrarily chosen thresholds and to apply allergen/drug-specific decision cutoff points.

Neuromuscular blocking agents

Neuromuscular blocking agents remain the most common cause (58%) of adverse reactions during general anesthesia [27]. Most of these reactions are immediate-type reactions and are induced by an IgE-mediated allergic response or a nonspecific histamine release. Although skin tests represent a primary tool for diagnosing these reactions, the BAT has been proven to be particularly relevant in cases where skin tests yield equivocal or negative results [28]. TABLE 2 summarizes the most relevant studies using the BAT in allergy to NMBA. It appears that the BAT is

highly specific but not very sensitive. As already exemplified, this rather low sensitivity could relate to the arbitrarily chosen decision thresholds. Variations in sensitivity probably reflect differences in timing between the reaction and the performance of diagnostic tests, differences in applied drug concentrations and in selection of patients/controls.

Alternatively, the BAT is useful in the identification of cross reactivity, which occurs more frequently in the aminosteroid-derived than in the benzylisoquinolone-derived NMBA [29]. As we advocate that the BAT can detect cross reactions not detected with skin tests and vice-versa, skin test and BAT are complementary [30].

β -lactam antibiotics

Diagnosis of allergy to β -lactam antibiotics generally relies upon skin testing and quantification of sIgE [31]. However, as addressed by Blanca *et al.* in particular cases novel instruments such as the BAT are gaining importance [31].

There are currently five studies that have evaluated the BAT in the diagnosis of β -lactam-allergic reactions with an appropriate number of well-characterized patients (TABLE 3). As for NMBA, the BAT appears to be highly specific but not very sensitive.

Quinolones

The diagnosis of immediate reactions to quinolones is still a matter of debate. The value of skin prick and intradermal testing is controversial [32,33]. The preferential diagnostic approach, therefore, is the DPT, which is not free of risk. Recently, the diagnostic value of the BAT in allergy to quinolones was studied by Aranda *et al.* in 38 patients, confirmed by history and/or provocation [34]. However, as three different types of quinolones (ciprofloxacin, moxifloxacin and levofloxacin) were analyzed together, more studies need to be carried out before the BAT can be validated in quinolone allergy.

Aspirin & NSAIDs

Hypersensitivity reactions to NSAIDs are very common in patients with asthma or chronic urticaria. It is commonly accepted that the majority of these reactions are not IgE mediated and correspond to a pharmacological mechanism caused by the inhibition of COX-1, which results in depletion of prostaglandin E2 with unstrained synthesis of cysteinyl leukotrienes and mediator release from basophils, mast cells and eosinophils. Controversy exists as to whether the BAT can be useful in the diagnosis of NSAID hypersensitivity (TABLE 4) [35–40].

However, a minority of the adverse reactions to NSAIDs appear to be 'real' IgE-mediated reactions and patients react exclusively to a single NSAID family [41]. So far, two studies have been published on selective NSAID reactors to pyrazolones [42,43].

Table 1. Characteristics of the basophil activation markers CD63, CD203c, CD300a and phosphorylated p38 MAPK.

Characteristics	Cell surface activation markers			Intracellular activation markers
	CD63	CD203c	CD300a	Phosphorylated p38 MAPK
Synonym	Gp53 (LAMP-3)	E-NPP3	IRp60	Absent
Family	Tetraspanins	NPP3	Immunoglobulin superfamily	Kinases
Resting basophils	Barely detectable	Constitutively expressed	Constitutively expressed	Barely detectable
Lineage specific	No	Yes	No	No
IgE-activated basophils	Upregulation ~5 min Bimodal expression	Upregulation ~3 min Unimodal expression	Upregulation ~5 min Unimodal expression	Upregulation ~3 min Unimodal expression
Relation with anaphylactic degranulation	Yes	No [†]	Under investigation	No [†]

[†]Additional CD63 measurement is necessary to establish the nature of the degranulation process. Adapted from [6]. Additional data from [7,8,49–52].

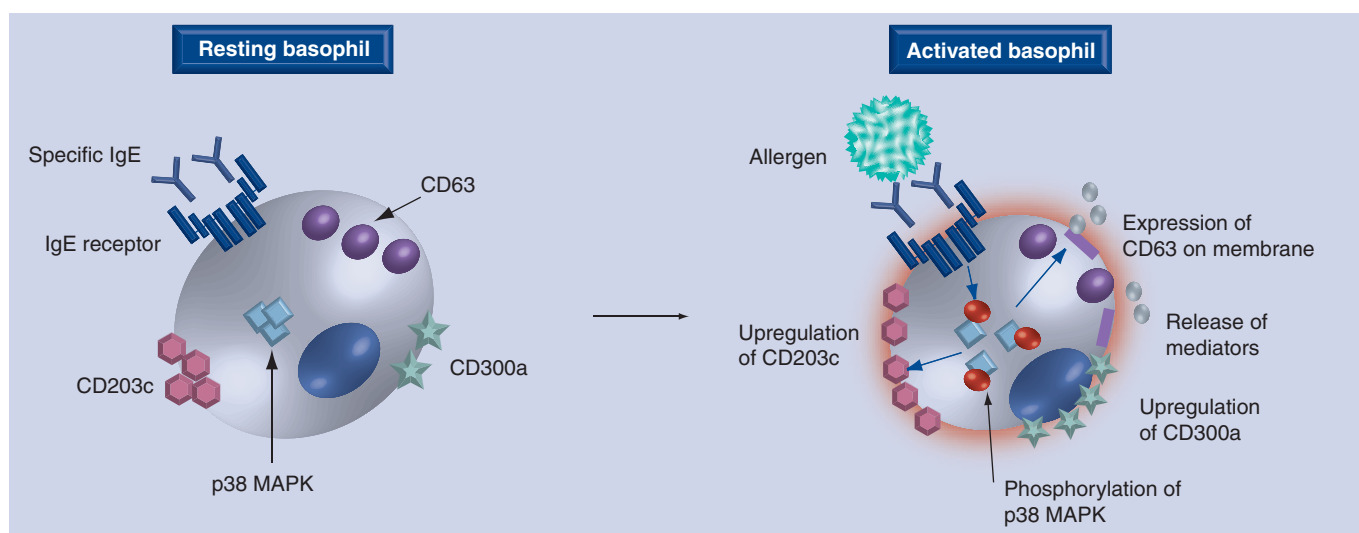


Figure 1. Principle of the basophil activation assay. Upon cross-linking of membrane-bound IgE, basophils upregulate the expression of specific activation markers such as CD63, CD203c and the expression of the inhibitory receptor CD300a. The exact mechanisms that govern basophil degranulation remain elusive, but it has been demonstrated that phosphorylation of p38 MAPK exerts a pivotal role in cell activation. Adapted from [53].

In conclusion, hypersensitivity reactions to NSAIDs are very common but the majority of these reactions are not IgE mediated. Consequently, in most patients BAT will not be useful to document 'hypersensitivity' to NSAIDs.

Expert commentary

The BAT provides the physician with an additional instrument to diagnose drug allergy and to tailor possible safe alternatives. However, additional studies are needed before the BAT can enter mainstream use. As with all studies of this kind, particular interest should be paid to several issues that might affect the outcome.

First, owing to the small number of patients, most researchers make use of arbitrary cutoff points to set the threshold for positivity (e.g., >15% activation and a stimulation index >2). However, considering only a minority of the basophils are activated in drug allergy, calculation of drug-specific thresholds is mandatory. Therefore, the use of larger groups of well-defined patients and appropriate controls is necessary. In the absence of a reference test or a gold standard, it is, however, difficult to include many well-diagnosed patients.

Second, interest should also be paid to some confounding factors. Different studies showed a decrease in sensitivity of the BAT with increasing time interval between the reaction and the

Table 2. Diagnostic performance of the basophil activation test in the diagnosis of neuromuscular blocking agent allergy.

Study (year)	Stimulus	Reference test	Activation marker	Sensitivity (%)	Specificity (%)	Patients and control individuals (n)	Ref.
Abuaf <i>et al.</i> (1999)	NMBA	H	CD63 CD45	64 43	81 96	28	[12]
Monneret <i>et al.</i> (2002)	NMBA	H ± ST	CD63	54	100	56	[16]
Sudheer <i>et al.</i> (2005)	NMBA	H	CD63 CD203c	79 36	100 100	31	[13]
Kvedariene <i>et al.</i> (2006)	NMBA	H + ST	CD63	36–86 [†]	93	92	[14]
Ebo <i>et al.</i> (2006)	Rocuronium [†] nonresponders: 76	H + ST	CD63	92	100	22	[30]
Sainte-Laudy and Orsel (2008)	NMBA	H ± ST ± IgE	CD63	60	100	49	[15]

[†]Increasing sensitivity when only the reactions that occurred during the last 3 years before performance of the basophil activation test are taken into account. H: History; NMBA: Neuromuscular blocking agent; ST: Skin test.

Table 3. Diagnostic performance of the basophil activation test in the diagnosis of allergy to β -lactam antibiotics[†].

Author (year)	Reference test	Activation marker	Sensitivity (%)	Specificity (%)	Patients and controls (n)	Ref.
Sanz <i>et al.</i> (2002)	H	CD63	50	93	88	[17]
Torres <i>et al.</i> (2004)	H $\pm$ ST $\pm$ IgE $\pm$ DPT	CD63	49	91	110	[18]
Abuaf <i>et al.</i> (2008)	H $\pm$ ST	CD203c CD63	52 22	100 79	41	[19]
Eberlein <i>et al.</i> (2010)	H $\pm$ ST $\pm$ IgE	CD63-CCR3 ⁺ CD63-IgE ⁺	55 53	100	39	[20]
De Weck <i>et al.</i> (2009)	H	CD63	50	89–97	262	[21]

[†]Stimulus used was β -lactam antibiotic.

*Selection marker.

DPT: Drug provocation test; H: History; ST: Skin test.

performance of the BAT: NMBA [16], NSAIDs [42] and β -lactam antibiotics [44]. Usual recommendations on the interval between the reaction and the performance of BAT are 6–12 months. However, more longitudinal follow-up studies are necessary to determine the optimal time of performing the BAT for each drug.

Another potential confounding factor is the medication used by the patient at the time the blood sample is collected,

cross-linking, fMLP can therefore not be used to control whether the basophil is responsible for IgE-mediated activation. Although nonresponders represent 5–10% of patients and control individuals tested, they are often not reported. However, nonresponders not only influence sensitivity and specificity of the assay, but most importantly nonresponsiveness can result in false-negative results with potentially dangerous implications for the individual patient.

where special attention should be paid to antihistamines and glucocorticosteroids. Nevertheless, Sturm *et al.* could not prove a significant influence of antihistamines or glucocorticosteroids after an incubation of 3 h, however, considering this does not correlate well with the clinical situation, no hard conclusions can be drawn [45,46]. Exactly how *in vivo* treatment with anti-allergic drugs alters basophil reactivity also awaits further investigation.

Finally, using the BAT must contend with the issue of nonresponders, that is, individuals whose cells are unresponsive to Fc ϵ RI cross-linking, with anti-IgE or anti-IgE-receptor I, and fail to upregulate expression of CD63 and/or CD203c. Importantly, as activation of the basophil with *N*-formyl-methionine-leucine-phenylalanine (fMLP) does not act via Fc ϵ RI

Table 4. Diagnostic performance of the basophil activation test in the diagnosis of allergy to NSAIDs.

Author (year)	Stimulus	Reference test	Activation marker	Sensitivity (%)	Specificity (%)	Patients and controls (n)	Ref.
Gamboa <i>et al.</i> (2003)	Metamizol	H $\pm$ DPT	CD63	42	100	56	[43]
Gomez <i>et al.</i> (2009)	Pyrazolones	H $\pm$ IDT $\pm$ DPT	CD63	55	86	107	[42]
Malbran <i>et al.</i> (2007)	Diclofenac	H	CD63	No significant difference in CD63 expression between patients and controls (IgE-independent basophil degranulation)	No significant difference in CD63 expression between patients and controls (IgE-independent basophil degranulation)	26	[37]
Gamboa <i>et al.</i> (2004)	NSAID	H $\pm$ DPT	CD63	15–55	74–100	90	[40]
Bavbek <i>et al.</i> (2009)	ASA Diclofenac	H $\pm$ DPT	CD63 CD203c CD63 CD203c	34 17 17 22	79 100 92 100	42	[35]
Celik <i>et al.</i> (2009)	ASA	H $\pm$ DPT	CD63 CD203c	30 70	40 45	20	[36]
Rodriguez-Trabado <i>et al.</i> (2008)	NSAID	H	CD63	43	100	72	[54]

ASA: Aspirin salicylic acid; DPT: Drug provocation test; H: History; IDT: Intradermal skin test.

Multiple case reports and series concerning drug allergy and its diagnosis using BAT have been described, such as the use of the BAT in diagnosis of allergic reactions to contrast media [22,23], or allergic reactions to the antimicrobial agent chlorhexidine [24,25,47].

The BAT appears particularly useful in those cases where other *in vitro* tests are lacking and clinicians must rely on potentially dangerous provocation tests.

The potential to simultaneously investigate different drugs is also an important advantage of the BAT, especially in patients on multiple medications. Simultaneous testing of different drugs also offers the clinician a tool for the detection of cross-reactivity between drugs with similar chemical structures.

Five-year view

Although in the hands of experienced personnel, the BAT can constitute a reliable instrument to document drug allergy, it remains imperative to keep in mind that it does not provide absolute diagnostic accuracy. Theoretically, one can anticipate that the diagnostic performance of BAT might improve further with the application of novel gating such as CD203, with the combined use of extra- and intra-cellular activation markers such as p38 MAPK and CD300a (or combinations thereof) and with a better understanding of the association of CD63 and CD203c expression and the release of mediators such as histamine. Furthermore, it is hypothesized that the BAT, as it closely mirrors the *in vivo* situation leading to symptoms, might also be used for other purposes. For example, it might help to predict the potential of drugs (or related compounds) being developed for marketing, to trigger

basophil activation. The fact that in this context the BAT does not discriminate between IgE- and non-IgE-mediated reactions, should not be considered as a shortcoming.

Finally, it is anticipated that the BAT might help document allergies to newer drugs such as biologicals, particularly in the absence of other diagnostic tests.

With novel drugs such as biological agents currently entering clinical practice, novel patterns of drug hypersensitivity reaction might be observed and further challenge the diagnostic management [48].

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- The basophil activation test (BAT) is not a primary diagnostic tool; it is a test complementary to the skin test and quantification of allergen-specific IgE.
- The BAT is currently mostly applied in the diagnosis of allergy to β -lactam antibiotics and neuromuscular blocking agents.
- The BAT is particularly useful to confirm clinical suspicion without performing dangerous provocation tests in cases where no alternative test is available.
- Since it allows simultaneous testing of different drugs, the BAT can contribute in the identification of cross-reactive substances/safe therapeutic alternatives.
- In order to obtain optimal predictive values, it is mandatory to apply drug-specific decision thresholds that can be obtained from receiver operating characteristic-analysis between well-defined patients and controls (exposed to the drug).
- Finally, the BAT can be applied to assess the allergic potential of novel drugs that have recently entered the market.

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