In vitro Diagnosis of Immediate Drug Hypersensitivity: Should We Go with the Flow?

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Key Words

Basophil activation · Flow cytometry · HistaFlow[®] · Immunoglobulin E · Immediate drug allergy

Abstract

Background: Diagnosis of immediate drug hypersensitivity reactions (IDHRs) is based upon history taking, skin prick or intradermal tests and quantification of specific immunoglobulin E (IgE) antibodies. Unfortunately, this is often insufficient to correctly identify patients with IgE-mediated IDHRs and is impossible in the case of non-IgE-mediated IDHRs. Drug provocation tests (DPT) are considered the 'gold standard' diagnostic but are not always possible, for ethical and practical reasons. Therefore, the validation of new cellular tests such as basophil activation testing (BAT) was necessary. This review focuses on the applications of BAT in IDHRs. **Methods:** A literature search was conducted, using the words basophil, flow cytometry, immediate drug allergy and drugs; this was complemented by the authors' own expertise. **Results:** BAT/HistaFlow[®] is a useful diagnostic tool in IDHRs, mainly used to diagnose allergy to neuromuscular blocking agents (NMBAs), antibiotics, nonsteroidal anti-in-

flammatory drugs (NSAIDs) and iodinated radiocontrast media. Its sensitivity varies between 50 and 60%, and specificity attains 80%, except for with quinolones and NSAIDs. **Conclusions:** The diagnostic utility of BAT (and to lesser extent HistaFlow) has been demonstrated and is mostly applied in IDHRs. However, larger-scale collaborative studies are necessary to optimize test protocols and validate the entry of BAT as a diagnostic instrument in drug allergy.

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Introduction

Although drug provocation tests (DPT) are considered the 'gold standard' diagnostic for immediate drug hypersensitivity reactions (IDHRs), such tests are impeded by the risk of life-threatening reactions and might even be contraindicated (i.e. in patients on beta-blockers or ACE-inhibitors) or, simply, be impossible [i.e. in patients with hypersensitivity to neuromuscular blocking agents (NMBAs)]. Besides, DPT do not exhibit absolute predictive values. DPT have not entered routine clinical practice. Diagnosis of IDHRs generally starts with a thor-

ough history-taking complemented by skin prick tests (SPT) or intradermal testing (IDT) and/or quantification of specific immunoglobulin E (sIgE) antibodies when an IgE-mediated activation of mast cells and basophils is suspected. Unfortunately, only a very small number of drug-sIgE assays are available and almost none have been clinically validated. In addition, it has to be kept in mind that IDHRs with release of mediators by circulating basophils and tissue resident mast cells do not involve IgE/FcεRI-cross-linking per se, but might result from alternative mechanisms that are undetectable by sIgE antibody assays. The latter reinforces the need for the development and validation of new cellular tests such as basophil activation testing (BAT). These tests can indeed be informative for IDHRs that are basophil-mediated and IgE-independent. This review focuses on the current applications of BAT in IDHRs. Starting with the authors' expertise, some misconceptions, shortcomings and unmet needs in in vitro IDHR diagnosis are highlighted as well. As with any subject attended by many questions, the alternative interpretations and some explanations or hypotheses expressed here may not find universal acceptance.

Basophils and Mast Cells

Basophils represent less than 1% of the peripheral blood leukocytes. They develop from CD34+ pluripotent progenitor stem cells and exhibit a segmented nucleus. They are identifiable by metachromatic staining with basic dyes (e.g. toluidine blue). Together with tissue-resident mast cells, basophils can be triggered in ways that are IgE-dependent and IgE-independent. Cross-linking of the surface-bound high-affinity IgE receptor (FcεRI) will generally occur via (glyco)proteins, chemical allergens or autoantibodies mounted against the FcεRI or membrane-bound IgE antibodies. If not IgE-dependent, activation will mainly result from the coupling of receptors with endogenous (e.g. cytokines, anaphylatoxins, chemokines, IgG and neuropeptides) or exogenous (e.g. pathogen-associated molecular patterns) substances. However, degranulation of basophils and mast cells can also result from other, largely unknown mechanisms (e.g. direct mast cell degranulation by opiates, iodinated contrast media, vancomycin and quinolones). Upon activation, basophils and mast cells will release a myriad of mediators that are responsible for early- and late-phase manifestations of the immediate allergic reaction.

BAT and HistaFlow®

The foundations of current flow-assisted BAT were laid a quarter of a century ago [1]. In the meantime, the technique has completely supplanted mediator-release assays that rely upon quantification of mediators released in the supernatant. The principles and requirements of BAT have been detailed elsewhere [2]. Traditional BAT relies on a flow cytometric analysis of activation and degranulation markers on the surface membrane. These changes can be detected and quantified on a single-cell level using specific monoclonal antibodies conjugated with different LASER-excitable fluorochromes. For example, basophils are traditionally identified by markers such as CCR3 (CD193)/CD3, CD123/HLA-DR or IgE/CD203c. Of these markers, only CD203c, the ectonucleotide pyrophosphatase/phosphodiesterase family member 3 enzyme (E-NPP3), is lineage-specific. After activation, the appearance or upregulation of surface activation and/or degranulation markers, such as CD63 and/or CD203c, is quantified. Alternative methods to quantify basophil activation imply quantification of the expression of surface inhibitory receptors such as CD300a [3], the phosphorylation of signaling molecules such as p38 mitogen-activated protein kinase (MAPK) [4] and signal transducer and activator of transcription 5 (STAT5) [5]. In addition, it was recently shown that histamine release can be quantified with flow cytometry. In this technique, designated as HistaFlow, the intracellular content of histamine and its release are analyzed by an enzyme affinity method using the histaminase diamine oxidase [6, 7]. Figure 1 and figure 2 show a HistaFlow dotplot of a cefazolin-reactive patient. Unlike the basophils of the patient displayed in figure 2, the cells of the patient in figure 1 display an anaphylactic degranulation with the appearance of CD63 and release of histamine.

BAT and HistaFlow in Immediate Drug Hypersensitivity

For about a decade now, BAT is being increasingly and successfully incorporated into the diagnostic management of IDHRs. As displayed in tables, the technique has mainly been applied to document allergy to NMBAs (table 1) [8–16], antibiotics like β-lactam antibiotics and quinolones (table 2) [17–30], aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs; table 3) [31–45], and iodinated radiocontrast media (RCM; table 4) [46–48]. In general, the sensitivity of the assay varies be-

Fig. 1. HistaFlow plots in a cefazolin-reactive patient showing clear degranulation of basophils in response to cefazolin (100 µg/ml). **a–c** Quiescent cells stimulated with buffer. **d–f** Responses to positive control stimulation with anti-IgE. **g–i** Response upon stimulation with the antibiotic. Note that only CD203c++CD63+ cells release histamine (decrease of DAO, **c, f, i**).

Fig. 2. HistoFlow plots in a cefazolin-reactive patient showing only activation of basophils (no clear anaphylactic degranulation of cells in response to 100 $\mu\text{g/ml}$ cefazolin). **a–c** Quiescent cells stimulated with buffer. **d–f** Responses to positive control stimulation with anti-IgE. **g–i** Response upon stimulation with the antibiotic. Note that there was only upregulation of CD203c (CD203c++CD63– cells). No release (decrease of DAO) could be measured (data not shown). **a, d, g** are the same as **b, e, h** but ungated, showing the clear isolation of basophils.

Table 1. BAT in immediate NMBA hypersensitivity

Stimulus	Reference test	Activation marker	Sensitivity, %	Specificity, %	N	Ref.
Various NMBAs	H	CD63	64	81	26	8
		CD45	43	96		
Various NMBAs	H ± ST	CD63	54	100	56	9
Various NMBAs	H	CD63	79	100	31	10
		CD203c	36	100		
Various NMBAs	H ± ST	CD63	36–86 ¹	93	92	11
Rocuronium	H ± ST	CD63	92 ²	100	22	12
Various NMBAs	H ± ST ± IgE	CD63	60	100	49	13
Rocuronium	H	CD63	80	96	104	14
Various NMBAs	H + ST	CD63	68	100	56	15
Atracurium	H ± ST	CD63	71 ³	100	75	16

H = History; N = number of patients and control individuals; ST = skin test.

¹ When only the reactions that occurred during the 3 years were taken into account, sensitivity increased.

² Taking into account the nonresponders, sensitivity was 76%. ³ Taking into account the nonresponders, sensitivity was 63%.

tween 50 and 60%, whereas specificity easily attains 80%, except for quinolones and NSAIDs (see below). From some of these studies, it seems that BAT/HistaFlow allows a simultaneous testing of different compounds including both active components and excipients such as Cremophore EL [49] and clavulanic acid in amoxicillin/clavulanic acid [50].

Apart from testing drugs, BAT seems to also be useful for testing related compounds such as antiseptics (e.g. chlorhexidine [51]), dyes (e.g. patent blue [52]), plasma-expanders (e.g. succinylated gelatin [53] and hydroxyethyl starch [54]).

Importantly, BAT not only facilitates identification of the culprit drug but also provides the opportunity to study cross-reactivity and the tailoring of safe alternatives for the future. For example, we have observed the contribution of BAT in the study of cross-reactivity amongst NMBAs. Here, a technique was demonstrated to complement skin testing in identifying the cross-reactivity of aminosteroid and benzyloquinoline NMBAs [12, 14, 16].

We recently showed that the HistaFlow technique can be applied to demonstrate histamine release induced by small chemicals such as drugs. Although HistaFlow will probably not enter mainstream diagnostic application, the technique might constitute an important research asset for future studies on the pathomechanisms of IDHRs. However, larger studies are necessary to confirm its potential as, for the time being, the largest series about

HistaFlow in IDHRs comprises only 10 patients who suffered from anaphylaxis to rocuronium [55].

Another recently published asset of BAT/Histaflow is the possibility to predict safe administration of codeine, which is structurally similar to pholcodine, in patients with an allergy to the antitussive pholcodine [56]; until these results emerged, skin testing and quantification of sIgE antibodies were unable to do this. It remains to be established in larger collaborative studies whether this indicates that such basophil experiments constitute a breakthrough in genuine opiate hypersensitivity. Recently, we observed that the opiates morphine and codeine do not trigger basophil degranulation in opiate-tolerant individuals, who tested positive in a solid-phase morphine sIgE antibody assay and could therefore erroneously have been diagnosed as opiate-allergic [57].

The diagnostic management of IDHRs to β -lactam antibiotics generally relies on SPT, IDT and the quantification of drug-sIgE antibodies. However, as addressed by Blanca et al. [58], in particular cases, BAT provides significant opportunities. Hitherto, 9 studies have investigated BAT as a diagnostic tool in IDHRs to β -lactam antibiotics, mainly amoxicillin. Compared with the quantification of sIgE antibodies, BAT shows a higher sensitivity (about 50%) [17, 19, 21, 24, 25] and specificity (approx. 90%) [17–19, 21]. This indicates that BAT with β -lactam antibiotics should be considered when skin tests and sIgE serology yield negative or equivocal results.

Table 2. BAT in immediate β -lactam and quinolone hypersensitivity

Stimulus	Reference test	Activation marker	Sensitivity, %	Specificity, %	N	Ref.
<i>β-lactam antibiotics</i>						
β -lactam	H	CD63	50	93	88	17
β -lactam	H + DPT	CD63	39	93	53	18
β -lactam	H $\pm$ ST $\pm$ IgE $\pm$ DPT	CD63	49	91	110	19
Amoxicillin	H $\pm$ ST	CD203c	52	100	41	20
		CD63	22	79		
β -lactam	H	CD63	50	89–97	262	21
β -lactam	H $\pm$ ST $\pm$ IgE	CD63-CCR3 ^a	55	100	39	22
		CD63-IgE ^a	53			
Amoxicillin	H	CD63	29	/	patients: 14 controls: 0	23
Amoxicillin	H $\pm$ ST $\pm$ DPT	CD63	50	/	patients: 61 controls: n.a.	24
Amoxicillin	H $\pm$ ST	CD63	50	/	patients: 30	25
<i>Quinolones</i>						
Various quinolones	H + DPT	CD63	0 ^b	/	4	26
Various quinolones	H + ST $\pm$ DPT	CD63	0 ^b	100	18	27
Various quinolones	H	CD203c	100	100	5	28
Various quinolones	H $\pm$ DPT	CD63	71	/	73	29
Various quinolones	H $\pm$ DPT	CD203c	n.a.	100	34	30

DPT with moxifloxacin was only performed in control individuals to establish tolerance status. H = History; N = number of patients and control individuals; n.a. = not available; ST = skin test.

^a Different selection marker. ^b Note that all CD63-based assays yielded entirely negative results, except in the Aranda series [29].

Another illustration of the potential of BAT is in allergy to quinolones. These drugs can cause severe allergies, with moxifloxacin being the most important culprit [59]. BAT can be of extra value, since the diagnosis of quinolone allergy can pose a significant challenge. First, there are still no well-validated drug-sIgE antibody assays readily available and controversy has arisen about the specificity of some methods [60]. Furthermore, quinolone SPT and IDT are associated with uncertainty, and positive skin responses have been reported in individuals without overt quinolone allergy. For example, we observed that moxifloxacin skin testing yielded a positive predictive value of 36% and a negative predictive value of 25% [61]. Studies on BAT with quinolones (table 2) reveal quite divergent but highly interesting findings, mainly with respect to the sensitivity of the test. All CD63-based assays yielded negative results [26, 27], except for in the study of Aranda et al. [29]. This suggests that fluoroquinolones might trigger basophil activation that cannot readily be depicted by traditional CD63-based assays. Al-

ternatively, the more consistent results with CD203c up-regulation [28, 30] could indicate that mediator release in response to quinolones could result from alternative degranulation pathways [62, 63].

IDHRs to NSAIDs are extremely common and it is generally accepted that a large majority of these reactions are independent from IgE/Fc ϵ RI cross-linking but correspond to a pharmacological mechanism caused by the inhibition of cyclo-oxygenase (COX)-1, which finally results in the depletion of prostaglandin E2 with unstrained synthesis of cysteinyl leukotrienes and mediator release from basophils, mast cells and eosinophils. Consequently, as addressed in other studies [64], BAT generally displays insufficient sensitivity (about 20–40%) to diagnose IDHRs to NSAID hypersensitivity. However, a minority of IDHRs to NSAIDs appear to be ‘genuine’ IgE-mediated reactions, and, in such cases, patients appear to react exclusively to a single NSAID family. So far, 5 publications have reported on BAT in selective hypersensitivity to pyrazolones [31, 32, 36, 43, 44], with BAT yielding a

Table 3. BAT in immediate NSAID hypersensitivity

Stimulus	Reference test	Activation marker	Sensitivity, %	Specificity, %	N	Ref.
Metamizol	H ± DPT	CD63	42	100	56	31
Metamizol	H	CD63	42.3	100	56	32
Various NSAIDs	H ± DPT	CD63	15–55	74–100	90	33
Diclofenac	H	CD63	No significant difference in CD63 expression between patients and controls (IgE-independent basophil degranulation)		26	34
Various NSAIDs	H	CD63	43	100	72	35
Pyrazolones	H ± IDT ± DPT	CD63	55	86	107	36
ASA	H ± DPT	CD63	34	79	42	37
Diclofenac		CD203c	17	100		
		CD63	17	92		
		CD203c	22	100		
ASA	H ± DPT	CD63	30	40	20	38
		CD203c	70	45		
Diclofenac	H ± DPT	CD63	0	/	22	39
Aspirin	H + DPT	CD63	80 ^a 78 ^b	83 ^a 50 ^b	59	40
Various NSAIDs	H + DPT	CD63	61	91	29	41
Various NSAIDs	H	CD63	37	90	80	42
Metamizol	H	CD63	0	/	patients: 6 controls: 0	43
Metamizol	H ± ST	CD63	70	100	30	44
Various NSAIDs	H ± DPT	CD63	100	20	91	45

ASA = Acetylsalicylic acid; H = history; N = number of patients and control individuals; ST = skin test.

^a For anaphylaxis. ^b For asthma/rhinoconjunctivitis.

Table 4. BAT in immediate hypersensitivity to RCM

Stimulus	Reference test	Activation marker	Sensitivity, %	Specificity, %	N	Ref.
Various RCM	H + ST	CD63	100	100	patients: 3 controls: n.a.	46
Various RCM	H ± ST	CD63	46–62% ^a	89–100% ^a	40	47
Various RCM	H ± ST ± DPT	CD63	63	100	28	48

H = History; N = number of patients and control individuals; n.a. = not available; ST = skin test.

^a Depending on the cut-off value.

sensitivity of 42–70% and a specificity of 86–100%; 1 study showed a significantly lower sensitivity, thereby contradicting the other studies [43].

IDHRs to RCM have been described, but their prevalence is low and is estimated at 0.02% for nonionic RCM and 0.4% for ionic RCM. Direct histamine release by

means of nonspecific RCM binding to surface receptors on mast cells or basophils and indirect cell activation by means of the complement or kinin cascade outnumber the genuine IgE-mediated reactions that might be overlooked with skin testing [47, 65]. These reactions are believed to account for approximately 4% of the IDHRs to

RCM. Up to now, 3 studies have reported on the value of BAT in the diagnosis of IDHRs to RCM [46–48]. These studies demonstrate a sensitivity of 46–63%, depending on the chosen threshold, and a specificity of 89–100%. Furthermore, it seems that the results of BAT and skin testing are complementary [47].

Commentaries and Perspectives

Already from the earliest days of BAT, it was obvious that this technique would become an asset in the allergic diagnostic instrumentation. Actually, the diagnostic utility of BAT (and to a lesser extent, HistaFlow) has repeatedly been demonstrated in both IgE-mediated allergy (e.g. aeroallergens, food, hymenoptera venom and *Hevea* latex) and autoimmune urticaria, a condition that results from the production of autoantibodies against FcεRI or IgE itself [2]. However, the utility and usefulness of BAT/HistaFlow needs to be reevaluated thoroughly, in view of the difficulties inherent to the correct preparation and storage of allergen extracts, optimizing and standardizing stimulation conditions and also the potential of alternative, more easily accessible diagnostic instruments such as component-resolved diagnosis, currently only available for food [66] and hymenoptera venom [67]. Conse-

quently, at present, the diagnostic application of BAT/HistaFlow has mainly to be sought in the domain of IDHRs, particularly when diagnosis cannot be established by other means. However, additional, collaborative, large-scale studies are needed to verify whether BAT/HistaFlow holds promise as well as to optimize and harmonize protocols, avoid the instigation of cynicism and skepticism and enable and justify the introduction of these tests to routine diagnostic application. Some of the requirements of these validation studies have recently been detailed elsewhere [68]. Clearly, these studies should adopt standardized and harmonized clinical and laboratory protocols, in order to avoid erroneous conclusions that might impede appropriate clinical validation of the assays.

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