Mast cell versus basophil activation test in allergy : current status

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Short running title: Mast cell versus basophil activation test

Didier G Ebo[1,2,*,£], Rajia Bahri[3,] *, Chiara Tontini[3] , Athina L Van Gasse[1,4], Christel Mertens[1] , Margo M Hagendorens[1,4], Vito Sabato[1], Jessy Elst[1]

* Didier G Ebo and Rajia Bahri should be considered joint first author

[1] Immunology – Allergology – Rheumatology, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp University Hospital and Infla-Med Centre of Excellence Antwerp University, Antwerpen, Belgium

[2] Immunology – Allergology AZ Jan Palfijn Ghent, Belgium

[3] Lydia Becker Institute of Immunology and Inflammation, Division of Musculo-skeletal and Dermatological Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, Core Technology Facility, University of Manchester, 46 Grafton Street, Manchester M13 9WU, UK

[4] Paediatrics, Faculty of Medicine and Health Sciences, University of Antwerp, Antwerp University Hospital and Infla-Med Centre of Excellence Antwerp University, Antwerpen, Belgium

£ Correspondence

D.G. Ebo, MD PhD

University of Antwerp

Faculty of Medicine and Health Sciences

Immunology -Allergology - Rheumatology

Campus Drie Eiken T5.95

Universiteitsplein 1

B-2610 Antwerpen, Belgium

Tel: ++ 32 (0) 3 2652595

Fax: ++ 32 (0) 3 2652655

immuno@uantwerpen.be

ORCID

Didier Ebo: 0000-0003-0672-7529

Rajia Bahri: 0000-0001-5969-5941

Chiara Tontini: 0000-0002-8437-8697

Athina L. Van Gasse: 0000-0002-3434-4333

Christel Mertens: 0000-0003-2359-0771

Margo Hagendorens: 0000-0001-6361-9503

Vito Sabato: 0000-0002-1321-314X

Jessy Elst: 0000-0003-3506-8200

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Abstract

In the past two decades, we witnessed the evolution of the basophil activation test (BAT) from mainly research applications to a potential complementary diagnostic tool to document IgE-dependent allergies. However, BAT presents some technical weaknesses such as the non-responder status observed in around 10-15% of tested patients, immediate post-event negative results. Moreover, the use of fresh basophils, ideally analysed within 4 hours of collection, restricts the number of tests that can be performed per sample, and is especially limiting when conducting batch analyses and interlaboratory comparisons to harmonize BAT practices among . All these limitations significantly hinder its wider application and urge the development of alternative testing, such as the mast cell activation test (MAT).

The essential difference between BAT and MAT is the heterogeneity of the starting material used to perform the assays. Since MC are tissue-resident cells, current alternatives to sourcing cells directly from tissues for functional studies are generating primary human MCs differentiated from donor progenitor cells or using immortalized mast cell lines. Hence, the methodological approach for mast cell-based functional studies is not only vastly different from BAT, but also different among MAT protocols currently developed.

This review summarizes the advantages and disadvantages of BAT and MAT assays, dedicating special attention to elucidating the key differences between the cellular sources used, and provides an overview of studies hitherto performed comparing both techniques in the diagnosis of IgE-mediated food and drug allergies.

Key words: allergy, basophils, mast cells, flow cytometry, food allergy, peanut allergy, drug allergy, anaphylaxis

Key messages

- BAT and pMAT are useful tools in the study of allergic and non-allergic responses.
- Comparative studies on BAT and pMAT are still insufficient and limited to food and drug allergies.
- Difficulties in performing comparative studies are due to the difference in the protocols used.

Introduction

In daily practice, clinicians predominantly rely upon skin tests and quantification of specific IgE antibodies (sIgE) to confirm the suspicion of an IgE-mediated allergy (1). However, none of these tests show absolute diagnostic reliability (2, 3) and, especially in the context of immediate drug hypersensitivity (IDH), a positive skin test is not necessarily indicative of an underlying IgE-mediated process (4). For more than half a century, functional *in vitro/ex vivo* assays have mainly focused on basophil histamine and sulphidoleukotriene release tests (5-8). However, the time-consuming and costly two-step approach, i.e., (i) cell incubation and (ii) quantification of released mediators in the supernatant, limited their mainstream use. The foundations of modern flow cytometry (FCM)-based basophil activation test (BAT) date back to 1991, with the discovery of the lysosomal-associated membrane protein (LAMP)-3, or CD63, as basophil degranulation marker (9). At present, BAT is a widely used allergy diagnostic tool, and different protocols have been developed to allow cell identification and quantification of activation/degranulation (e.g., via detection of surface markers/intracellular expression changes, and exteriorization of granule content) (10). However, traditional BAT using patients' own cells presents several disadvantages. First, although analyses can be performed up to 24 hours (11-13), BAT should be ideally performed within 4 hours of sampling (13, 14). Second, around 10-15% of tested patients show complete non-response to *in vitro* stimulation (complete non-responder status) (15-17). Third, there is evidence of false negative results observed immediately after an anaphylactic event (e.g. for β-lactam antibiotics) (18, 19). Fourth, the need to perform experiments on the day of sample collection poses some difficulties in executing batch analyses and organizing interlaboratory comparisons, also known as round robin testing (20).

Importantly, BAT protocols are not harmonized among laboratories in terms of identification/activation markers, procedures (e.g., in-house developed *versus* commercially available BAT methods), allergen sources and concentrations used, resulting in not equally interchangeable interpretations between studies and potentially conflicting results (21, 22).

In an attempt to circumvent the limitations of standard BAT testing, different groups have focused on the development of passive BAT (pBAT, also referred to as indirect BAT, iBAT) (23-26). In pBAT, donor basophils are stripped and subsequently passively sensitized with patients' sera before stimulation with the relevant allergen(s). Although the pBAT is a step forward, some limitations remain. First, pBAT seems less sensitive than conventional BAT, with cells responding only when incubated with sIgE titres exceeding 1-3.5 kUA/L (23-25). The need for such high amounts of sIgE for sensitization is an important limitation, as sIgE levels in the blood can be quite low, especially for drugs and related compounds, such as antiseptics. Second, the outcome of pBAT is highly dependent on the donor, whose cellular responder status is unpredictable and can only be determined *ad hoc*. Third, pBAT and the standard BAT do not allow to study direct activation by occupation of the Mas-related G protein coupled receptor X2 (MRGPRX2) by the offending drug. To this end, cells need to be conditioned as described by Toscano *et al.* (27).

In parallel to the concerted efforts to harmonize BAT practices among different laboratories (20), different authors have explored the potential of FCM-based *in vitro* mast cell (MC) activation tests (henceforth called MAT) to overcome the known limitations of BAT (28-33). In the MAT, mast cell lines (e.g., LAD2) or cultured primary human MCs (hMCs) can be activated either directly, or indirectly after passive sensitization with patients' sera (pMAT) (34). Importantly, unlike BAT which uses resting viable patients' basophils as cellular source for testing, there is currently no standardized "MC" source material for MAT. Consequently, when reporting MAT results and its performance, a precise description of the source material is critical for correct interpretation and appropriate comparisons with other diagnostic tests (33), as results obtained with mast cell lines are not necessarily translatable to cultured primary MCs, and *vice versa* (35-38). Furthermore, one-to-one comparison between BAT and MAT performance is currently limited to one study (33). Starting from clinical needs and laboratory experience, the primary objective of this work is to provide a status update on comparisons between BAT and pMAT applied to food and drug allergy diagnostics. As with

147 other techniques still under development, the interpretations, hypotheses, reappraisals, or
148 explanations expressed here may not find universal acceptance. Nevertheless, we believe that
149 our opinion might i) benefit future evaluations of the diagnostic accuracy of IgE-mediated food
150 and drug allergies and ii) facilitate the widespread adoption of these techniques (34, 39, 40).
151
152 **Basophil and mast cell-based assays: similar yet different**
153 As shown in Figure 1-2 and summarized in Table 1, the essential difference between BAT and
154 MAT is the heterogeneity of the starting material used to perform the assays. As starting point
155 for the experiments, although different basophil cell lines are available (36, 38), BAT is usually
156 performed on resting basophils sourced from peripheral blood of tested patients, as these
157 cells are easily accessible for *ex vivo* experiments in clinical practice. Conversely, since MCs
158 are tissue-resident cells not found in blood, the approach for MC-based functional studies is
159 vastly different. Ideally, functional hMC studies should be performed on MCs sourced from
160 tissues of interest. However, such studies are hindered by a number of technical issues,
161 namely isolating sufficient numbers of viable, mature and functionally competent hMCs, the
162 poor *ex vivo* cellular expansion , and the possible influence of the isolation technique used on
163 residual MC functionality. To circumvent these limitations, different strategies were adopted,
164 including culturing primary hMCs starting from donor progenitor cells, and generating human
165 MC lines (e.g., HMC-1, LAD1/2, LUVA, ROSA and MCPV-1 cells). Primary hMCs can be obtained
166 by culturing $CD34^+$ and/or $CD117^+$ immature mononuclear cell progenitors isolated from
167 different source materials, such as cord blood, bone marrow, foetal liver cells and, more
168 recently, peripheral blood (PB) (for a detailed review of different primary hMCs generation
169 methods see (34, 38)). However, culturing hMCs from progenitor cells poses several
170 difficulties and challenges, namely the availability of source material, the cost of isolation and
171 *in vitro* differentiation, the time required to generate mature hMCs, and donor- and protocol-
172 specific biological variations. To circumvent these disadvantages, several research groups
173 have developed different protocols for culturing sizeable numbers of primary MCs from
174 different progenitor cells of diverse origins (28-32, 41). These protocols mainly differ in regard
175 to the cytokines used to induce maturation and the duration of cell culture, but all ultimately
176 produce hMCs which closely resemble naturally produced MC subsets, showing phenotypic
177 and, most importantly, functional changes in MC behaviour that can meet specific research
178 and diagnostic needs. For example, certain protocols could favor the maturation of cells

especially suited to study IgE-mediated processes, while other protocols generate cells strongly responsive to MRGPRX2 activation. Hence, the current MC heterogeneity due to the distinct maturation protocols used should not be considered a hindrance to further development and validation of hMC-MAT, but rather a valuable asset.

A concern raised by Chirumbolo *et al.* (42) is that hMCs are usually generated from precursors sourced from healthy subjects, which might not be equally representative of MCs sourced from patients with different allergic manifestations. However, as addressed elsewhere (43), there is little, if any, evidence supporting this concern. Krohn *et al* (44), showed MCs cultured from asthmatics and controls to respond similarly to recombinant Der p 2, the major allergen from house dust mite (*Dermatophagoides pteronyssinus*). Furthermore, Cop *et al.* showed that $CD117^{+}$ hMCs cultured from subjects with birch pollen allergy and healthy donors displayed similar CD203c and high affinity IgE receptor (FcεRI) expression densities (45), as well as MCs of patients with IgE/FcεRI-independent anaphylaxis to rocuronium showed similar MRGPRX2 expression and function to MCs of patients with IgE/FcεRI-mediated anaphylaxis (46).

Another drawback of hMC cultures is that they cannot be maintained for an extensive period of time, with new samples required for each assay. This contributes significantly to make hMC generation time consuming, expensive, and difficult to standardise as diagnostic test for clinical use. The use of immortalised MC lines expressing fully functional FcεRI could possibly provide an alternative approach (47).

Currently available hMC lines derive either from healthy MCs immortalized by producing targeted KIT or *Ras* gene mutations (LUVA, MCPV-1, $ROSA^{KIT\ WT}$), or sourced from donors with mast cell leukaemia with KIT mutation and/or other karyotype changes (HMC-1, LAD1/2). As a result, immortalized MCs can double in culture within weeks, either spontaneously or with the help of specific cytokines, thus obtaining enough cells displaying similar proprieties, making them ideal for repetitive testing over an extended period of time. However, not all MC lines are created equal, especially for functional testing, as not all express viable FcεRI (48), nor their receptor expression and responsiveness to IgE/FcεRI-mediated activation is stable over time. In fact, cell lines of tumour origin such as LAD2 cells (49) are considered intermediately differentiated (35) and unstable, since they eventually lose their capacity to undergo FcεRI-mediated degranulation (38), and become less responsive to anti-IgE under the

same conditions (33). In addition, as these cell lines are either patented or owned by selected laboratories, some additional costs can be incurred for use for diagnostic purposes.

If one has the means to generate hMCs from progenitor cells, a third choice could be to either immortalize hMCs, or invest in strategies extending survival/prolonging their lifespan. Using transgenic mice for the human high-affinity IgE, Zbaren *et al.* engineered a conditional homeobox B8 (Hoxb8)-immortalized progenitor line from bone marrow cells. The mature Hoxb8 mast cells in their study seem promising tools for testing IgE/FcεRI-mediated allergies, however further comparisons with existing diagnostic approaches are lacking (50).

BAT versus MAT in food allergy

To our knowledge, the first one-to-one comparative analysis between BAT and pMAT in IgE-mediated food allergy dates back to 2018 (33). In this study, Bahri *et al.* showed that pMAT is an elegant tool to diagnose peanut allergy and peanut sensitization. The pMAT used FCM-based analysis and quantification of primary hMCs generated from $CD117^{+}$ peripheral blood precursors. hMCs were sensitized with sera of children and adolescents with peanut allergy, following a positive or negative double-blind placebo-controlled food challenge (DBPCFC), and hMCs were subsequently stimulated with peanut extract. The blinded comparison between hMC-pMAT and existing diagnostic tools (including component-resolved diagnostics and BAT) revealed hMC-pMAT's superior diagnostic performance and proved useful to explore differences in effector cell function between basophil and MC responses during allergic reactions. The hMC-pMAT proved also to be a very sensitive assay, with significant upregulation of surface expression of the degranulation marker CD63 after stimulation with peanut at concentrations up to 2-log lower than what is required for BAT (51). A second indirect comparison between pMAT and BAT in peanut allergy/sensitization was made by Santos *et al.* (52). In this study, the authors investigated whether peanut-induced MC activation could be elicited by passive sensitization using LAD2 cells (LAD2-pMAT) (49). Activation of LAD2 cells sensitized with plasma from children with confirmed peanut allergy after stimulation with peanut extract was greater than the activation when sensitized with plasma from peanut-sensitized children or non-allergic children. In contrast to the one-to-one blinded comparison between hMC-pMAT and BAT conducted by Bahri et al. (33), data of LAD2-pMAT were indirectly compared with the outcomes of BAT from a study conducted in 2014 (51).

In both studies, BAT and MAT offered better accuracy in the diagnosis of peanut allergy compared to peanut-specific IgE or Ara h 2 (20,24,13). However, unlike Bahri *et al.* (33), Santos *et al.* reported that BAT showed greater diagnostic accuracy compared to LAD2-pMAT, due to its higher sensitivity (51) (Table 2). However, the comparison between pMAT and BAT used BAT observations from an earlier study, in which non-responders were excluded for both i) calculation of performance metrics in the diagnosis of peanut allergy (whole primary study population) and ii) external validation of BAT diagnostic cut-offs (51). This is not in line with the recommendations of a 2015 position paper, recently updated (12), on BAT's clinical utility, suggesting to treat non-interpretable invalid BAT results from non-responding patients as false negatives to calculate the test performances (53). As argued elsewhere (17, 54), complete BAT non-responders, i.e., cases with basophils not responsive to stimulation with the positive control and allergen, should not be discarded when assessing the overall test performance. Furthermore, as the LAD2-pMAT was not tested in parallel with alternative MCs (i.e., cultured primary hMCs, other human MC lines) in the Santos *et al.* study, the poor performance of LAD2-pMAT compared to BAT should not be generalized nor inferred to other pMATs (32, 33, 41).

In clinical practice, physicians need to unambiguously identify the causative food with unequivocal and congruent positive and negative results between allergen-specific IgE (sIgE) and skin tests. In this respect, interestingly, both studies (33, 52) included a small distinctive subgroup of peanut-sensitized individuals which was used to ascertain whether the pMAT could discriminate between allergy and mere sensitization, as reflected by a positive sIgE and/or skin test but negative challenge. In the study by Bahri *et al.* (33), based upon the findings in children and adolescents with a sIgE peanut < 15 kUA/L (55) or skin prick test response < 8 mm (56), hMC-pMAT continued to provide superior discrimination compared to other diagnostics, including traditional BAT. In the study by Santos *et al.* (52), the LAD2-pMAT was able to discriminate between peanut-allergic and peanut-sensitized children showing similar levels of peanut sIgE, with a strong correlation with BAT results.

Collectively, these findings are highly relevant, as the discrimination between allergy and sensitization in pollen-associated food allergy syndromes is one of the most challenging problems encountered during the investigation of IgE/FcεRI-mediated food allergy in clinical practice (57).

In conclusion, hitherto comparative studies on BAT and pMAT focused primarily on peanut allergy in children and adolescents, and several open questions related to these models still need to be addressed before conclusions on the diagnostic superiority of available tests in IgE-mediated food allergy can be drawn.

BAT versus MAT in immediate drug hypersensitivity and allergy

As reviewed elsewhere (10, 58, 59), over the past two decades BAT has become a widespread diagnostic tool, especially useful for the diagnosis of immediate drug hypersensitivity and allergy and proving to be beyond a simple diagnostic aid. *Ex vivo* basophil testing might deepen our insight into immune (allergic) and nonimmune (non-allergic) processes, such as the occupation of the MRGPRX2 receptor by different drugs, help with the identification of antibody recognition sites, and improve our understanding of cross-reactivity and desensitization strategies (4, 27, 59).

The first attempt to explore the utility of pMAT in immediate drug hypersensitivity was reported in 2015 (Master thesis of D. Ludwig available from: https://eprints.soton.ac.uk/416617/). The addition of prilocaine hydrochloride, paracetamol, benzylpenicillin, lidocaine, propofol, cefuroxime sodium, rocuronium bromide and bupivacaine hydrochloride to LAD2 cells failed to directly stimulate β-hexosaminidase release from non-sensitized cells. In contrast, when using chlorhexidine, a net release of around 10% of the total stored β-hexosaminidase was observed when added at a concentration of 0.03 mM, and approximately 30% at 0.1 mM. However, this “release” was supposedly the result of a direct cytotoxic effect of the biguanide antiseptic. Preliminary attempts to passively sensitize LAD2 cells with IgE from patients followed by stimulation with relevant allergen were not successful. Although FcεRI appeared to be expressed on the cell surface, cells changed during culture so that either intact FcεRI was no longer expressed, or there was a defect in the coupling of the receptor to the degranulation process. For this reason, no further studies involving sensitization with human sera were performed. This disappointing finding with the LAD2-pMAT in immediate drug hypersensitivity/allergy is in stark contrast with the promising results obtained when using hMC-pMAT (60-62), which could become a novel instrument to explore the MC-activating capacity of drug-reactive IgE antibodies. For this purpose, the utility of hMC-pMAT was explored in IgE-mediated allergy to chlorhexidine and rocuronium, two predominant causes of peri-operative hypersensitivity, to try to connect as closely as possible

to the current expertise with BAT (63), although not designed for a head-to-head comparison between hMC-pMAT and BAT. To ensure the broadest possible evaluation of hMC-pMAT, archival sera were selected to perform MC experiments, which considered the different BAT outcomes to allow further stratification into subgroups (i.e. with positive, negative, or non-responder BAT). We observed that, in general, the results obtained through hMC-pMAT largely paralleled BAT findings, and that the hMC-pMAT could therefore i) become an attractive alternative to BAT and ii) help overcome the problems associated with non-responder basophils, although there is still room for improvement in the rocuronium allergy test protocol. Taken together, based on the comparison between definitive chlorhexidine-allergic patients (i.e. patients with a compelling history and combined congruent positivity for sIgE, skin test, and BAT) and control individuals, and considering both chlorhexidine concentrations, we found hMC-pMAT with chlorhexidine to be a reliable diagnostic tool (sensitivity 92.3%, specificity 100%, positive predictive value 100%, and negative predictive value 95.2%) (60, 61). For rocuronium, when considering definitive rocuronium allergic patients (congruent positive sIgE, skin test and BAT), sensitivity would be around 70%, and when considering rocuronium tolerant individuals (congruent negative sIgE, skin test and BAT) specificity 100% (62). Collectively, these findings indicate the need for drug-specific activation protocols, since the optimal stimulation conditions (time, concentration) used for BAT might not be directly translatable to the hMC-pMAT.

The diagnostic approach for immediate drug hypersensitivity and allergy cannot be considered complete if it fails to address drug cross-reactivity or identify safe drug alternatives for patients to use. Therefore, a second step in the exploration of BAT and hMC-pMAT in drug allergy was the demonstration that both techniques can provide information on cross-sensitization profiles (24, 64, 65). To appreciate the relevance of these findings, it is important to understand the limitations of currently available methods in assessing potential drug cross-reactivity. A significant part of our knowledge of drug cross-reactivity profiles stems from sIgE binding and hapten inhibition studies. However, these approaches are hindered by difficulties in solid phase coupling of studied drugs, or by the masking of relevant antigenic epitopes. Furthermore, results of sIgE inhibition studies are not necessarily predictive of the clinical outcome during subsequent exposure (66, 67) in contrast to skin tests, the current preferred predictors for the clinical significance of potential drug cross-reactivity. However, being an *in vivo* procedure, skin tests, particularly intradermal tests, can be dangerous (68-70), and a

positive skin response does not necessarily indicate an underlying sIgE/FcεRI-dependent mechanism (4). A positive skin test might also reflect an irritant response, or non-specific histamine release by MRGPRX2 occupation, as this receptor is abundantly expressed by skin MCs (71). In light of these difficulties, basophils and MCs constitute attractive complementary alternatives to explore both functionally relevant drug cross-reactivity patterns, and to better discriminate between IgE-dependent (allergic) and MRGPRX2-dependent (non-allergic) immediate drug hypersensitivity (4). For example, when studying the cross-reactivity profile of chlorhexidine using pBAT and hMC-pMAT, only MC-based experiments were capable to demonstrate the MRGPRX2 agonistic activity of polyhexamethylene biguanide (24, 65).

Conclusions, unmet needs and perspectives

The major challenge of allergy diagnosis lies in the development of safe, accessible and reliable diagnostics, capable of correctly predicting the clinical outcome following exposure to the offending allergen(s) and cross-reactive structures. Over the past three decades, evidence has accumulated that FCM-assisted analysis and quantification of *ex vivo*-activated basophils in BAT might meet these requirements (10, 12, 39). Moreover, there is ever-increasing evidence that BAT can be more than a diagnostic aid, with potential non-diagnostic applications such as therapeutic monitoring, tracking the natural progression of diseases over time, and the identification of allergenic recognition sites and their changes (59). However, it is becoming increasingly clear that BAT requires to understand degranulation metrics and their limitations for the correct interpretation of its results (10, 12, 72). As already illustrated in the introductory paragraph, while BAT produces results quickly, hence making it suitable for routine clinical use, the non-responder status and the difficulty in standardizing the technique are major setbacks to its wider application. To overcome these limitations, numerous alternatives have been developed, such as the pBAT and pMAT.

The development of the pBAT is undoubtedly an asset, but even this test has some limitations. For instance, it is clearly less performant at lower sIgE concentrations, hence less sensitive than the traditional BAT, and difficult to standardise because of donor variability and the rather limited number of experiments that can be performed over a 24-hour window using single donors (23, 24). Furthermore, basophils are not suitable for detecting activation via MRGPRX2 binding.

The pMAT is a practical solution to the aforementioned limitations of both BAT and pBAT, and a promising diagnostic tool in the field of IgE-mediated allergy. This technique has virtually no non-responders, succeeds in demonstrating degranulation with low sIgE concentrations, and can be performed on a large scale using single/pooled hMCs donors or MC cell lines, which facilitates standardisation. However, after 5 years since its seminal description, we still have limited data coming mostly from peanut allergy and peri-operative hypersensitivity causing severe anaphylaxis from chlorhexidine and rocuronium. Admittedly, both the hMC-pMAT and LAD2-pMAT present some general and more specific limitations, but not insurmountable. First, in contrast to BAT/pBAT, pMAT techniques require laboratory facilities for cell culture, which are expensive and time-consuming. However, pMAT uses serum samples that can be easily frozen, stored and shipped to a reference centre experienced in MC lines and cultures, capable of offering batch testing at a reasonable cost.

As described elsewhere (34), the main limitations of the LAD2-pMAT are the slow growth rate, the intermediate differentiation, the loss of FcεRI responsiveness over time, and variable expression of the MRGPRX2 receptor compared to human tissue-derived MC. This could make the assay less sensitive to IgE cross-linking or MRGPRX2-based stimulation, and less suitable for studying allergic and non-allergic processes over time. Furthermore, LAD2 cells express very low levels of tryptase and chymase, and are less efficient in cytokine generation, making these cells not ideal for broader functional assays.

Conversely, the weaknesses of the hMC-pMAT are the elevated cost, the time required to generate mature hMCs (ranging from 4 to 10 weeks depending on the protocol), and the donor-dependent variation in response, as observed in BAT. However, an optimal culture can yield several million cells, allowing to conduct a considerable number of experiments with single donor hMCs. Alternatively, pooling different hMC donors can also help reduce inter-assay variation. Unlike LAD2, attempts to immortalise or freeze cultured hMC have yet proven unsuccessful (unpublished data). In summary, basophil- and MC-based techniques present each their advantages and disadvantages, which are major key determinants for their appropriate use. While we suggest being mindful and thorough in the exploration of the limitations of each assay, the application of these novel techniques should be expanded to additional allergens/drugs research, diagnostic settings, and the monitoring of allergic diseases in the future.

Although BAT and pMAT are gaining increasingly more relevance in allergy diagnostics and starting to appear regularly in guidelines and expert reviews, any speculation on the superiority of one methodology over the other should be supported by evidence from solid prospective one-to-one comparative studies, which are still insufficient to this day. However, comparative analyses might not be straightforward, as reproducibility is difficult for a plethora of different reasons, primarily caused by the lack of standardization of BAT and/or pMAT protocols. The biggest hurdles most difficult to standardize are the difference in source materials used (LAD2, hMCs obtained from different donors and via different culture protocols), markers used for cell identification, activation and degranulation, assay execution (e.g., priming, stimulation time and concentration), the type of allergenic material tested (native extract, purified or recombinant component), as well as the reporting and interpretation of data. Furthermore, as with any study of this nature, another major difficulty is accurately identifying patients and control individuals without over-reliance on other diagnostics that lack sensitivity and specificity. Moreover, the limitations of both diagnostic tools, including factors affecting the test performance, like age, geography, and allergenic cross-reactivity that could influence or confound the outcome are, in our opinion, still not properly elucidated yet.

In conclusion, both BAT and pMAT are promising methods for allergy research and diagnosis, each showing unique pros and cons. However, both BAT and pMAT require more analysis and standardization before entering clinical practice as valid and reliable day-to-day tools.

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Tables

Table 1: Summary of key differences, strengths and limitations between BAT and pMAT.

CHARACTERISTICS	BAT	LAD2-pMAT	hMC-pMAT
Non-responders (%)	10-15%	No	No
Ideal timing from sample collection	4 hours	Time-independent	Time-independent
Batch testing possible	No	Yes	Yes
Passive sensitization required	No[#]	Yes	Yes
Donor-dependent variability	Yes	No	Yes
Standardized protocols	No	No	No
Cellular source of clonal origin	No	Yes	No
Stable FcεRI response over time	Yes	No	Yes
MRGPRX2 testing possible	No[*]	Yes	Yes
Mediator release	++	+	+++
Easy technical execution	+++	+	+
Cost of the technique	+	++	+++

except in pBAT (23-25)

* forced expression of the MRGPRX2 receptor can be obtained through stimulation or cell purification (27)

+= Low

++= Medium

+++= High

Abbreviations:

FcεRI: high-affinity IgE receptor; MRGPRX2: Mas-related G protein-coupled receptor 2.

Table 2: Performance of BAT and pMAT allergy tests in the diagnosis of peanut allergy

BAT		pMAT					
%CD63+ (AUC ROC)		%CD63+ (AUC ROC)			Peanut sIgE	Ara h 2 sIgE	
Whole studied population (95% CI)	Equivocal SPT/sIgE results subgroup (95% CI)	Whole studied population (95% CI)	Equivocal SPT/sIgE results subgroup (95% CI)	MC type used	Threshold (kUA/L)	Threshold (kUA/L)	**Ref.**
0.97 (0.93-1.0)	0.92 (0.8-1.0)	0.874 (nd)	nd	LAD2 cell line	<0.4	<0.2	(51) (52)
0.94 (0.87-1.0)	0.84 (0.67-1.0)	0.99 (0.96-1.0)	0.97 (0.90-1.0)	PB-derived hMCs	<0.5	<0.21	(33)

Abbreviations
AUC ROC: area under the curve receiver operator characteristics curve; hMC: human mast cells; nd: not disclosed; PB: peripheral blood; sIgE: allergen-specific IgE; SPT: skin prick tests.

Figure captions

Figure 1: Human basophils and mast cells express different receptors and are highly compartmentalized.

Summary of key differences between basophils and mast cells in receptor expression and main location of mature cells found in the human body.

Abbreviations: IgE-FcεRI: Immunoglobulin IgE-high affinity IgE receptor complex; MRGPRX2: Mas-related G-protein coupled receptor type X2. Figure created with Biorender.com.

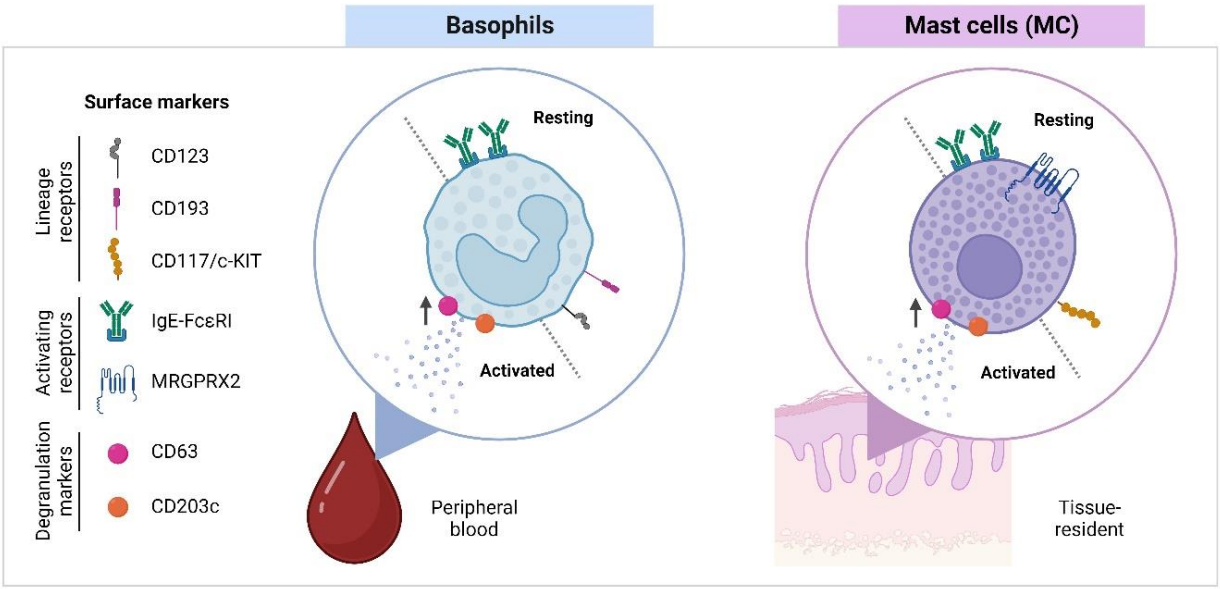
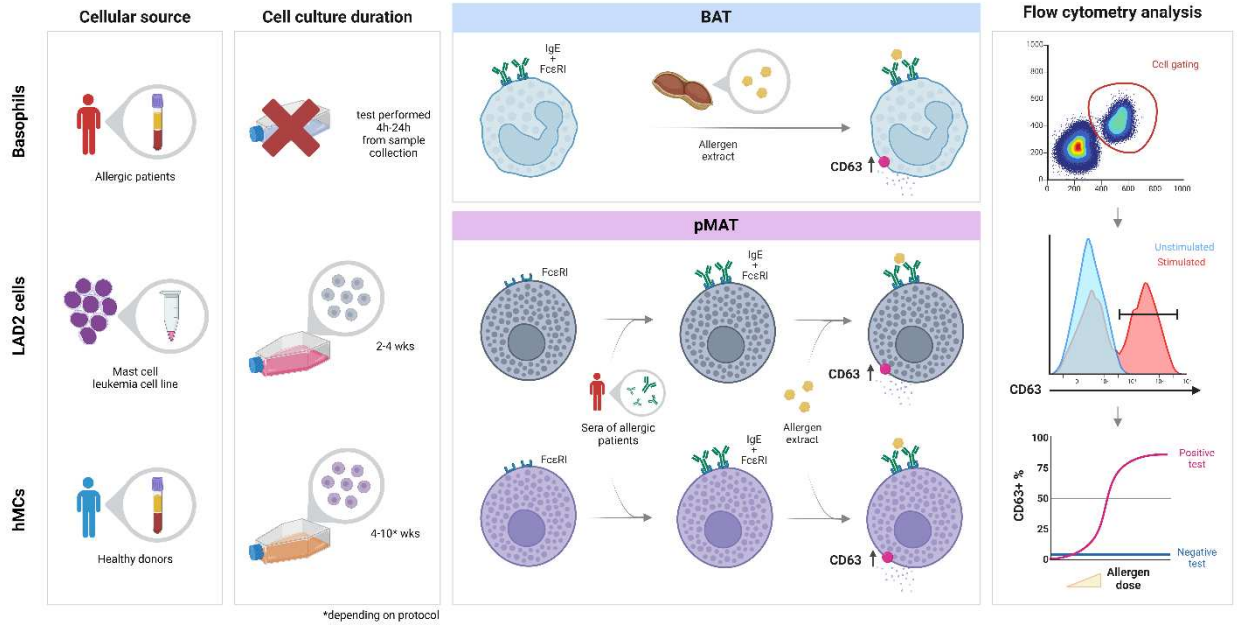


Figure 2: BAT and pMATs: summary of currently described techniques

A simplified depiction of described methods used to perform BAT and pMAT in IgE-mediated allergies. Figure created with Biorender.com.



Graphical abstract