
CLINICAL APPLICATIONS OF BASOPHIL ACTIVATION TEST IN ALLERGY DIAGNOSIS

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ABSTRACT

Currently, IgE-mediated allergy diagnosis is based mainly on skin prick tests, but these tests are not recommended for patients that had previously experienced a severe allergic reaction to an allergen source. Serum specific IgE quantification has also its flaws. Therefore, *in vitro* cellular assays, such as the basophil activation test (BAT) are a good diagnosis alternative. Basophil activation test measures the IgE-mediated hypersensitivity to allergens without exposing the patients to a risk of anaphylaxis. BAT is a functional assay that measures the expression of activation markers such as CD63 and CD203c on the surface of basophils. It is widely used in allergy research but recent clinical validations and standardisations made this test available also for routine testing. In this study, we describe the mechanisms behind the basophil activation test and its clinical application in food, respiratory, venom and drug allergy diagnosis.

Keywords: Allergy, Basophil activation test, Allergy diagnosis, CD63, CD203c

INTRODUCTION

IgE-mediated hypersensitivity reactions are usually diagnosed after a careful clinical anamnesis and confirmed by skin prick testing, measurements of serum specific IgE and, when necessary, provocation tests is. Skin testing performed with commercially available extracts is not always possible (e.g. anti-histamines intake) or can be contraindicated (history of life-threatening anaphylaxis). Furthermore, challenge tests cannot be recommended in daily practice due to ethical considerations, practical reasons and variations in sensitivity and specificity [1]. Therefore, clinicians confirm their clinical suspicions after specific IgE quantification. Unfortunately, these assays do not have absolute diagnostic reliability, especially when it comes to pan-allergens with high potential of cross-reactivity. Also, there might be clear discrepancies between sensitization, revealed by serum IgE and the clinical manifestation of that sensitization [1]. Basophil activation test is a good *in*

vitro alternative, mimicking an allergic reaction in a test tube. It determines the ability of an allergen to induce basophil activation, measuring the expression of specific markers using flow cytometry. BAT has the advantage of being a functional test, positive results occur only after successful IgE cross-linking, not by monovalent binding as in IgE assays [2]. In this review, we describe the principle of basophil activation test and its clinical applications in diagnosing food, respiratory, venom and drug allergy.

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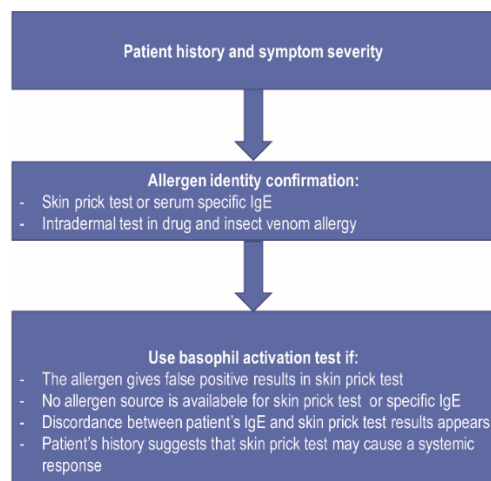


Fig. 1. Diagnostic algorithm for allergic disease (adapted after Hoffmann *et al*, 2015 [3])

BASOPHIL ACTIVATION TEST PRINCIPLES

The main characteristic of allergic effector cells in immediate-type allergy is allergen-specific IgE bound to the high affinity IgE receptor, i.e., FcεRI, from the cell surface. Binding of allergens to surface IgE results in FcεRI cross-linking and elicits the acute phase of the allergic response that causes the sudden release of vasoactive mediators into the tissue and/or circulation. This process is called anaphylactic degranulation (and may induce life-threatening anaphylaxis)[3]. In contrast, in piecemeal degranulation, cells secrete granule contents without exocytosis [4]. Both mast cells and basophils share these key characteristics. Mast cells reside in the tissue and are considered primary allergic effector cells [5]. Basophils are peripheral blood granulocytes, with secretory granules containing histamine and are capable of secreting proteases, cytokines, chemokines, and lipid mediators. Basophils present on their surface unique markers depending upon whether the cells undergo anaphylactic or piecemeal degranulation, which can then be measured by flow cytometry [6]. Basophil

activation is a process that starts with intracellular changes (signal transduction from FcεRI by means of phosphorylation of p38MAPK and calcium influx, followed by changes on the cell membrane level (CD203c) with final degranulation of the basophils (CD63) [1](Figure 2).

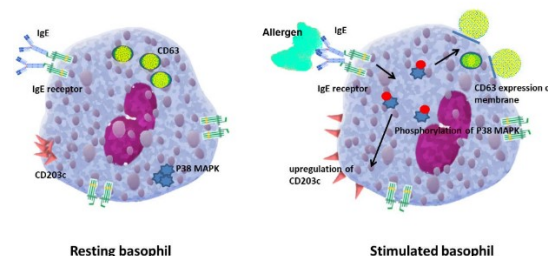


Fig. 2. Comparison between a resting and a stimulated basophil. Cross-linking of membrane-bound IgE from basophil induces mediators secretion and up-regulates the expression of specific activation markers such as CD63 and CD203c (modified after Hausmann *et al*, 2009 [7]).

Basophil activation test measures an *in vitro* activation of patients' basophils in the test tube. After blood sample preparations (whole-blood assay or leukocyte isolation) and preincubation with interleukin IL-3 (depending on the allergen of interest) or prewarming of blood samples and reagents to 37°C, the allergen is added, and basophils are incubated for 15 minutes to 1 hour at 37°C in the water bath (depending on the allergen of interest) (**Stimulation**). Crude allergen extract, purified or recombinant allergens can be used as stimulants. After stimulation, the samples are stained with monoclonal antibodies coupled to fluorochromes (**Staining**). Another step is the lysis of contaminating erythrocytes (**Red blood cell lysis**), followed by **data acquisition** (using a flow cytometer) and **data analysis** (dedicated software) (**Figure 3**).

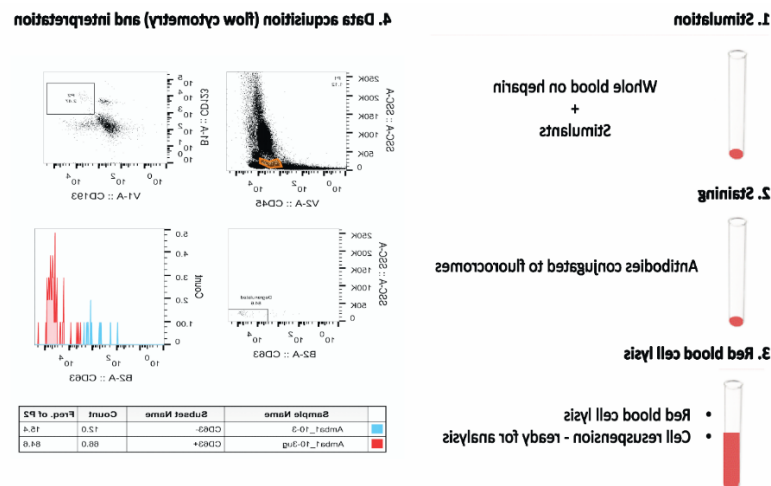


Fig. 3. Basophil activation test includes several steps: stimulation, staining, red blood cell lysis, data acquisition and interpretation

The minimal number of basophils to be analyzed should be 200 per test tube for accurate quantification of basophil activation, but in order to minimize the variation of results caused by equipment measurement error, it is preferred that 600 to 1000 basophils to be evaluated. To select basophils from

the whole leukocyte population, several positive and negative selection strategies are possible. CD123, CCR3, CRTH2, IgE can be used as identification markers, while CD63, CD203c, CD107a and b, CD13, CD164, CD69, p38 MAPK, STAT5 represent activation markers (Table I) [7].

Table I. Basophil activation test identification and activation markers (modified after Hemmings *et al*, 2018 [2])

	Marker	Cell expression	Description
Identification markers	CCR3	Basophils, mast cells, Th2 lymphocytes	Stable marker for identification [8], [9]
	CD123	Basophils, plasmacytoid dendritic cells	Highly expressed in these cells [10]
	IgE	Basophils, monocytes, dendritic cells	Other cells expressing high-affinity IgE receptor FcεRI [9], [11]
	CRTH2	Basophils, eosinophils, Th2 lymphocytes	Differentiation from eosinophils by side scatter or T lymphocytes by CD3 is reported [12]
Activation markers	CD63	Basophils, mast cells, platelets, macrophages	Widely used activation marker and an accurate marker of anaphylactic degranulation [13]

CD203c	Basophils, mast cells, CD34 ⁺ progenitor cells	Upregulation has been reported to be representative of piecemeal degranulation also used as identification marker and expressed in low levels of resting cells [14]
CD107a , CD107b	Basophils, mast cells, T cells and NK cells	CD107a and CD107b was found to be only expressed by activated basophils and its upregulation was similar to CD63.
CD13	Basophils, myeloid cells	CD13 and CD164 has an expression profile comparable to CD203c [15]
CD164	Basophils, CD34 ⁺ progenitor cells	
CD69	Basophils, lymphocytes, neutrophils, monocytes, eosinophils	Expressed progressively when stimulated with IL-3; however, found to be weakly expressed to IgE mediated stimulation [16]
p38 MAPK, STAT5	Various cell types	Basophilic phosphorylation of intracellular molecules can alternatively be used to measure basophil activation [17]

Currently, BAT diagnostic kits use CD63 as activation marker (Flow CAST®, Buhlmann Diagnostics Corp), while some kits measure CD203c (Allergenicity Kit, Beckman Coulter). CD63 is a 53kDa protein from the transmembrane-4 superfamily, known also as lysosomal-associated membrane glycoprotein-3 (LAMP-3). When it comes to a resting basophil, this protein is located on the membrane of intracellular secretory granules. After stimulation by FcεRI, these granules fuse with the plasma membrane and thus, CD63 is expressed on the surface of degranulated basophils [13]. Early studies suggested that this marker is associated with granules containing histamine, suggesting that CD63 up-regulation can be used as an indirect marker of histamine release [13][18]. However, recent studies suggest that histamine release may be the sum of both anaphylactic and piecemeal degranulation, and up-regulation of CD63 may be representative of only anaphylactic degranulation [19].

CD203c is another basophil activation marker present on CD34⁺ progenitor cells, basophils and mast cells. It is a glycosylated type II transmembrane molecule that belongs to the family of ectonucleotide pyrophosphatase/phosphodiesterase enzymes [20]. CD203c is constitutively expressed in low levels on the basophil surface membrane and is

quickly upregulated upon cell activation via allergen (**Figure 2**) or more slowly via IL-3 [21]. This up-regulation differs from CD63 in terms of the inciting stimuli and early signalling events, which suggests that CD203c may be associated with piecemeal degranulation [6].

The quality of basophils obtained is confirmed using for stimulation fMLP, a bacterial peptide. Anti-IgE antibodies can be used as IgE-mediated positive control, while buffer can be used as a negative control. The results of BAT can be displayed as a percentage of basophils expressing the defined activation marker (in the case of CD63) or in terms of mean fluorescence intensity (MFI) (in the case of CD203c), by calculating the stimulation index (SI), represented by the ratio between MFI of the sample and the MFI of the negative control [22].

BAT APPLICATION IN FOOD ALLERGY

When it comes to diagnosing food allergy, oral food challenge (OFC) is the gold standard. However, it requires ingestion of food that can induce an acute allergic reaction and needs to be performed in a supervised environment, capable of treating anaphylaxis. Thus, specialists prefer, when it is possible, to diagnose food allergy based on clinical history, skin prick test and/or serum specific IgE.

These tests have high sensitivity but low specificity. BAT can improve the diagnosis of food allergy proving to be more accurate than IgE sensitization tests with a specificity ranging between 75 and 100% and sensitivity between 77 and 98% [23]. This high specificity may be able to reduce the number of OFC performed. Also, BAT can distinguish between individuals that are clinically allergic to those who are tolerant.

Basophil activation test is also used for monitoring the clinical response to immune-modulatory treatment of food allergy. Studies with immunotherapy for peanut and egg show that the basophil reactivity to those allergens decreased during the treatment [24, 25].

BAT APPLICATION IN RESPIRATORY ALLERGY

Skin prick tests and serum levels of specific IgE, combined with clinical history, were considered sufficient to diagnose respiratory allergies, but allergic reactions to inhaled allergens can be complex due to the diversity of potential sources to which the patients are exposed to and the number of tissues that can be affected. Therefore, patients who report local allergic rhinitis can have negative skin prick tests and undetectable levels of specific IgE, being hard to differentiate between allergic and non-allergic rhinitis. Despite the absence of serum allergen-specific IgE, for these patients, BAT has proved more sensitive and able to diagnose IgE-mediated allergy [26].

Since basophil sensitivity is a reproducible measure [27] it can be used to determine the efficacy of allergen-specific immunotherapy (AIT) to aeroallergens. BAT was used for monitoring of patients undergoing AIT with birch [28] and timothy [29] and showed reduced allergen sensitivity during the treatment. This reduction may be due to blocking factors competing (specific IgG) for the allergen with the cell-bound specific IgE [28], [29].

BAT APPLICATION IN VENOM ALLERGY

In the case of Hymenoptera venom allergy diagnosis, basophil activation test report both high specificity (83-100%) and sensitivity (85-100%) [30]–[32]. And even though testing for specific IgE to the major bee and wasp allergens (Api m 1 and Ves v 5) solves many suspect cases, BAT is used in diagnosing unique issues. For example, a small percentage of patients with a clinical history of venom allergy report undetectable specific IgE and negative skin tests. Since sting provocation tests are complicated and

have an unethical nature, BAT has proven effective for 80% of these patients [33].

Another issue with the diagnosis of Hymenoptera venom allergy is the double-positive results for bee and wasp venom, approximately 60% of patients exhibit specific IgE to both. This double positivity may be caused by a co-sensitization to both venoms, cross-reactivity due to sequence homology or irrelevant recognition of cross-reactive epitopes [3]. And, in order to prescribe venom immunotherapy (VIT), determination of the sensitizing allergen is crucial [34], especially if the patient had an anaphylactic reaction to only one insect. BAT brings extra information when it comes to detecting the dominant allergen and the real source of venom sensitization [35] and has the lowest rate of double positivity of diagnostic tests for Hymenoptera allergy [36]. In the case of patients double-positive in BAT, the allergen that induces the highest degranulation is considered to be the primary sensitizing allergen [35].

BAT APPLICATION IN DRUG ALLERGY

In the context of an allergic reaction to drugs or related compounds, several studies validate BAT for the diagnosis of an IgE-mediated allergy from neuromuscular blocking agents (NMBAs) [37], beta-lactam antibiotics and clavulanic acid as a β -lactamase inhibitor [38]–[40], iodinated radio-contrast media (RCM) [41].

In case of small molecular weight drugs, called haptens, which are not capable of Fc ϵ RI cross-linking themselves (hapten concept [42]) a conjugation to carrier molecules is required. The carriers are usually proteins, for eliciting an immune reaction in susceptible individuals. Moreover, after the drug metabolism, some reactive intermediates may be formed (pro-hapten concept; [43], [44]). Therefore, for the investigation of drug hypersensitivity reactions the use of drug metabolites and hapten-carrier conjugates has been promoted [45]. For example, in the case of propylphenazone (PP) hypersensitivity, basophils reacted in BAT only after stimulation with the drug-carrier conjugate not when they were stimulated with pure PP [46]. Nevertheless, as a consequence of lacking knowledge regarding relevant determinants, metabolic intermediates, their reactive functions, BAT is most frequently performed with solutions of plain drugs [46].

BAT presents some limitations listed in **Table II** alongside with possible solutions. One of these limitations is the stability of blood samples. Using EDTA as anticoagulant means that the sample is stable only for a few hours, so a new method has been developed – passive sensitization, but this method is

applied only in research. The main steps of passive sensitization are peripheral blood mononuclear cells (PBMC) isolation from a healthy donor, with well-characterized basophils; removal of IgE from the receptor with lactic acid (pH 3.9), incubation with

patient serum (1 hour, 37° C) followed by a regular BAT, as previously described. So the patient's samples are represented by serum, which can be stored for a longer period at 4° C [7].

Table II. Limitations of basophil activation test and possible solutions [7], [47]

Limitations	Possible solutions
Reduced basophil reactivity over time	Perform the basophil activation test within max. 24h from blood collection.
Changes in temperature can affect basophil reactivity	A good transportation system that ensures a stable temperature of the sample.
Allergen exposure, chronic inflammation and infection can induce higher basophil reactivity	Avoid basophil activation test after allergen exposure or during infections.
Immunosuppressors, including oral corticosteroids, may increase basophil reactivity	Avoid immunosuppressors before blood collection for basophil activation test.
Determining optimal allergen concentration	A dose-response curve with patients and healthy (exposed) controls for the exclusion of unspecific stimulation.
Difficulties in establishing the thresholds for positivity	Analysis of allergens in question with confirmed allergic patients and healthy (exposed) controls instead of arbitrarily chosen thresholds.

CONCLUSIONS

Basophil activation test is a functional test that mimics an allergic reaction *in vitro*, proving to be a useful and safe diagnostic method that can be used to evaluate IgE-mediated hypersensitivity reactions. It can be considered a surrogate for provocation test and oral food challenge. Currently, it is applied mostly in research because, in order to be accepted and used in the clinical routine, it needs clinical validation. This

means standardised laboratory procedures, standardised allergen concentrations and optimal allergen positivity thresholds. Recent clinical validations and standardisations made this test available also for routine testing of several allergens.

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APLICAȚIILE CLINICE ALE TESTULUI DE ACTIVARE A BAZOFILELOR ÎN DIAGNOSTICUL BOLILOR ALERGICE

REZUMAT

În prezent, diagnosticul alergiei mediate IgE se bazează în principal pe testul cutanat prick, dar această testare nu este recomandată pacienților care au avut anterior o reacție alergică severă la sursa alergenică testată. Cuantificarea IgE seric specific are, de asemenea, neajunsurile sale. Prin urmare, testele celulare *in vitro*, cum ar fi testul de activare a bazofilelor (BAT) sunt o bună alternativă de diagnostic. Testul de activare a bazofilelor măsoară hipersensibilitatea mediată IgE la diferiți alergeni fără a expune pacienții la un risc de anafilaxie. BAT este un test funcțional care măsoară expresia markerilor de activare precum CD63 și CD203c de pe suprafața bazofilelor. Este utilizat pe scară largă în cercetare, dar validările clinice recente și standardizările au făcut disponibil acest test și pentru testarea de rutină. În acest studiu, descriem mecanismele testului de activare a bazofilelor și utilizarea clinică a acestuia în diagnosticul alergiilor alimentare, respiratorii, la venin și la medicamente.

Cuvinte cheie: alergie, test de activare a bazofilelor, diagnostic alergie, CD63, CD203c