

Diagnostic Tests Based on Human Basophils: Potentials, Pitfalls and Perspectives

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Key Words

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Abstract

Human basophils are important tools for studying immediate-type hypersensitivity reactions since they release a variety of mediators (e.g., histamine, leukotriene C₄, IL-4 and IL-13) following allergen triggering. Several diagnostic tools have been introduced that measure either leukotriene production or the upregulation of surface markers (CD63 and CD203c) from these cells after antigen stimulation. However, a broad variability in basophil activity exists between different basophil donors and different antigens within one donor. This manifests itself in terms of their reactivity (maximum secretory response), based on the intracellular signaling of the basophils studied, and in terms of their sensitivity. The latter is governed by the number of IgE receptors per basophil, the ratio of antigen-specific IgE to total IgE, and by the number of cell surface antigen-specific IgE molecules for half-maximal responses, termed 'intrinsic sensitivity'. These variables give rise to shifts in the dose-response curves which, in a diagnostic setting where only a single antigen concentration is employed, may produce false-negative

data. Thus, in order to meaningfully utilize the current basophil activation tests for diagnostic purposes, each allergen should be pre-evaluated separately in order to determine a suitable stimulation range. Additionally, anti-IgE or anti-FcεRIα antibodies should serve as positive controls, bearing in mind that 10–20% of basophil donors are not responsive to IgE-mediated stimulation. Diagnostic studies using CD63 or CD203c in hymenoptera, food and drug allergy are critically discussed. Basophil-based tests are indicated for allergy testing in selected cases but should only be performed by experienced laboratories.

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Introduction

Human basophils have suffered from being regarded as diminutive counterparts of their tissue-fixed mast cell relatives. This is partly due to a lack of appreciation of the differences in both number and function of basophils and mast cells between rodents and humans, and also because of previous deficiencies in basophil-specific markers and tools for their enrichment. Despite this, the mechanisms of IgE-dependent effector cell function have mainly been discovered using these easily accessible blood cells rather than mast cells [for a review, see ref. 1,

2]. However, in terms of their biological importance, the misconceived redundancy of basophils is far from objective, especially in the light of their enhanced capacity to secrete T helper cell 2 type cytokines compared with human mast cells and the evidence of their presence in tissues affected by allergic inflammation, as shown using new markers for basophils. Well endowed with allergen-specific IgE on their surface and a comprehensive inflammatory mediator machinery, basophils might be responsible for acute allergic and anaphylactic reactions, particularly when histamine but no tryptase is involved [3]. Therefore, the basophil is increasingly attracting clinical scientists, ultimately serving as a diagnostic tool for IgE- or even non-IgE-mediated responses.

In recent years, new diagnostic tests have been proposed for the allergy laboratory, combining this old principle with state-of-the-art technology: after allergen stimulation, basophil activation can now be detected by flow cytometry, indirectly indicating an IgE-dependent sensitization. This kind of basophil activation test (BAT), monitoring CD63 [4] or CD203c [for a review, see ref. 5] on basophils, appears to be more elegant and feasible for many laboratories that have access to a flow cytometer, circumventing the need for specialized measurement of secondary mediators (such as histamine or sulfidoleukotrienes). After decades of skin and IgE testing, this new technology is a welcome tool in the allergy laboratory, providing insights into a patient's ability to react to allergens because of its analytical sensitivity. The opportunity to use a functional basophil-based assay, being superior in terms of standardization and quantification compared with functional in vivo tests, has raised particular interest in its diagnostic potentials.

Since these new technologies have been reviewed [6, 7], it is time to carefully assess the utility of basophil-based tests in the diagnostic laboratory. Many of the basic ideas regarding basophil function have been addressed in

the past but have been overlooked in the context of the present discussion. Here, we reappraise some of these forgotten aspects in view of minimizing errors of judgment and maximizing the potential diagnostic use of basophil tests.

Basics of Basophil Activation

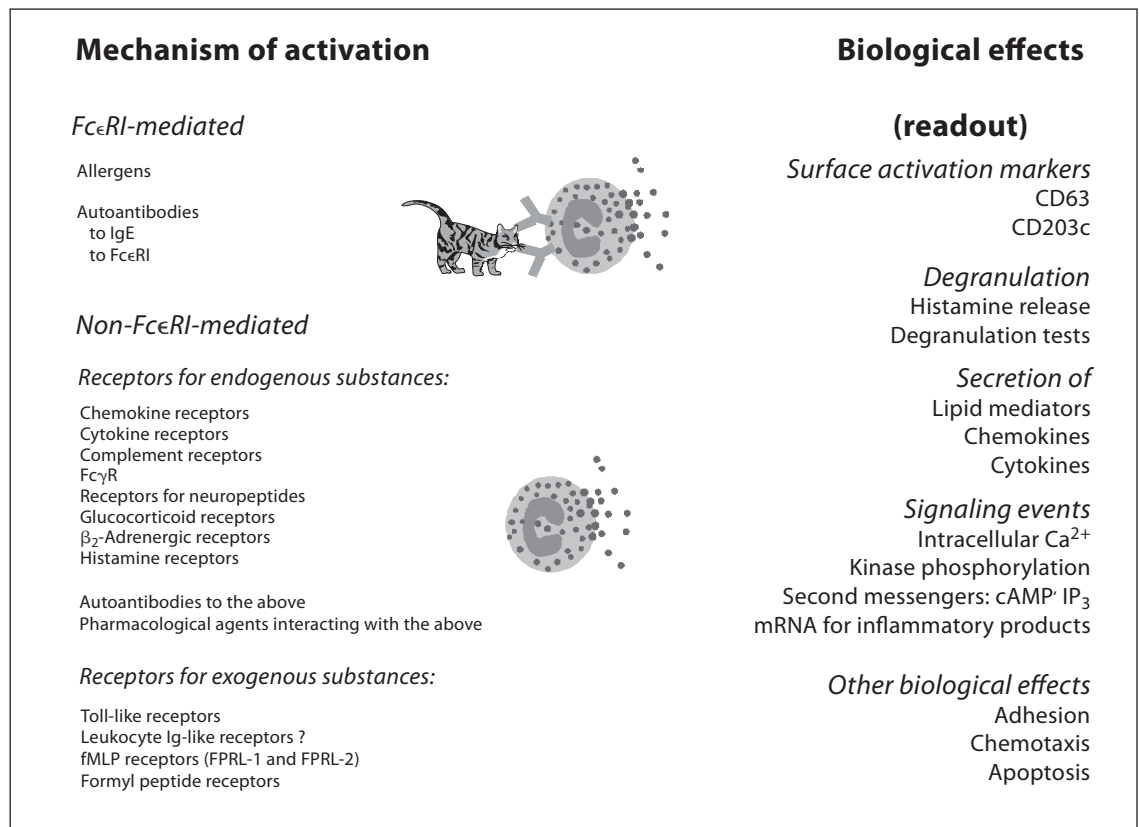
The basophil granulocyte can be activated by a number of pathways, which may be differentiated by whether or not they involve the high-affinity IgE receptor, and monitored by various readouts (fig. 1).

IgE Receptor-Mediated Activation and Subsequent Signals

IgE-dependent stimulation involves aggregation of high-affinity IgE receptors, FcεRI (table 1), caused by crosslinking of receptor-bound IgE molecules on the cell surface by allergens. This form of activation can also be caused by autoantibodies directed against both IgE and its receptor, as has been implicated in the pathogenesis of a subgroup of patients with chronic idiopathic urticaria, termed 'autoimmune urticaria'. Furthermore, certain plant lectins, superantigens, as well as parasitic products can cause IgE receptor triggering by binding to IgE irrespective of the need for antigen-specific IgE, indicating a potential role of these cells in innate immunity. FcεRI receptor activation results in a phosphorylation of Syk and Lyn kinases (which are closely associated with the β and γ chains of the receptor) leading to a complex cascade of signal transduction events (fig. 2) culminating in degranulation, lipid mediator production and cytokine synthesis. Syk and Lyn appear to regulate phosphatidylinositol (PI) 3-kinase which then leads to the activation of phospholipase C and a subsequent mobilization of intracellular calcium by inositol triphosphate (IP₃). This

Table 1. Basophil FcεRI receptor medians and ranges

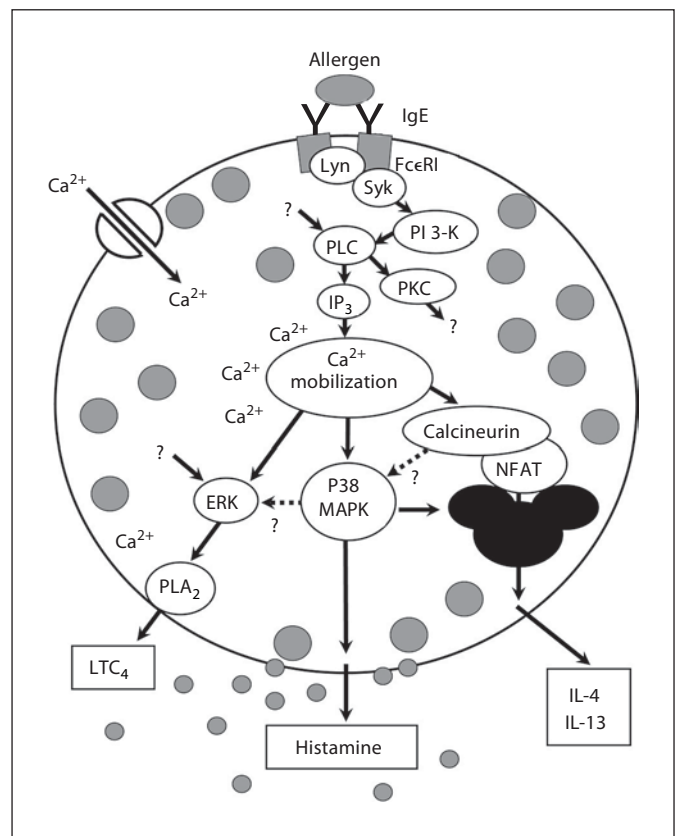
	Median	Range
IgE receptors	100,000 (whole population) 250,000 (atopic population)	5,000–1,000,000
Open receptors	8,000	5,000–15,000
IgE receptors for 50% of the maximum response (intrinsic sensitivity)	2,000	1,000–10,000 (90% of the population) minimum 200 maximum >30,000



1

Fig. 1. Pathways of basophil activation and functional readouts.

Fig. 2. Schematic representation of basophil activation caused by IgE receptor triggering. Crosslinking of high-affinity IgE receptors following allergen binding leads to the phosphorylation of Lyn and Syk tyrosine kinases which activate PI 3-kinase and phospholipase C (PLC). While PI 3-kinase may activate certain isoforms of phospholipase C, others may be activated by tyrosine kinases still needing to be identified in basophils. Following phospholipase C activation, IP₃ generation leads to a release of calcium stores into the cytosol which then facilitates a further rise in intracellular calcium caused by extracellular influx of the ion. IP₃-mediated calcium mobilization is essential for the control of production and secretion of all main basophil mediator types. Less is known regarding the other product of phospholipase C activity, namely diacylglycerol and subsequent activation of protein kinase C (PKC), which exists in numerous isoforms having varied actions on mediator release. Calcium mobilization activates p38 MAPK, which plays a universally important role in governing the release of all mediator classes in basophils; however, its exact interplay with calcineurin and ERK is still unclear. ERK is essential in the synthesis of LTC₄ in basophils, but has less effects on controlling other mediators. Basophil histamine and cytokine releases are extremely sensitive to the actions of calcineurin antagonists, suggesting a role for this enzyme in basophil signaling, but this is based solely on pharmacological evidence. For the sake of visual simplicity, arrows sometimes denote either a direct activation/modulation or indirect influence.



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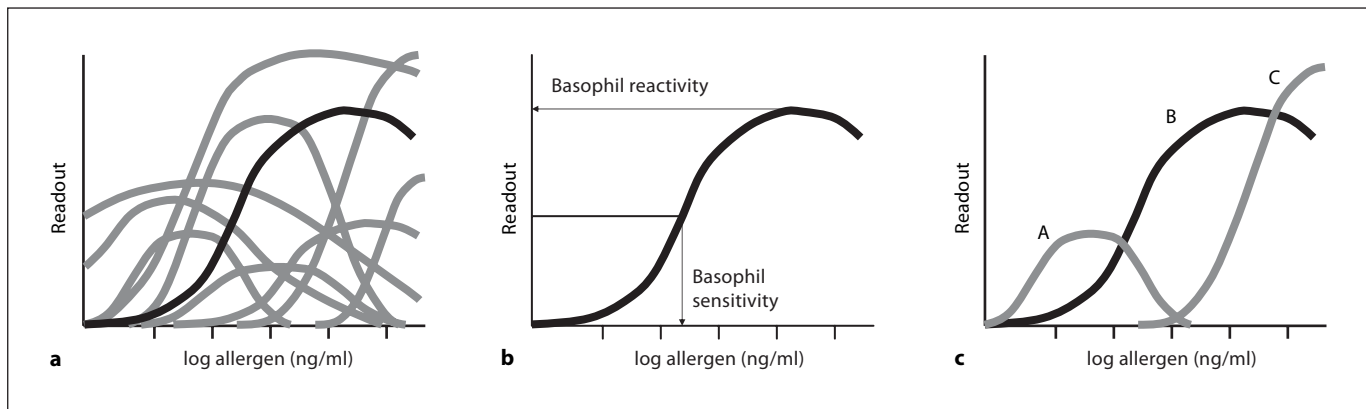


Fig. 3. Variability and quantification of basophil responses. **a** IgE-/FcεRI-mediated basophil functions demonstrate highly variable dose-response curves between different donors or within the same donor applying different stimuli (i.e. allergens). **b** Basophil reactivity (maximum of the basophil response) and basophil sensitivity, the (allergen) concentration inducing a distinct response, are used to analyze IgE-/FcεRI-mediated basophil responses. **c** Both variables, basophil reactivity (in this example $C > B > A$) and basophil sensitivity ($C < B < A$) are independent from each other [19].

controls further calcium influx into the cells and is a necessary step in order for mediator secretion to occur. The signaling cascade downstream of these events involves p38 MAPK, calcineurin pathways and nuclear factor of activated T cells as a transcription factor for IL-4. Other members of the mitogen-activated protein kinase family, such as c-Jun NH₂-terminal kinase and extracellular signal-regulated kinase (ERK), do not appear to play such a prominent role in basophil mediator signaling compared with mast cells, although ERK in basophils does regulate leukotriene C₄ (LTC₄) generation (but not degranulation or cytokine production).

Priming of the Basophil: Enhanced Signals of Activation

IgE-dependent basophil activation can be either enhanced or inhibited by various mediators. For example, histamine itself can act as an autocrine inhibitor via histamine H₂ receptor binding on basophils leading to sustained cAMP production and a lowered calcium response. On the other hand, the presence of IL-3, nerve growth factor or other priming factors can considerably enhance basophil activation, though the precise mechanisms are yet to be elucidated [8].

Priming by coinubation with IL-3 has been introduced to maximize the analytical sensitivity of basophil assays. However, IL-3 will not lead to a shift of resulting dose-response curves, but rather to an enhanced response to various stimuli.

Variability of IgE-Mediated Responses: Complex Interdependence

Definitions for FcεRI-Dependent Basophil Responses

IgE-mediated basophil responses are characterized by a broad variability, occurring between different donors or within the same donor applying different allergens (fig. 3a). Individual bell-shaped dose-response curves, extensively analyzed in histamine release studies during the last 3 decades, can vary by several orders of magnitude (depending on the allergen and the donor) with regard to the concentration, inducing a distinct response [9], termed 'basophilic cellular sensitivity' (fig. 3b) [10]. Independently, the individual maximum response, defined as 'basophilic cellular reactivity' (fig. 3b) [10], varies between 0 and 100% and is determined by early signaling events after IgE-mediated stimulation of basophils (e.g., sustained phosphorylation of basophil Syk kinases) [11, 12].

Non-IgE Receptor-Mediated Activation

A number of endogenous substances, such as the activated complement products, C3a and C5a, may trigger basophils via receptors, and with an appropriate source of complement, basophils may be used in test systems for complement activation by endogenous or exogenous substances. Exogenous substances that are not allergens can be tested for direct or indirect effects on basophils. Bacterial peptides, such as formyl-methionyl-leucyl-phenylal-

anine (fMLP), can cause basophil activation and histamine release (but less cytokine secretion) by IgE-independent mechanisms as well as by several types of drugs such as opioids, X-ray contrast media and many small peptides. However, some agents, including mastoparan, peptide 401, polymyxin B and substance P, which activate connective tissue-like mast cells (such as those that are found in the skin), do not activate basophils and may be an important limitation to BAT in some cases.

Variables of Allergen-Driven Basophil Responses

A number of parameters accounting for the broad variability of allergen-dependent IgE-mediated basophil responses (fig. 4) are listed below:

(1) total basophil IgE receptor cell surface density, i.e. number of FcεRI receptors/cell (table 1) [13–16];

(2) fraction of membrane-bound allergen-specific IgE antibodies (proportion of allergen-specific IgE antibodies/cell compared with total IgE) [13, 17, 18];

(3) intrinsic cellular sensitivity of the basophils, i.e. number of IgE molecules required for 50% of the maximal cellular response (table 1) [18, 19];

(4) cellular reactivity of the basophils (maximum cellular response after optimal IgE-mediated stimulation, i.e. with polyclonal anti-IgE) [15, 19, 20];

(5) biochemical and structural features of the allergen (number and proportion of single molecules in a complex allergen mixture; number of IgE-binding epitopes of a single molecule);

(6) nature of the aggregates formed by complexes of (single) allergens and IgE (i.e. dimers, trimers, oligomers) [18].

Relations between Variables Determining Basophil Responses

Some of these variables are interdependent or connected with serological parameters, whereas others are not (fig. 4):

(1) IgE receptor number is directly regulated by total serum IgE [16, 21–23].

(2) Fraction of membrane-bound allergen-specific IgE depends on the ratio of allergen-specific IgE to total serum IgE and on point 1.

(3) Cellular sensitivity is an intrinsic feature of the donor's basophils and accounts in part for variable shifts of the dose-response curves in different individuals (even in the case of similar total and antigen-specific IgE serum concentrations) [19].

(4) Cellular reactivity is independent of point 3 [19] (fig. 3c, 4), is quantitatively determined by early signaling

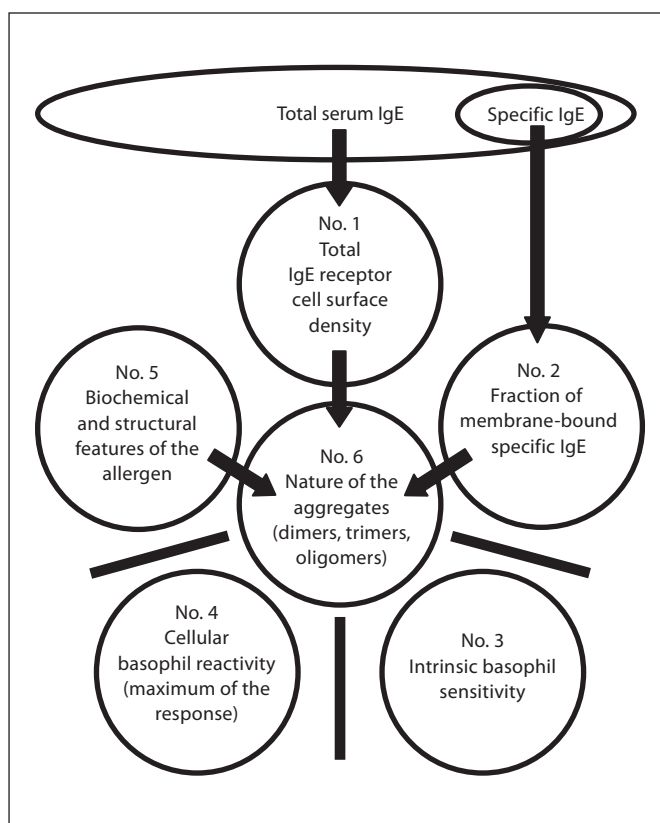


Fig. 4. Variables determining basophil responses. Arrows indicate direct influence of one variable on another. Straight lines indicate independence of neighboring variables from each other.

events of the basophils (Syk kinases, PI 3-kinase products) [12] and, being positive above the assay threshold, has no further diagnostic value for the detection of allergen-specific sensitizations.

(5) Allergen structure will influence point 6, i.e. the nature of the aggregates, as will points 1 and 2.

Subsequently, these variables – first highlighted by one of the authors in a series of detailed studies [18, 19, 24] and reviewed in MacGlashan et al. [2] – determine the test outcome and performance of cellular basophil-based tests in comparison with other tests (i.e. IgE measurements). All parameters, which have been comprehensively studied using histamine release, form the basis of the test outcome, independently of the final test readout, i.e. histamine, sulfidoleukotriene release or surface markers of activation (CD63, CD203c). In conclusion, promoting cellular tests for the evaluation of IgE-mediated sensitizations has the caveat of introducing a number of interrelated variables on top of simple IgE serum concentrations.

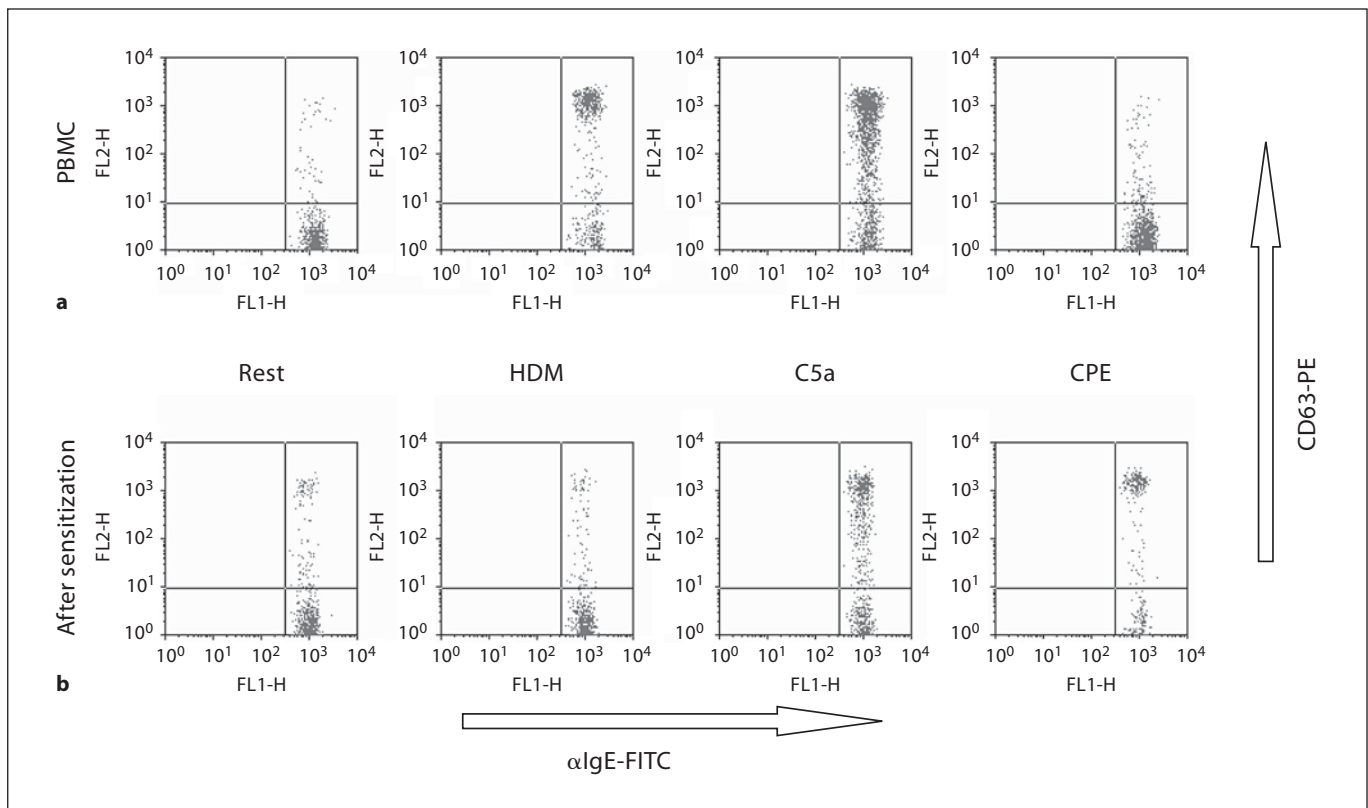


Fig. 5. Activation of basophils monitoring CD63 by flow cytometry. Basophils from a house dust mite allergic patient were stimulated for 30 min at 37°C with house dust mite (HDM), C5a, crude peanut extract (CPE), or buffer control (rest) before (**a**) or after (**b**) stripping and sensitization of these basophils with serum from a peanut-allergic patient. The horizontal axis (FL1-H) depicts the binding of FITC-conjugated anti-IgE, the vertical axis (FL2-H) depicts the binding of phycoerythrin (PE)-conjugated CD63 antibody.

Consequences for Basophil-Based Biological Tests

The maximum of individual basophil activation, appearing below or above a certain threshold level, will determine the qualitative test outcome (i.e. negative vs. positive, non-responder vs. responder).

Further interpretation of the quantitative degree of basophil activation (amount of histamine released or sulfidoleukotrienes produced, quantitative expression of CD63 or CD203c) has presumably no clinical diagnostic value.

Before using basophil-based tests for diagnostic purposes, a suitable diagnostic concentration range has to be defined for each allergen source (or single allergen molecule, usually in the picogram/milliliter to microgram/milliliter range).

Multiple allergen concentrations, i.e. spanning a range of at least 10^4 , are required to cover varying individual responses; one single allergen concentration is not suffi-

cient to analyze basophil responses, even if only qualitative results will be reported.

Finally, allergic sensitivities to multimeric allergens determined by basophil-based tests are not necessarily biologically or clinically advantageous over IgE measurements or skin test reactions, since they are also only clinically relevant in case of corresponding symptoms. (There is one exception, namely IgE binding of crossreactive carbohydrate determinants without necessarily inducing cellular responses or clinical symptoms.)

Non-Responding Basophils (Anti-IgE Non-Releasers)

In the normal population, about 10–20% of donors possess basophils that have no, or a very poor, response to crosslinking of the IgE receptor [20, 25]. The reason for this phenomenon has recently been identified as a selective decrease in Syk expression in the non-releasing basophils [26, 27]. Culture of basophils with IL-3 can restore

the releasing phenotype in a subgroup of the low or non-responders [27]. To register these non-releasing basophils, it is important to include a positive control, such as a polyclonal anti-IgE antibody (monoclonals are in general poor inducers) or an anti-FcεRI antibody. It is not clear why in the flow assay stimulation test method non-responders are found at rather low percentages (<5%) [28]. If this is due to the anti-FcεRI antibody used, one should be aware that one could potentially obtain false-positive responses from the use of such a potent antibody, while responses due to crosslinking IgE by anti-IgE or allergen might be very low in anti-IgE non-responders.

BAT for Diagnostic Purposes

Biology of CD63 and CD203c: Novel Markers Allow Elegant Readout

The original finding of CD63 binding in human basophils included immunoelectron microscopic analysis of the CD63 epitope in resting and activated basophils [29]. In this way, it was demonstrated that CD63 antibodies bind to the inside of basophilic granules. After cloning of the CD63 antigen, four putative transmembrane regions were described [30]. This suggests that the CD63 antigen is anchored in the granule membrane and that fusion of the granules with the plasma membrane causes the exposure of the CD63 antigen on the outside of the basophils. The relation with degranulation was further highlighted by a strong association between the percentage of CD63-binding basophils after activation and histamine release, as well as the identical time courses of these activation parameters after stimulation with anti-IgE and fMLP. However, supraoptimal stimulation with anti-IgE leads to further increase in fluorescence with anti-CD63, while histamine release decreases [31]. The underlying reason for the separation of both readouts under these conditions remains unclear. Expression of CD63 and CD107a, another recently described activation-dependent cell surface glycoprotein [28], seems to reflect basophil degranulation. However, they are not specific to basophils: CD107a is expressed by peripheral blood mononuclear cells and CD63 by all leukocytes of the myeloid hematopoietic lineage, as well as by thrombocytes. In these cell types, activation also increases CD63 binding to the plasma membrane.

In contrast to CD63, the CD203c antibody selectively binds to basophils, mast cells and their progenitors [32]. The CD203c antigen recognizes the type II transmembrane ectoenzyme E-NPP3 [33]. Relatively moderate

changes in the plasma membrane result in the increased extracellular exposition of the CD203c antigen on basophils. In our hands, density gradient centrifugation of basophils already induced increased CD203c expression, while degranulation did not occur. Moreover, CD203c expression has been demonstrated to be increased after the addition of IL-3, which is a priming and not a degranulating stimulus for basophils [34]. Basophil antigens with similar behavior include CD13 and CD164, resembling early signals after basophil priming or activation [28].

In conclusion, antibodies to both CD63 and CD203c demonstrate increased binding to activated basophils. However, CD63 (and CD107a) expression is associated with degranulating basophils, while CD203c (and probably CD13 or CD164) expression is found on primed and activated basophils.

Pitfalls in the Analysis of CD63 Binding on Basophils

A problem in assessing the CD63 surface expression on basophils is that it is not selective to basophils, and thus, an extra basophil-selective marker or the analysis of pure basophils is required. As specific marker, anti-IgE can be used. Binding of anti-IgE to monocytes and blood dendritic cells is often found in allergic individuals, but these cells can be removed from the flow cytometric analysis on basis of their scatter characteristics. When basophils are activated through FcεRI, care should be taken due to reduced binding of anti-IgE to these activated basophils. Alternatively, basophils can be labeled by using CD203c, as a basophil-specific marker, in addition to CD63. Another option is to include CD123, which is a relative selective marker for basophils.

Several studies have demonstrated that CD63 analysis is more sensitive than quantifying histamine release in the determination of basophil activation by allergic compounds [35, 36]. This is remarkable, taking into account the strong correlation between CD63 binding and histamine release [29]. A possible explanation can be the false-positive CD63 binding on activated thrombocytes [37] which bind to basophils through P-selectin [38]. Especially, when leukocytes are separated by density centrifugation, platelet adhesion to basophils is likely to occur, and only countercurrent elutriator centrifugation in the absence of Ca²⁺ and Mg²⁺ will result in thrombocyte detachment [Knol et al., unpubl. data; 31]. In addition, when basophils in whole blood are examined, many thrombocytes will be present and potentially adhere to basophils [31]. When basophils were analyzed after a single density centrifugation for in vivo activation, it was demonstrated

that the increased CD63 expression on basophils was due to adhering platelets expressing CD63 [Knol, unpubl. observations; 31]. Several studies have demonstrated that the adhesive properties of basophils increase independently of degranulation [39, 40]. In the case when adhesion of thrombocytes to activated basophils might disturb CD63 analysis of degranulating basophils, it is important to monitor thrombocyte adhesion by platelet-specific antibodies such as CD41 or CD42.

Clinical Issues in BATs: Quality of Study Groups – Selection Is the Key!

Flow cytometric analysis of in vitro activated basophils using CD63 has been applied in an increasing number of clinical studies with different allergens and other stimuli, e.g., several indoor and outdoor inhalants, food allergens, natural rubber latex, hymenoptera venoms, as well as drugs [for a review, see ref. 6]. It has also been proposed that the BAT might be helpful in diagnosing non-IgE-mediated reactions such as immediate-type reactions to non-steroidal anti-inflammatory drugs (NSAID) [41]. Since most inhalant allergies are simply diagnosed by case history, skin test, specific IgE detection and provocation test, without an urgent need for further diagnostic procedures, three more complex topics in allergy will be discussed below: hymenoptera allergy, food allergy and drug hypersensitivity.

As with any study of this nature, a major task is to accurately identify a group of patients with clinically significant allergy and to compose appropriate control groups. Therefore, some questions remain with regard to the general validity of the results of these past studies.

BAT May Increase Diagnostic Sensitivity in Hymenoptera Allergy

In many studies, such as the analysis of hymenoptera allergy [36, 42], calculation of sensitivity for the BAT was based on clinical history (used as the 'gold standard'). Selecting patients by clinical history is one way of composing study groups, in particular if confirmatory provocation testing to validate clinical history is not feasible. For instance, diagnostic sting challenges in hymenoptera allergy are not recommended due to the potential risk for the patient [43]. The overall sensitivity of in vitro diagnoses increased using CD63-based BAT since it was positive in a number of patients with low total IgE and specific IgE below the usual detection limit [42]. Sainte-Laudy et al. [36] also demonstrated a high sensitivity of the BAT as compared with serological proof of specific IgE. It is not clear whether these results might reflect platelet (co)-ac-

tivation (see above) or true IgE-mediated sensitization, which can indirectly be demonstrated in rare cases of extremely low total and allergen-specific IgE due to the high analytical sensitivity of basophil-based tests.

BAT Should Be Further Validated with Controlled Challenges in Food Allergy

In clinical studies on food allergy, double-blind placebo-controlled food challenges are recommended to confirm clinical history. To date, there is only one study validating the CD63-based BAT in patients with food allergy confirmed by double-blind placebo-controlled food challenges [44]. In contrast, in two more studies, patients with pollen-associated oral allergy syndrome were selected on the basis of clinical history [45, 46]. Since this type of food allergy is easily recognized by clinical history, selection of patients by clinical history is acceptable in this subgroup of food-allergic reactions. Although the BAT proved to be sensitive in detecting IgE-mediated food allergy in all reports, more studies are desirable validating the BAT in patients with different food allergies confirmed by double-blind placebo-controlled food challenges.

The Role of the BAT in Drug Hypersensitivity Remains to Be Elucidated

There is still limited experience applying the BAT in IgE-mediated drug hypersensitivity. One major problem in drug allergy is that besides IgE-mediated penicillin allergy and some other drug hypersensitivities, with most other drugs, the allergenic determinants are unknown. In 58 patients with a positive history of penicillin allergy and positive skin tests with penicillins, sensitivity of the CD63-based BAT was 50% [47]. Since the CD63 BAT was only performed in patients with positive skin tests to penicillins, the yield of the BAT is rather low diagnostically, although higher than specific IgE (38%).

It remains to be elucidated whether the BAT is useful in diagnosis of hypersensitivity reactions to NSAID, which are not considered to be IgE mediated. In a study of 60 patients with a clinical history of an NSAID hypersensitivity syndrome, sensitivity of the BAT ranged between 11.7% (paracetamol) and 54.8% (naproxen) [41]. However, in this study, provocation testing was performed to confirm clinical history in only 18 out of 60 patients. The authors performed the BAT in all patients with a panel of 5 NSAID but did not test the culprit drug in all cases. If a BAT with 1 NSAID was positive, the authors considered this as successful proof of NSAID hypersensitivity, even if the culprit drug did not induce any

basophil activation or was not tested. The authors provide no explanation why in many subjects the culprit drug does not induce any basophil activation in contrast to other NSAID. They also provide limited explanation of the suspected underlying pathomechanism. A positive BAT (cutoff 10% CD63+ basophils) was demonstrated in another study in 30% of patients (n = 20) with NSAID hypersensitivity [48]. However, as long as it remains unclear whether the BAT can be used in elucidating non-IgE-mediated reactions, further studies are needed to clarify their role in NSAID hypersensitivity. At present, the BAT does not seem to render provocation testing with NSAID unnecessary.

Clinical Utility of the Basophil Laboratory

The question where to place basophil-based tests in the clinical setting has to consider various points: (1) the allergen source in question (i.e. routine, occupational or exotic allergen source), (2) the diagnostic value (compared with other tests), (3) logistic considerations (pre-analytical handling: transport and rapid utilization of fresh blood cells), (4) laboratory requirements (technical skills and experience with cellular tests), and (5) expenses (compared with other tests).

Diagnostic and clinical studies, addressing points 1 and 2, will also take care of the other parameters (points 3 and 4) and cover the costs (point 5). In the routine setting, a specialized laboratory has to ensure that logistic and laboratory requirements (points 3 and 4) can be fulfilled, while the medical community has to decide upon re-embursement for a new diagnostic test by health care providers (point 5).

Indication for Use of Basophil-Based Tests

Various diagnostic situations will serve as examples to highlight the use of basophil-based tests.

Suggestive History of IgE-Mediated Allergy, Negative Skin Test and Negative IgE

Due to their extremely high analytical sensitivity, basophil-based tests can indirectly indicate allergen-specific IgE on the cell, especially if allergen-specific serum IgE levels are low (below the threshold) due to a rather low total IgE (<10 kU/l). This is rarely the case in atopic individuals, but can be found in non-atopic individuals with IgE-mediated sensitizations to non-atopic allergens (hymenoptera venom, IgE-inducing drug allergens, some occupational allergens).

Example: patient with an anaphylactic reaction after an insect sting, displaying negative skin tests and negative allergen-specific serum IgE results combined with a rather low total serum IgE level.

History of IgE-Mediated Allergy with Important Therapeutic Implication, Discordance between Skin Test and IgE Measurement

Basophil-based tests can serve as another indirect indicator (besides skin tests) for IgE-mediated reactions and can subsequently confirm important therapeutic decisions.

Example: patient with a severe allergy to a pet at home (e.g., a cat), with a small positive skin test, but a negative IgE result. A positive basophil test would confirm the IgE-mediated reaction, whereas a negative basophil result would show that an IgE-mediated sensitization is less likely to exist.

Example: patient with a severe reaction to an occupational allergen source with questionable diagnostic results (skin test or IgE).

History of IgE-Mediated Reaction, if No Appropriate Allergen for IgE Detection Is Available

In some instances, an allergen preparation is not available for allergen-specific IgE measurements, but an aqueous solution for skin tests and subsequent basophil tests might exist. Positive test results could indirectly suggest an IgE-mediated sensitization.

Example: patient with a severe allergic immediate-type reaction to a natural, presumably protein-containing (i.e. plant) extract after exposure through inhalation, ingestion or direct (mucosal) application. Serial dilutions for skin tests and basophil tests could provide diagnostic hints in terms of an IgE-mediated reaction. Additional appropriate negative controls with non-affected individuals have to be demonstrated. This is particularly important in the case of non-characterized, possibly contaminated, allergen preparations with irritative potential, since basophil tests represent bioassays based on live cells.

Performance of Basophil Tests and Crucial Controls

Diagnostic tests with basophils consist of three steps: (1) preanalytical handling, (2) stimulation of the basophils, including appropriate controls, and (3) readout of the activation signal.

Basophil-containing samples should be handled with care. Reactivity and viability will continuously decline within hours; therefore, transportation and subsequent experimentation should be carefully organized.

Proper stimulation requires experience in handling basophil leukocytes. Inadequate steps like vigorous vortexing, delayed workup and wrong temperatures can easily ruin an experiment. Similar to other diagnostic tests in allergy, the source and quality of the applied stimuli (i.e. allergens) will play a crucial role in the outcome of the result.

The diagnostic window of basophil stimulation tests is defined by a positive and a negative control. The latter is usually a buffer identical to the diluent of the stimuli to be tested. For IgE-dependent stimulation, a positive control such as anti-IgE or anti-FcεRIα antibodies have been introduced. Currently, the best anti-IgE antibody is not a mouse monoclonal, but rather a polyclonal antibody preparation.

IgE-independent stimuli, such as fMLP combined with C5a, can be used as additional positive controls to verify cell functionality and basophil test performance. This approach is particularly useful in the case of a negative response to anti-IgE or anti-FcεRIα.

Interpretation of Basophil-Based Tests

Different outcomes are to be expected after BAT. A positive BAT indicates an IgE-mediated sensitization (1) if appropriate allergens have been applied, (2) if negative controls with the same allergens and non-affected individuals are available, and (3) if positive parallel controls (anti-IgE or anti-FcεRI-antibodies) have demonstrated a positive response.

A negative BAT can rule out an IgE-mediated sensitization if appropriate allergens have been applied, which have demonstrated a positive response in other affected individuals, and if positive parallel controls with anti-IgE or anti-FcεRI antibodies have demonstrated a positive response.

Inconclusive results have to be considered if positive parallel controls (anti-IgE or anti-FcεRIα antibodies) did not induce a positive response, regardless of the response to the allergen in question, while IgE-independent controls (i.e. fMLP plus C5a) have demonstrated a positive response (indicating sufficient cell viability and robust general test performance).

No results (due to preanalytical or laboratory failure) can be considered if all positive controls (anti-IgE or anti-

FcεRIα antibodies as well as fMLP plus C5a) have failed to induce a positive response, indicating insufficient cell viability or failure in performing the test.

Additional Applications of BAT

The various BATs mentioned above use blood from a sensitized patient, which limits the practical possibilities for running the method to patient-near centers, since the cells must be used within several hours after drawing of the blood. This obstacle can be overcome by combining basophils from a non-sensitized person with serum containing specific and relevant IgE antibodies. In its original form, basophils from adult donors were stripped of their original IgE by a brief treatment with low pH, followed by incubation with the sensitizing serum [49, 50]. More recently, cord blood basophils have been used as recipient cells, which has the advantage of eliminating the low pH IgE dissociation step, which may interfere with the biological functions of the cells [Dr. Per Stahl Skov, pers. commun.]. In this way, it is possible to use basophils from normal donors, together with stored serum samples from patients with the relevant specific IgE [51].

It is conceivable that basophil cell lines, such as the KU812 [52], or animal cell lines transfected with the human IgE high-affinity receptor (FcεRI) [53] may also be used as recipient cells for this purpose, though these experimental systems are, at present, generally less sensitive than using primary basophils.

Albeit not a clear-cut diagnostic test, BAT or basophil mediator assays may be used for the determination of allergens provided that there are good basophil donors or – in the case of passive sensitization – sera with IgE to the relevant allergen. This technique may be useful for the characterization or quality assurance of allergen extracts, or for the quantification of the biological activity (proper folding, conformational integrity, functional epitopes) of new allergens [54, 55]. For histamine release, it has been demonstrated that such biological tests may be at least as sensitive as immunochemically based tests, such as radioallergosorbent test inhibition [56]. In addition, basophil stimulation tests with allergens are based on functional crosslinking and go beyond the demonstration of IgE binding with serological assays.

Basophil-based tests might also be used in patients on treatment with omalizumab. An abolished response to a relevant allergen, previously demonstrated as an activator of basophils, would indicate successful removal of al-

lergen-specific IgE from the cell surface. In case of an unchanged allergen-driven basophil activation, the omalizumab dose may need to be increased to ensure proper treatment. This approach might be particularly useful, since a commercial test for free IgE does not exist, hampering the control of sufficient IgE removal.

Perspectives

Basophil-based tests represent bioassays with extraordinary high analytical sensitivity. Subsequently, trace amounts of antigens can be compared in terms of potency and IgE-binding capacity. Basophil-based tests are excellent research tools to understand inherent problems of

signal-response coupling, i.e. IgE-mediated signaling, activation and degranulation. Valuable insights in both stimulatory and inhibitory signals after FcεRI-mediated activation might disclose new treatment options for allergic reactions and diseases.

Compared with the detection of IgE in serum, basophil tests have the advantage in demonstrating functional responses: IgE-mediated activation will only occur after successful crosslinking and not by incomplete antigens binding monomeric IgE. However, the proper handling of basophils and the extraordinary variability of their responses represent inherent difficulties, limiting the potentials of basophil-based tests being useful only as complementary diagnostic tools in selected cases.

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