

Review Article**Basophil Activation Test by Flow Cytometry: Present and Future Applications in Allergology****D. G. Ebo, C. H. Bridts, M. M. Hagendorens, N. E. Aerts, L. S. De Clerck, and W. J. Stevens***

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The diagnosis of allergic reactions in clinical practice rests upon both clinical history and the demonstration of specific immunoglobulin E (sIgE), either in the serum or via skin tests. However, for various reasons, identification of the offending allergen(s) is not always possible. Moreover, not all allergies are IgE-mediated. In an attempt to find reliable methods to investigate hypersensitivity reactions, histamine and sulfidoleukotriene release tests have long been introduced. However, relatively few comprehensive quality reports have been published so far. Upon challenge with a specific allergen, basophils not only secrete quantifiable bioactive mediators but also upregulate the expression of different markers which can be detected efficiently by flow cytometry using specific monoclonal antibodies. This review addresses the principals, particular technical aspects and pitfalls as well as the clinical and research applications of flow-assisted analysis of in vitro activated basophils © 2008 Clinical Cytometry Society

Key terms: allergy; basophil; diagnosis; flow cytometry

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INTRODUCTION

Allergy diagnoses generally rely upon an evocative clinical history and appropriate confirmatory in vitro and/or in vivo tests such as quantification of specific IgE (sIgE) antibodies, skin tests, and eventually challenge tests. However, for different reasons, correct identification of the precipitating agent(s) is not always straightforward with equivocal, contradictory, or false negative test results. In an attempt to find more sensitive and specific tests to investigate the cause of allergic reactions, functional in vitro tests have been developed. Traditionally, those functional in vitro tests have focused on basophil-mediator release assays such as histamine and leukotriene release tests (1,2). However, as addressed in these reviews, several technical and practical shortcomings and pitfalls have restricted their validation and hampered entrance in mainstream diagnostic use.

With the discovery of the 53 kDa tetraspanin CD63 (3,4), also known as lysosomal-associated membrane glycoprotein-3 (LAMP-3), and the observation that this secretory granule-associated protein is upregulated concomitantly with basophilic degranulation (5), the start was set for the development of a flow-assisted technique

for the detection of the plasma membrane expression of CD63 to quantify basophil activation, because numerous papers on the different clinical applications of the test have been published. In the search for optimization of the technique, other cellular markers with interesting potential in the identification of basophils or detection of their activation have been described. More recently, the attention was drawn to the intracellular mechanisms of basophil activation, and a proof of concept was provided flow cytometry to constitute a unique tool to study signal transduction in the basophil at a single cell level (6).

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This review will summarize on some general principles, technical issues, and mostly focus on the current and future clinical and research applications of the basophil activation test (BAT).

PRINCIPLES

Basophils are granulocytes that develop from CD34+ pluripotent progenitor stem cells, differentiate, and mature in the bone marrow, and circulate in the periphery where they represent less than 1% of the leukocytes. Basophils exhibit a segmented nucleus and are identified by means of metachromatic staining with basic dyes, such as toluidine blue. Human basophils express a variety of cytokine receptors for interleukins, chemokines, complement, prostaglandins, and immunoglobulin Fc receptors (FcεRI and FcγRII) [for review, see (7)].

The basophil can be activated by a number of stimuli, which are mediated by the high-affinity IgE receptor (FcεRI) or not and give rise to distinct activation pathways. The conditions required for effective FcεRI cross-linking and subsequent degranulation are explained elsewhere (8). It is clear that both cellular (FcεRI-IgE) and allergen characteristics determine the final outcome. Electron microscopic studies have revealed that basophil activation may follow two main pathways: (1) the so-called anaphylactic degranulation (AND), which is characterized by fast morphological changes, exocytosis of the intracellular granules and release of preformed mediators and (2) the piecemeal degranulation (PMD), characterized by morphological changes and secretion of granule content without exocytosis (9).

Anyhow, upon challenge with specific allergens that cross-link FcεRI, basophils not only secrete and generate a broad variety of quantifiable bioactive mediators, but also upregulate the expression of distinct activation markers. These alterations can be immunophenotyped on a single cell level by flow cytometry using specific monoclonal antibodies. At present, the most commonly applied activation markers are CD63 and CD203c [Table 1].

CD63 (gp53, LAMP-3) is a member of the transmembrane-4 superfamily (tetraspanins) and is expressed by different cell types such as basophils, mast cells, platelets, and macrophages (3,4). In resting basophils, CD63 is anchored to the intracellular granules and only barely expressed on the surface membrane, both in healthy subjects as well as in patients with allergies. In contrast, as a result of fusion between the granule and the membrane during exocytosis, CD63 is expressed with a high density on activated basophils (5).

CD203c (neural cell surface differentiation antigen, E-NPP3) is a glycosylated type II transmembrane molecule that belongs to the family of ectonucleotide pyrophosphatase/phosphodiesterases (E-NPPs) enzymes that catalyze the hydrolysis of oligonucleotides, nucleoside phosphates, and NAD (10). In haematopoietic cells, surface E-NPP3 is exclusively and constitutively expressed by basophils (11,12).

The upregulation of CD63 and CD203c in response to specific activators and inhibitors appears to follow different kinetics and seems to be directed through alternative signal transduction pathways (13–16). The expression of CD63 seems to be more closely related to AND than CD203c (15).

p38 MAPK (mitogen-activated protein kinase) is an archetypical member of the third MAPK-related pathway that relays a variety of environmental stresses and inflammatory cytokines to intracellular responses. The MAPKs co-ordinately regulate cell proliferation, differentiation, motility, and survival [for review, see (17,18)]. In basophils, p38 MAPK has been repetitively demonstrated to be a pivotal regulator of cell activation following stimulation with different secretagogues (6,19–21).

TECHNICAL ASPECTS AND PITFALLS

Sampling and Preservation

Correct sampling and preservation of the blood is of critical importance to obtain optimal cellular viability and functionality especially with basophils. Ideally, analy-

Table 1
Characteristics of the Activation Markers CD63, CD203c and Phosphorylated p38 MAPK

	CD63	CD203c	Phosphorylated p38 MAPK
Synonym	Gp53 (LAMP-3)	E-NPP3	
Family	Tetraspanins	NPPs	MAPK
Resting basophils	Barely detectable (need for additional marker(s) to identify cells)	Constitutively expressed	Barely detectable
IgE-activated cells	Upregulation ~3 min Expressed at high density (> 1 log scale) Bimodal expression ^a	Upregulation ~5 min Expression less prominent Unimodal expression ^b	Upregulation ~3 min Expressed at high density (> 1 log scale) Unimodal expression ^b
Sensitive to IL-3 priming	No	Yes	Yes
Parallel expression	CD107a (LAMP-1), CD107b (LAMP-2)	CD13, CD164	

LAMP, lysosome-associated membrane protein; E-NPP, Ectonucleotide pyrophosphatase/phosphodiesterases; MAPK, mitogen activated protein kinase.

^aExpression only on subpopulation of cells.

^bExpression in almost all cells.

sis should be performed within 3 h after sampling in preservative and endotoxin-free tubes. Under such conditions, tubes (heparin or EDTA) can be kept at room temperature. For longer preservation (recommended maximum 24 h), EDTA and storage at 4°C could be of use, but currently comprehensive data on this topic are lacking. When cell-isolation techniques are used, blood is normally anticoagulated with EDTA or ACD.

Whole Blood or Isolated Basophils

Flow-assisted analysis of in vitro-activated basophils can be applied either on whole blood or on isolated cells. White blood cells can be easily isolated using centrifugation techniques or density gradient techniques.

The use of whole blood has the advantage of being more practical with simpler manipulation (less centrifugation steps), and the presence of all blood components might better mirror the physiological or pathological in vivo conditions. Additionally, interference with other serum components (anti-IgE, antireceptor antibodies, circulating immune complexes, complement factors, and drugs) cannot be ruled out entirely. When pure basophils are needed, more advanced techniques such as magnetic bead isolation and cell sorting will be needed, as basophils could have different densities. However, cell separation is generally accompanied by loss of basophils and in vitro activation of cells might occur.

Stimulation Conditions

Basophils react immediately upon stimulation, and this is highly dependent on the conditions, requiring optimal temperature, optimal incubation times, as well as appropriate composition of buffer in which reagents have to be diluted. We have demonstrated that prewarming of blood as well as reagents for 15 min at 37°C to markedly enhance onset (within 3 min) and magnitude of anti-IgE mediated CD63 upregulation but not to alter the kinetics of phosphorylation of signaling molecule p38 MAPK (6).

Priming with IL-3

Some authors have shown short preincubation with IL-3 might increase sensitivity of CD63-based assays (22,23), but this seems to be not crucial for potent proteinaceous allergens (24) and stimulation with serum of patients with autoimmune urticaria (25). Alternatively, when investigating allergens that elicit relative little basophil activation such as drugs, increase of assay sensitivity might be relevant. Priming with IL-3 might allow to widen the sometimes narrow gap between specific stimulation and unspecific activation or cytotoxicity as IL-3 primed basophils may react to allergen concentrations a 10–100-fold lower than those for nonprimed cells (26). However, in CD203c (23) and p38 MAPK-based (6) assays, priming with IL-3 causes a nonspecific increase in the test result. The differential influence of IL-3 priming on CD63 and CD203c might (to some extent) explain contradictory results and contribute to the con-

troversy in the literature regarding which is the “best” marker for measuring basophil activation.

Negative and Positive Control Stimulation

Negative control stimulation to assess spontaneous expression of the activation markers, generally implies incubation of the cells with stimulation buffer at the same volume as for the allergen stimulation.

Correct interpretation of the BAT needs relevant negative and positive control stimulation to be included. Traditionally, the positive control is a polyclonal or monoclonal anti-IgE antibody. An alternative positive stimulant is monoclonal anti-FcεRI antibody. In the absence of positive control stimulation, it becomes impossible to correctly interpret negative results of allergen challenges. For these “non-responders,” the BAT is lost as a diagnostic instrument, whereas, for study purposes, patients with unresponsive basophils should be recorded as false negatives. Approximately 5–10% of the individuals tested fail to upregulate CD63 and CD203c with regard to IgE-mediated basophilic activation. False-negative results might also be explained by other causes such as recent exposure to the allergen with temporary refractoriness of the cells and/or transiently reduced levels of allergen-specific circulating and membrane-bound IgE. On the other hand, sensitivity of the assay might significantly decrease over time (27). Therefore, it is recommended to perform analysis between 6 weeks and 12 months after the acute event.

False negative results can also have technical causes such as improper handling and storage, inappropriate challenge of the cells with poorly identified allergens that contain cytotoxic or inhibitory components, application of cytotoxic stimulation concentrations.

As formyl-methionylleucylphenylalanine (fMLP) acts via a G-protein-coupled receptor (FPR-1) that activates MAPK pathways and phospholipase C and does not mimic an IgE-mediated activation of the basophil, it cannot be recommended as an appropriate positive control. However, fMLP can eventually be applied as a tool to assess viability of the cells.

Allergen Stimulation

Proper selection of allergen is a prerequisite that cannot be overemphasized. Currently, flow-assisted analysis and quantification of in vitro activated basophils generally relies upon the use of natural allergen extracts that have proven to be rather satisfactory. Nevertheless, it should be kept in mind that extracts prepared from natural source material might be heterogeneous with varying composition, presence of nonspecific stimulatory and/or inhibitory components such as preservatives, endotoxins, and lectins. Potency of extracts might significantly decrease over time, due to spontaneous degradation of the constituent proteins. Therefore, extreme care must be taken in preparing and conserving allergen extracts. It is mandatory that these extracts should be properly and repeatedly characterized.

To some extent, applying isolated or recombinant allergens can circumvent these issues. Flow-assisted allergy diagnosis, using isolated and/or recombinant allergens, might be a highly reliable assay for component-resolved allergy diagnosis (23,28–31).

Commercially available skin test extracts are rarely suitable for basophil activation experiments, as they contain cytotoxic and/or inhibitory preservatives. To some extent, dialyzing the extract might solve this problem.

For drugs, the injectable (intravenous and/or intramuscular) formulation is recommended.

It is of note that although some companies provide numerous allergen extracts for basophil activation experiments, these are rarely clinically validated on sufficient numbers of well-characterized patients and appropriate control individuals.

Dose-Finding Experiments

As addressed recently by Kleine-Tebbe (32), basophil responses can be extremely heterogeneous between different individuals. However, allergen-specific phosphorylation of p38 MAPK and upregulation of CD63 and CD203c is generally not restricted to a single stimulation concentration but implies different stimulation concentrations that span several log scales (6,28,29,33–36). This provides the opportunity to restrict basophil activation experiments to an “optimal” concentration that discriminates between patients and control individuals. Unfortunately, this might not apply to all allergens. Drugs might show a considerably smaller range of basophil activation concentrations (37).

Another important reason to perform dose-response experiments is to establish the challenge concentrations that elicit submaximal and maximal stimulation. As demonstrated in Figure 1, and already published in this Journal, the BAT can monitor venom immunotherapy, provided the cells are stimulated submaximally (36).

Identification Markers

Most studies have currently applied an anti-IgE antibody to identify basophils in peripheral blood. It has been argued that other cells (e.g., monocytes) that also express FcεRI might interfere with the test and that exposure of basophils to anti-IgE before allergen stimulation may alter their responsiveness. Compared with basophils, the average cell-surface FcεRI expression on monocytes is low. In difficult cases, an additional staining with anti-HLADR can help to discriminate between HLA-DR[−] basophils and HLA-DR⁺ monocytes. Alternatively, anti-IgE antibodies applied for basophil characterization do not elicit cell activation. In classical basophil activation assays, cells are exposed to an anti-IgE for flow cytometric identification only after the stimulation phase and presumed additional activation by anti-IgE is avoided by fixing, cooling, or adding EDTA containing buffer.

In peripheral blood, CD203c expression is exclusively and constitutionally expressed on the surface of resting

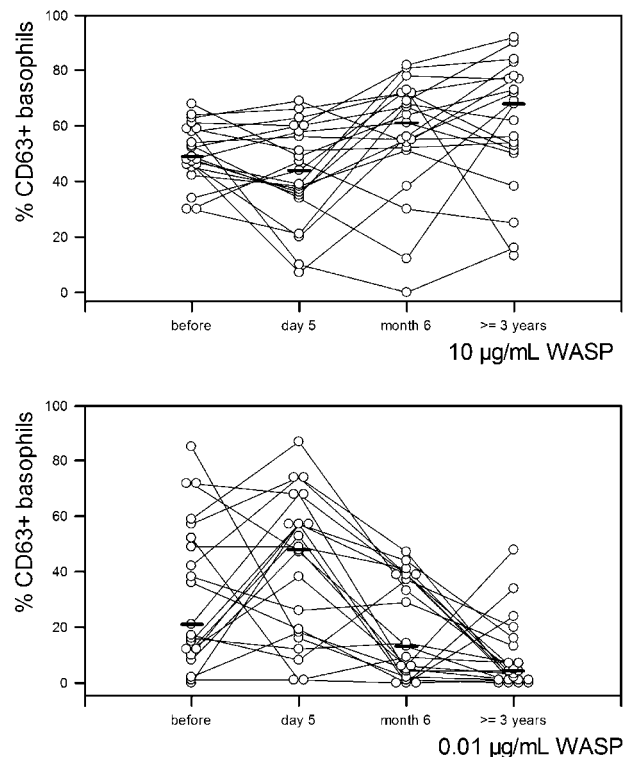


Fig. 1. Effect of venom immunotherapy on a longitudinal survey of 36 months. Percentages of CD63-positive basophils in 21 wasp venom allergic patients before treatment (day 0), after semrush hyposensitization (day 5) and after 6 months (month 6) as well as 36 months (month 36) of maintenance therapy. Upper and lower panel represent the data for stimulation at 10 and 0.01 µg/mL, respectively. The bold lines denote the medians. From this figure, it emerges that prolonged maintenance venom-specific immunotherapy (over 6 and 36 months) to inhibit basophil responsiveness (assessed flow cytometrically by quantification of CD63 expression), provided the cells are challenged submaximally.

basophils. Therefore, it can be applied as an alternative marker for basophil identification. However, spontaneous expression of CD203c by resting basophils can be weak, hampering clear identification of the cells (23). Alternatively, as CD203c expression is quickly upregulated upon encounter of the basophil with specific allergen (see below); it offers the advantage to study basophil activation without additional staining in a so-called single color BAT.

Prostaglandin D₂ (PGD₂), a product of the arachidonic acid/cyclo-oxygenase pathway, is generated at sites of inflammation and is believed to exert a key regulatory function in inflammatory and immune responses. PGD₂ is predominantly released by resident mast cells and has a prominent role in allergic reactions due to its ability to induce extravasation of leukocytes and by acting as a chemoattractant for Th2 cells, eosinophils, and basophils. To date, two distinct G-protein-coupled receptors for PGD₂ have been described; the D prostanoid receptor (DP or PD1) (38) and the chemoattractant receptor-homologous molecule expressed by Th2 lymphocytes (CRTH2, PD2, CD294) (39,40). PGD₂ favors Th2 func-

tions via CRTH2 while restraining Th1 functions via DP (41,42). Basophils can readily be discriminated from CRTH2 carrying eosinophils and TH2 lymphocytes on the basis of light scattering and an additional staining with anti-CD3, respectively (43).

Another potential basophil characterization marker is the eotaxin CC chemokine receptor-3 (CCR3) (44). However, CCR3 is not lineage specific and is expressed on a variety of inflammatory cells associated with allergic responses such as basophils, mast cells, and TH2 lymphocytes, and resident tissue cells such as airway epithelium. Subsequently, as for CRTH2, it is best to apply an additional marker such as anti-CD3 to differentiate with TH2 lymphocytes, particularly in atopic patients.

Finally, some groups (35,37,45–48) have applied the combination of anti-CD123 and anti-HLADR to characterize basophils in peripheral blood. CD123 is a glycosylated type I transmembrane molecule that belongs to the cytokine receptor superfamily. CD123 is the primary low-affinity subunit of the IL-3 receptor that associates with CD131, the common β -chain of the IL-3, IL-5, and GM-CSF receptor, to form the high-affinity IL-3 receptor. The IL-3 receptor is involved in cell signaling for cell growth and differentiation. In peripheral human blood, the CD123 antigen is expressed at high levels only on plasmacytoid dendritic cells and basophilic granulocytes but at low levels also on monocytes, eosinophilic granulocytes, myeloid dendritic cells, and subsets of haematopoietic progenitor cells. However, in contrast to basophils, these cells stain positive with an anti-HLA-DR antibody. CD123 expression appears to be less variable than expression of IgE and is independent of the allergy status of the donor. For a review on CD123, the reader is referred elsewhere (49).

Activation Markers

At present, the most commonly used markers in basophil activation experiments are CD63 and CD203c. The major characteristics of CD63 and CD203c are summarized earlier and in Table 1.

The few comparative studies, that have been done, show that CD63 and CD203c are clearly different in their upregulation profile. Upregulation of CD63 is generally bimodal with a subpopulation of cells that express CD63 with a high intensity versus a population with lower CD63 expression (Fig. 2). Upregulation of CD203c is generally less prominent but often occurs in almost all cells. Consequently, results for CD63 expression are generally expressed as net percentage CD63⁺ basophils, whereas results for CD203c are mainly expressed as stimulation indices (SI) of mean fluorescence intensity (MFI) (14,15,23).

It has been argued that a fraction of CD63 might come from contaminating activated platelets that adhere to basophils. However, platelets from patients do not upregulate CD63 expression in response to stimulation with allergen or anti-IgE and a vast majority of anti-IgE⁺/anti-CD63⁺ cells have been phenotyped as CD45⁺,

CD14⁺, CD16⁺, and CD41⁺ (CD41 is gpIIB antigen expressed by platelets and megakaryocytes.)

Hennersdorf et al. (15) identified four additional markers of basophil activation (CD13, CD107a, CD107b, and CD164) and demonstrated that the lysosome-associated membrane proteins CD107a (LAMP-1), CD107b (LAMP-2), and CD63 (LAMP-3) are concomitantly upregulated, according to their localization on the membrane of intracellular granules. These granules contain the pre-synthesized bioactive mediators that are released upon Fc ϵ RI cross-linking, and AND is distinct from the small vesicles associated with PMD. PMD is associated with upregulation of the transmembrane glycoprotein sialomucin endolyn (CD164) and the ecto-enzymes CD13 (gp150) and CD203c.

The mechanisms that govern basophil activation and culminate in degranulation are complex and remain incompletely disentangled. Nevertheless, the different pathways involved in basophil activation and mediator release are propagated by consecutive phosphorylation cascades. Traditionally, analysis of signaling is based upon western blot and ELISA assay, but recently we provided the proof of concept flow cytometry to constitute a reliable tool to quantify phosphorylated p38 MAPK (6). Moreover, from this study, it appears phosphorylated p38 MAPK that is highly linked to the upregulation of CD63 to constitute a novel marker for flow-assisted allergy diagnosis.

CLINICAL APPLICATIONS

Analysis of Patient Basophils

Currently, flow-assisted analysis and quantification of in vitro activated basophils proved sensitive and specific for the diagnosis of several IgE-mediated allergies including classical inhalant allergens (house dust mite, cat epithelium, and pollen), hymenoptera venom allergy, natural rubber latex allergy, primary and secondary food allergies, and drugs (Table 2).

In individual patients, the BAT confirmed diagnosis of different food (68,69) and drug (70–72) allergies. Moreover, in some of the patients with drug allergy, the BAT contributed to establish the individual therapeutic alternative and/or allowed identification of potentially cross-reactive structures.

In venom allergy, it was demonstrated the BAT to take the sting out of difficult cases that yield equivocal or negative sIgE and/or skin test results (73).

In addition, the BAT allowed component resolved diagnosis of pollen allergy (28), venom allergy (74), and natural rubber latex allergy (30).

Finally, the technique was applied to evaluate passive IgE sensitization through blood transfusion (75).

Analysis of Nonatopic/Atopic Donor Basophils

Flow-assisted analysis and quantification of in vitro activated basophils can also be applied on cells obtained from nonatopic and/or atopic donor individuals. In a first application, frequently referred as the passive sensi-

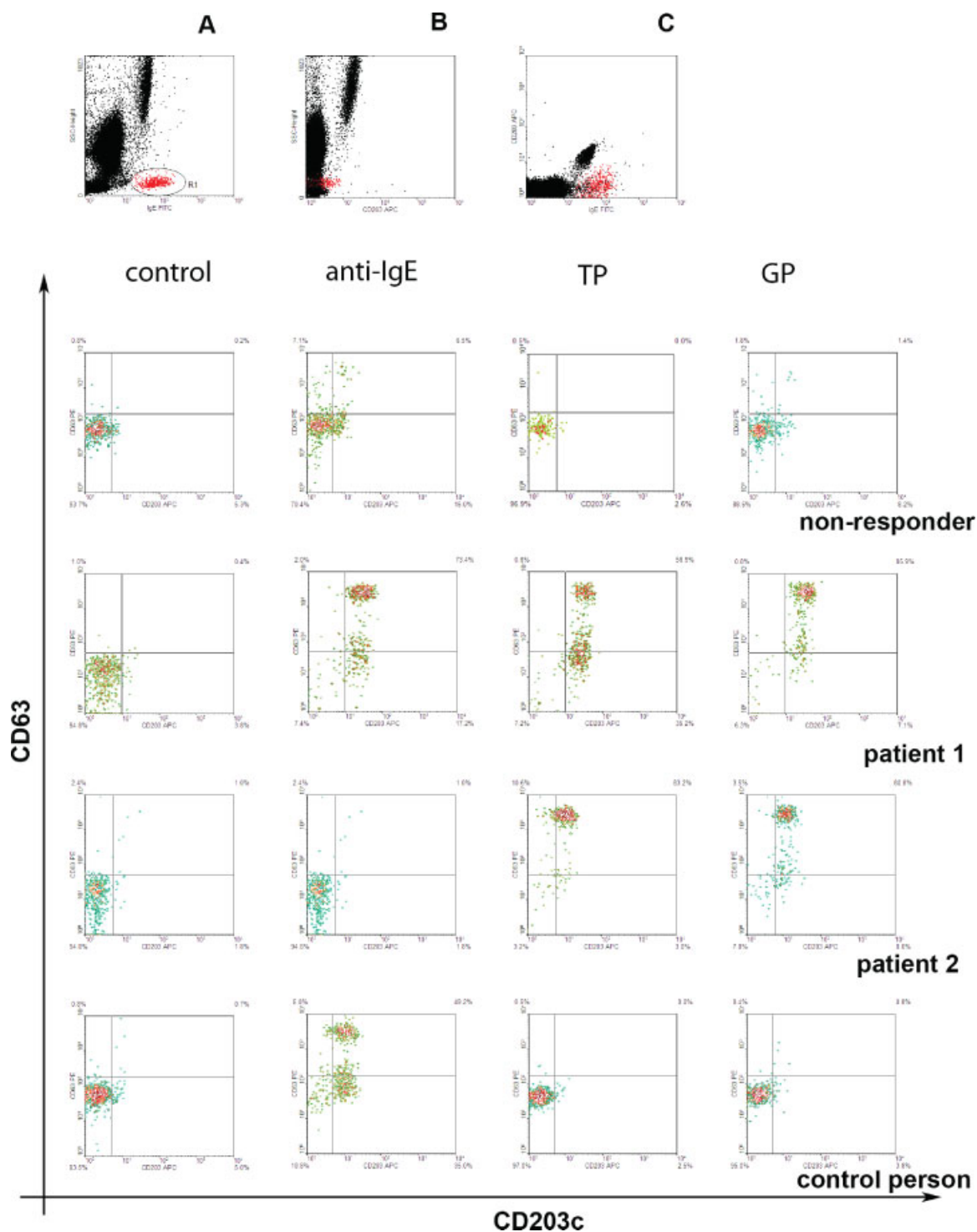


FIG. 2. Differences in distribution of CD63 and CD203c. Top panel: **A:** Selection of basophils according to side scatter characteristics (SSC) and IgE labeling. **B** and **C:** Show gated basophils (red) on CD203c and SSC (B) and IgE and CD203c plot (C). Bottom panel: triple stain (IgE, CD63, and CD203c) dot plots: Lane 1 shows a typical CD203c and CD63 plot in a patient allergic to tree and grass pollen with cells nonresponsive to stimulation with anti-IgE and allergen (tree pollen (TP) and grass pollen (GP)). Lanes 2 and 3 show typical CD203c and CD63 plots in two patients allergic to tree and grass pollen. Note the bimodal dual response of CD63, whereas the CD203c response is homogeneous in patient 1. Lane 4 shows a typical CD203c and CD63 response in a control individual without tree and grass pollen allergy.

Table 2
Clinical Applications (adapted from 50,51)

Stimulus	Reference test	Sens	Spec	N	Reference
<i>Inhalant allergens</i>					
House dust mite	H + IgE and/or ST	56–78 ^a	91–100	20	22
Dactylis glomerata	H + IgE and/or ST	73–100	100	20	22
Cypress pollen	H + ST + PT	91	100	75	52
HDM and Lolium perenne	H + IgE + ST	93	98	128	53
Cat epithelium (Fel d 1)	H + IgE and/or ST	100 ^a	95	39	23
<i>Latex</i>					
Latex	H + IgE + ST	93	92	102	54
Latex	H + ST	93	100	73	55
Latex	H + IgE and/or ST	80	97	79	56
Latex (Hev b5, Hev b 6.01, Hev b 6.02)	H	96	100	33	30
<i>Hymenoptera venom</i>					
Wasp	H	92	80	70	34
Wasp	H	85	83	87	35
Bee	H	91	90	87	35
Bee	H	100	100	12	57
Wasp	H	97	100	39	57
Wasp	H	99 ^b	100	94	36
<i>Drugs</i>					
NMBA	H	64	93	50	58
NMBA	H ± ST	54	100	60	59
NMBA	H	79	100	24	60
NMBA	H + ST	36–86 ^c	93	92	27
Rocuronium (NMBA)	H + ST	92	100	22	37
β-Lactam	H + ST	50	93	88	61
β-Lactam	H ± ST ± IgE ± PT	49	91	110	62
Metamizol	H ± PT	42	100	55	63
Aspirin and NSAID	H ± PT	15–55	74–100	90	64
<i>Food</i>					
Carrot, celery, hazelnut	H (OAS)	85–90	80–90	20	65
Apple	H (OAS)	100 ^d	100	59	33
Apple	H (OAS)	88	75 ^e	59	33
Apple (Mal d 1)	H (OAS)	75	68	54	66
Celery (Api g 1)	H (OAS)	75	77	54	66
Carrot (Dau c 1)	H (OAS)	65	100	54	66
Carrot (Dau c 1)	H (OAS)	65	100	54	66
<i>Autoimmune urticaria</i>					
Serum of patients with AIU	H + ASST	20	70	65	67
	H + ASST	96	91	64	48

Fel d 1, major allergen from cat (*Felis domesticus*); Sens, sensitivity; spec, specificity; N, total number of patients and control individuals; NMBA, neuromuscular blocking agent; OAS, oral allergy syndrome; AIU, auto-immune urticaria; H, history; ST, skin test; PT, provocation tests; ASST, autologous serum skin test.

^aIn the study by Cozon et al. (22) and Ocmant et al. (23) there is some evidence for IL-3 to increase sensitivity of the CD63-based BAT.

^bTaken into account the non-responders to anti-IgE control stimulation and allergen, the sensitivity of BAT is 84%.

^cIn the study by Kvedariene et al. (27) it is demonstrated sensitivity of the BAT to decrease over time.

^dTaken into account the non-responders sensitivity of the BAT is 90%.

^eIn an additional comparison between birch pollen allergic patients with and without apple-induced OAS.

tization procedure, (previously stripped, i.e., removal from membrane-bound IgE by acidic buffers) donor basophils, before allergen challenge, are preincubated with serum of the patient. These passive sensitization protocols are able to detect allergen-specific IgE in the patients' serum, however, are quite laborious, difficult to standardize and highly dependent on the reactivity of the donor cells. In addition, passive sensitization assays have been proven to be less sensitive than the direct basophil activation assays (76).

Apart from the limited diagnostic applications, passive sensitization protocols have also been applied to assess functional differences between peanut sIgE antibodies

(77) to functionally analyze cross-reactivity of sIgE antibodies (31,77–79) and to characterize allergens (31).

Given the disappointing results of passive sensitization protocols with (stripped) donor basophils, the development of mast cell lines or stable humanized (rat) basophil leukemia cell lines seems of particular interest.

A second application comprises demonstration of functional autoantibodies against the α-chain of the high-affinity IgE receptor (FcεRI), or less commonly, against IgE itself in patients with autoimmune chronic urticaria (25,48,67,80–82). These autoantibodies are able to induce histamine release from basophils and mast cells via direct cross-linking of adjacent IgE or IgE receptors (83).

RESEARCH APPLICATIONS

The mechanisms that govern basophil activation and finally culminate in mediator release are complex and remain incompletely elucidated. Analysis of signaling in basophils has been performed using Western-blot techniques that have provided fundamental insights into the different transduction pathways and regulatory mechanisms involved (19,20,84). We have recently demonstrated that flow cytometry can provide results concerning dose responses and kinetics of p38 MAPK phosphorylation qualitatively similar to the data obtained with the Western-blot techniques (6). However, the flow cytometric approach offers different opportunities that make it more suitable for clinical application. First, the technique allows to reduce the sampling volume considerably and to perform repetitive drawings in the same individual, thereby creating the opportunity to study the pharmacological effects of drugs or follow-up of treatment as for to the effect of venom-specific immunotherapy. Second, the flow cytometric technique does not require an a priori separation and purification of the basophils to ensure reliable measurements with negligible contribution from other cell contaminants. Flow cytometry enables to study the cells in their "natural" environment and to avoid potential interference from additional manipulations. Alternatively, the technique also significantly shortens the analysis time (3 h instead of 1–2 days) and thus renders it more accessible for clinical and research applications. Last but not the least, flow cytometry allows identification of cells with heterogeneity in responsiveness rather than providing data that represent a mean value for the total-isolated cell population.

The technique was also repeatedly applied to study and to compare the (residual) allergenicity of natural allergen extracts, chemically modified allergoids, and recombinant allergens (14,28,29,85–88).

CONCLUSION, PERSPECTIVES, AND SPECULATIVE VIEWPOINTS

After several improvements, the BAT has become a sensitive and specific instrument for in vitro diagnosis of immediate allergy, complementary to traditional quantification of specific IgE, and skin tests. The technique has been validated for several IgE-mediated allergies including indoor and outdoor allergens, primary and secondary food allergies, latex allergy, hymenoptera venom allergy, and some drug allergies. The present challenge is to harmonize and improve protocols in multicentre studies to allow entrance of the BAT in mainstream diagnostic application, at least in cases where routine allergy testing has lead to equivocal results.

Until recently, due to the very low number of basophils in human peripheral blood, assessment of intracellular signaling in these cells and pharmacological studies on these cells were difficult to perform. With the advent of novel monoclonal antibodies directed against phosphorylated epitopes and new intracellular staining techniques, it is anticipated that flow cytometry might constitute an

additional instrument to investigate intracellular signaling in basophils. Moreover, as the technique allows integrating immunophenotyping of individual cells, it might capture data that are inaccessible for techniques that are performed on homogenous cell populations and from which results represent an average of isolated cells analyzed.

Finally, as the BAT closely resembles the in vivo pathway leading to symptoms, we believe that the technique might further be adopted to differentiate between clinically relevant and irrelevant IgE antibodies, to study cross-reactivity, quantitative evaluation of (residual) allergenicity, and component-resolved diagnosis.

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LITERATURE CITED

1. Crockard AD, Ennis M. Basophil histamine release tests in the diagnosis of allergy and asthma. *Clin Exp Allergy* 2001;31:345–350.
2. Demoly P, Lebel B, Arnoux B. Allergen-induced mediator release tests. *Allergy* 2003;58:553–558.
3. Nieuwenhuis HK, van Oosterhout JJ, Rozemuller E, van Iwaarden IF, Sixma JJ. Studies with a monoclonal antibody against activated platelets: Evidence that a secreted 53,000-molecular weight lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. *Blood* 1987;70:838–845.
4. Metzelaar MJ, Wijngaard PL, Peters PJ, Sixma JJ, Nieuwenhuis HK, Clevers HC. CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J Biol Chem* 1991;266:3239–3245.
5. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991;88(3 Pt 1):328–338.
6. Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, de Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: A proof of concept. *Clin Exp Allergy* 2007;37:1668–1675.
7. Falcone FH, Zillikens D, Gibbs BF. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. *Exp Dermatol* 2006;15:855–864.
8. Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. *Mol Nutr Food Res* 2006;50:620–624.
9. Dvorak AM. Cell biology of the basophil. *Int Rev Cytol* 1998;180:87–236.
10. Goding JW, Grobbs B, Slegers H. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. *Biochim Biophys Acta* 2003;1638:1–19.
11. Bühring HJ, Simmons PJ, Pudney M, Muller R, Jarrossay D, van Agt-hoven A, Willheim M, Brugger W, Valent P, Kanz L. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood* 1999;94:2343–2356.
12. Bühring HJ, Seiffert M, Giesert C, Marxer A, Kanz L, Valent P, Sano K. The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3. *Blood* 2001;97:3303–3305.
13. Majlesi Y, Samorapoompichit P, Hauswirth AW, Scherthaner GH, Ghannadan M, Baghestanian M, Rezaie-Majd A, Valenta R, Sperr WR, Bühring HJ, Valent P. Cerivastatin and atorvastatin inhibit IL-3-dependent differentiation and IgE-mediated histamine release in human basophils and downmodulate expression of the basophil-activation antigen CD203c/E-NPP3. *J Leukoc Biol* 2003;73:107–117.
14. Bühring HJ, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004;133:317–329.
15. Hennersdorf F, Florian S, Jakob A, Baumgartner K, Sonneck K, Nordheim A, Biedermann T, Valent P, Bühring HJ. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* 2005;15:325–335.

16. Hauswirth AW, Sonneck K, Florian S, Krauth MT, Böhm A, Sperr WR, Valent R, Scherthaner GH, Printz D, Fritsch G, Bühring HJ, Valent P. Interleukin-3 promotes the expression of E-NPP3/CD203C on human blood basophils in healthy subjects and in patients with birch pollen allergy. *Int J Immunopathol Pharmacol* 2007;20:267-278.
17. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: A family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004;68:320-344.
18. Cuenda A, Rousseau S. p38 MAPK-kinases pathway regulation, function and role in human diseases. *Biochim Biophys Acta* 2007;1773:1358-1375.
19. Miura K, Schroeder JT, Hubbard WC, MacGlashan DW Jr. Extracellular signal-regulated kinases regulate leukotriene C₄ generation, but not histamine release or IL-4 production from human basophils. *J Immunol* 1999;162:4198-4206.
20. Gibbs BE, Plath KE, Wolff HH, Grabbe J. Regulation of mediator secretion in human basophils by p38 mitogen-activated protein kinase: Phosphorylation is sensitive to the effects of phosphatidylinositol 3-kinase inhibitors and calcium mobilization. *J Leukoc Biol* 2002;72:391-400.
21. Plath KE, Grabbe J, Gibbs BE. Calcineurin antagonists differentially affect mediator secretion, p38 mitogen-activated protein kinase and extracellular signal-regulated kinases from immunologically activated human basophils. *Clin Exp Allergy* 2003;33:342-350.
22. Cozon G, Ferrandiz J, Peyramond D, Brunet J. Detection of activated basophils using flow cytometry for diagnosis in atopic patients. *Allergol Immunopathol (Madr)* 1999;27:182-187.
23. Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandene L. Flow cytometry for basophil activation markers: The measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods* 2007;320:40-48.
24. Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, de Clerck LS, Stevens WJ. Comments regarding marked improvement of the basophil activation test by detecting CD203c instead of CD63⁺ by Boumiza et al. *Clin Exp Allergy* 2003;33:849-843.
25. Gyimesi E, Sipka S, Dankó K, Kiss E, Hídvégi B, Gál M, Hunyadi J, Irinyi B, Szegedi A. Basophil CD63 expression assay on highly sensitized atopic donor leukocytes: a useful method in chronic autoimmune urticaria. *Br J Dermatol* 2004;151:388-396.
26. de Weck AL, Stadler BM, Dahinden CA. New perspectives in the modulation of allergic inflammation. *Int Arch Allergy Appl Immunol* 1989;90 (Suppl 1):17-21.
27. Kvedariene V, Kamey S, Ryckwaert Y, Rongier M, Bousquet J, Demoly P, Arnoux B. Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006;61:311-315.
28. Hauswirth AW, Natter S, Ghannadan M, Majlesi Y, Scherthaner GH, Sperr WR, Bühring HJ, Valent R, Valent P. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol* 2002;110:102-109.
29. Kahlert H, Cromwell O, Fiebig H. Measurement of basophil-activating capacity of grass pollen allergens, allergoids and hypoallergenic recombinant derivatives by flow cytometry using anti-CD203c. *Clin Exp Allergy* 2003;33:1266-1272.
30. Sanz ML, Garcia-Aviles MC, Tabar AI, Anda M, Garcia BE, Barber D, Salcedo G, Rihs HP, Raulf-Heimsoth M. Basophil activation test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children. *Pediatr Allergy Immunol* 2006;17:148-156.
31. Wallowitz ML, Chen RJ, Tzen JT, Teuber SS. Ses i 6, the sesame 11S globulin, can activate basophils and shows cross-reactivity with walnut in vitro. *Clin Exp Allergy* 2007;37:929-938.
32. Kleine-Tebbe J, Erdmann S, Knol EF, MacGlashan DW Jr., Poulsen LK, Gibbs BE. Diagnostic tests based on human basophils: Potentials, pitfalls and perspectives. *Int Arch Allergy Immunol* 2006;141:79-90.
33. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, de Clerck LS, Stevens WJ. Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. *Cytometry B Clin Cytom* 2005;64B:28-33.
34. Erdmann SM, Sachs B, Kwiczen R, Moll-Slodowy S, Sauer I, Merk HF. The basophil activation test in wasp venom allergy: Sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004;59:1102-1109.
35. Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: A prospective study. *Allergy* 2004;59:1110-1117.
36. Ebo DG, Hagendorens MM, Schuerwegh AJ, Beirens LM, Bridts CH, de Clerck LS, Stevens WJ. Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. *Cytometry B Clin Cytom* 2007;72B:196-203.
37. Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, de Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006;61:935-939.
38. Boie Y, Sawyer N, Slipetz DM, Metters KM, Abramovitz M. Molecular cloning and characterization of the human prostanoid DP receptor. *J Biol Chem* 1995;270:18910-18916.
39. Abe H, Takeshita T, Nagata K, Arita T, Endo Y, Fujita T, Takayama H, Kubo M, Sugamura K. Molecular cloning, chromosome mapping and characterization of the mouse CRTH2 gene, a putative member of the leukocyte chemoattractant receptor family. *Gene* 1999;227:71-77.
40. Nagata K, Hirai H. The second PGD(2) receptor CRTH2: Structure, properties, and functions in leukocytes. *Prostaglandins Leukot Essent Fatty Acids* 2003;69:169-177.
41. Kostenis E, Ulven T. Emerging roles of DP and CRTH2 in allergic inflammation. *Trends Mol Med* 2006;12:148-158.
42. Pettipher R, Hansel TT, Armer R. Antagonism of the prostaglandin D₂ receptors DP1 and CRTH2 as an approach to treat allergic diseases. *Nat Rev Drug Discov* 2007;6:313-325.
43. Boumiza R, Debard AL, Monneret G. The basophil activation test by flow cytometry: Recent developments in clinical studies, standardization and emerging perspectives. *Clin Mol Allergy* 2005;3:9.
44. Ducrest S, Meier F, Tschopp C, Pavlovic R, Dahinden CA. Flowcytometric analysis of basophil counts in human blood and inaccuracy of hematology analyzers. *Allergy* 2005;60:1446-1450.
45. Olweus J, BitMansour A, Warnke R, Thompson PA, Carballido J, Picker LJ, Lund-Johansen F. Dendritic cell ontogeny: A human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci USA* 1997;94:12551-12556.
46. Varro R, Chen C-H. A no-wash 3-color basophil degranulation flow assay using CD123+HLA-DR- phenotype for basophil classification. *Cytometry* 2000;S(10):116.
47. Gonzalez-Munoz M, Luque R, Nauwelaers F, Moneo I. Detection of anisakis simplex-induced basophil activation by flow cytometry. *Cytometry B Clin Cytom* 2005;68B:31-36.
48. Frezzolini A, Provini A, Teofoli P, Pomponi D, De PO. Serum-induced basophil CD63 expression by means of a tricolour flow cytometric method for the in vitro diagnosis of chronic urticaria. *Allergy* 2006;61:1071-1077.
49. Moretti S, Lanza F, Dabusti M, Tieghi A, Campioni D, Dominici M, Castoldi GL. CD123 (interleukin 3 receptor α -chain). *J Biol Regulat Homeost Agents* 2001;15:98-100.
50. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, de Clerck LS, Stevens WJ. In vitro allergy diagnosis: Should we follow the flow? *Clin Exp Allergy* 2004;34:332-339.
51. Ebo DG, Sainte-Laudy J, Bridts CH, Mertens CH, Hagendorens MM, Schuerwegh AJ, et al. Flow-assisted allergy diagnosis: Current applications and future perspectives. *Allergy* 2006;61:1028-1039.
52. Paris-Köhler A, Demoly P, Persi L, Lebel B, Bousquet J, Arnoux B. In vitro diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). *J Allergy Clin Immunol* 2000;105(2, Pt 1):339-345.
53. Sanz ML, Sanchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, Diéguez I, de Weck AL. Allergen-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. *Clin Exp Allergy* 2001;31:1007-1013.
54. Ebo DG, Lechkar B, Schuerwegh AJ, Bridts CH, de Clerck LS, Stevens WJ. Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy* 2002;57:706-712.
55. Sanz ML, Gamboa PM, Garcia-Aviles C, Vila L, Dieguez I, Antepara I, de Weck AL. Flow-cytometric cellular allergen stimulation test in latex allergy. *Int Arch Allergy Immunol* 2003;130:33-39.
56. Hemery ML, Arnoux B, Dhivert-Donnadieu H, Rongier M, Barbotte E, Verdier R, et al. Confirmation of the diagnosis of natural rubber latex allergy by the Basotest method. *Int Arch Allergy Immunol* 2005;136:53-57.
57. Eberlein-König B, Schmidt-Leidescher C, Rakoski J, Behrendt H, Ring J. In vitro basophil activation using CD63 expression in patients with bee and wasp venom allergy. *J Invest Allergol Clin Immunol* 2006;16:5-10.
58. Abuaif N, Rajoely B, Ghazouani E, Levy DA, Pecquet C, Chabane H, Leynadier F. Validation of a flow cytometric assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol* 1999;104(2, Pt 1):411-418.
59. Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002;102:192-199.

60. Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005;60:251-256.
61. Sanz ML, Gamboa PM, Antepara I, Uasuf C, Vila L, Garcia-Aviles C, Chazot M, de Weck AL. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002;32:277-286.
62. Torres MJ, Padial A, Mayorga C, Fernandez T, Sanchez-Sabate E, Cornejo-Garcia JA, Antúnez C, Blanca M. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004;34:1768-1775.
63. Gamboa PM, Sanz ML, Caballero MR, Antepara I, Urrutia I, Jauregui I, González G, Diéguez I, de Weck AL. Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. *Allergy* 2003;58:312-317.
64. Gamboa P, Sanz ML, Caballero MR, Urrutia I, Antepara I, Esparza R, de Weck AL. The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004;34:1448-1457.
65. Erdmann SM, Heussen N, Moll-Slodowy S, Merk HF, Sachs B. CD63 expression on basophils as a tool for the diagnosis of pollen-associated food allergy: Sensitivity and specificity. *Clin Exp Allergy* 2003;33:607-614.
66. Erdmann SM, Sachs B, Schmidt A, Merk HF, Scheiner O, Moll-Slodowy S, Sauer I, Kwiecien R, Maderegger B, Hoffmann-Sommergruber K. In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. *Int Arch Allergy Immunol* 2005;136:230-238.
67. Wedi B, Novacic V, Koerner M, Kapp A. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression-inhibitory effects of anti-inflammatory drugs. *J Allergy Clin Immunol* 2000;105:552-560.
68. Ebo DG, Bridts CH, Mertens MH, Stevens WJ. Coriander anaphylaxis in a spice grinder with undetected occupational allergy. *Acta Clin Belg* 2006;61:152-156.
69. Ebo DG, Ahrazem O, Lopez-Torres G, Bridts CH, Salcedo G, Stevens WJ. Anaphylaxis from mandarin (*Citrus reticulata*): Identification of potential responsible allergens. *Int Arch Allergy Immunol* 2007;144:39-43.
70. Ebo DG, Haine SE, Hagendorens MM, Bridts CH, Conraads VM, Vorlat A, Vrints CJ, de Clerck LS, Stevens WJ. Hypersensitivity to nadroparin calcium: Case report and review of the literature. *Clin Drug Investig* 2004;24:421-426.
71. Ebo DG, Wets RD, Spiessens TK, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to patent blue. *Allergy* 2005;60:703-704.
72. Ebo DG, Goossens S, Opsomer F, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to hyaluronidase. *Allergy* 2005;60:1333-1334.
73. Ebo DG, Hagendorens MM, Bridts CH, de Clerck LS, Stevens WJ. Hymenoptera venom allergy: Taking the sting out of difficult cases. *J Invest Allergol Clin Immunol* 2007;17:357-360.
74. Binder M, Fierlbeck G, King T, Valent P, Buhring HJ. Individual hymenoptera venom compounds induce upregulation of the basophil activation marker ectonucleotide pyrophosphatase/phosphodiesterase 3 (CD203c) in sensitized patients. *Int Arch Allergy Immunol* 2002;129:160-168.
75. Johansson SG, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L, Söderström L, Stiller V, Oman H. Passive IgE-sensitization by blood transfusion. *Allergy* 2005;60:1192-1199.
76. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol* 1999;82:33-40.
77. Shreffler WG, Lencer DA, Bardina L, Sampson HA. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen. Ara h 2. *J Allergy Clin Immunol* 2005;116:893-899.
78. de Leon MP, Drew AC, Glaspole IN, Suphioglu C, Rolland JM, O'Hehir RE. Functional analysis of cross-reactive immunoglobulin E antibodies: Peanut-specific immunoglobulin E sensitizes basophils to tree nut allergens. *Clin Exp Allergy* 2005;35:1056-1064.
79. de Leon MP, Drew AC, Glaspole IN, Suphioglu C, O'Hehir RE, Rolland JM. IgE cross-reactivity between the major peanut allergen Ara h 2 and tree nut allergens. *Mol Immunol* 2007;44:463-471.
80. De Swert A, van den Keybus C, Kasran A, Cadot P, Neyens K, Coorevits L, Kochuyt AM, Degreef H, Ceuppens JL. Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD 63. *J Allergy Clin Immunol* 2005;116:662-667.
81. Yasnowsky KM, Dreskin SC, Efav B, Schoen D, Vedanthan PK, Alam R, Harbeck RJ. Chronic urticaria sera increase basophil CD203c expression. *J Allergy Clin Immunol* 2006;117:1430-1434.
82. Irinyi B, Szeles G, Gyimesi E, Tumpek J, Heredi E, Dimitrios G, Adány R, Hunyadi J, Szegedi A. Clinical and laboratory examinations in the subgroups of chronic urticaria. *Int Arch Allergy Immunol* 2007;144:217-225.
83. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;328:1599-1604.
84. Kopley CL, Wilson BS, Oliver JM. Identification of the Fc epsilonRI-activated tyrosine kinases Lyn, Syk, and Zap-70 in human basophils. *J Allergy Clin Immunol* 1998;102:304-315.
85. Reininger R, Swoboda I, Böhle B, Hauswirth AW, Valent P, Rumpold H, Valenta R, Spitzauer S. Characterization of recombinant cat albumin. *Clin Exp Allergy* 2003;33:1695-1702.
86. Grönlund H, Bergman T, Sandström K, Alvelius G, Reininger R, Verdino P, Hauswirth A, Liderot K, Valent P, Spitzauer S, Keller W, Valenta R, van Hage-Hamsten M. Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. *J Biol Chem* 2003;278:40144-40151.
87. Drew AC, Eusebius NP, Kenins L, de Silva HD, Suphioglu C, Rolland JM, O'hehir RE. Hypoallergenic variants of the major latex allergen Hev b 6.01 retaining human T lymphocyte reactivity. *J Immunol* 2004;173:5872-5879.
88. Ball T, Edstrom W, Mauch L, Schmitt J, Leistler B, Fiebig H, Sperr WR, Hauswirth AW, Valent P, Kraft D, Almo SC, Valenta R. Gain of structure and IgE epitopes by eukaryotic expression of the major Timothy grass pollen allergen. Phl p 1. *FEBS J* 2005;272:217-227.