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Update on the Performance and Application of Basophil Activation Tests

Emily C. McGowan, MD and
Johns Hopkins Asthma and Allergy Center, Division of Allergy and Clinical Immunology, 5501 Hopkins Bayview Circle, Baltimore, MD 21224, USA, Telephone: 410-550-2191, Fax: 410-550-2527

Sarbjit Saini
Emily C. McGowan: emcgowa4@jhmi.edu

Abstract

The basophil activation test (BAT) is a flow-cytometry-based functional assay that assesses the degree of cell activation after exposure to a stimuli. Though no standardized technique currently exists, recent advances have improved the performance of this assay, including identification of new basophil-specific markers and comparisons of the expression of CD63 to CD203c during activation. The basophil activation test has also been validated for many IgE-mediated disease conditions, which have been extensively reviewed elsewhere. This review focuses on the most recent applications of this test to the diagnosis of allergy to drugs, foods, venoms, and pollens, and the evolving role of the BAT in monitoring immunotherapy.

Keywords

Basophil activation test; Basophil; CD63; CD203c; CCR3; Food allergy; Drug allergy; Venom hypersensitivity; Immunotherapy

Introduction

The culprit agents in IgE-mediated hypersensitivity reactions are often detected by careful clinical histories and confirmed by skin prick testing, serum IgE measurements, and, if necessary, provocative challenges. There are many instances when these in vitro and in vivo assays are contradictory, inaccurate, or unethical to perform. Thus, there is a need for a reliable in vitro functional assay to shed light on these difficult cases and to avoid potentially dangerous provocative challenges.

Blood basophils, leukocytes that typically comprise <1 % of the circulating white blood cells, are a substrate for such an in vitro assay. Basophils are similar to mast cells in that they express the high-affinity IgE receptor and secrete cytokines and inflammatory

Address Correspondence to: Sarbjit Saini, MD, Johns Hopkins Asthma and Allergy Center, Division of Allergy and Clinical Immunology, 5501 Hopkins Bayview Circle, 2B71B, Baltimore, MD 21224, USA, Telephone: 410-550-2129, Fax: 410-550-2527, ssaini@jhmi.edu.

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mediators upon stimulation. Early studies of the role of basophils in allergic disease focused on mediator release, such as histamine [1, 2] and leukotrienes[3]. The development of a flow cytometry-based functional basophil assay became possible in 1991, when Knol et al. demonstrated that a surface marker, CD63, is up-regulated on basophils at the same time as basophil degranulation [4]. Over the past 20 years, many advances have been made in improving the basophil activation test and broadening its application in the clinical realm. The aim of this paper is to review some of the recent advances in the performance and application of these functional assays.

Principle of the basophil activation test

Human basophils have secretory granules containing histamine and are capable of secreting proteases, cytokines, chemokines, and lipid mediators. Activation of basophils occurs upon cross-linking of the high-affinity IgE receptor (FcεRI) by allergen or artificial cross-linkers. Basophils can also be activated via complement and chemokine receptors [5]. Electron microscopy has demonstrated two distinct pathways of basophil degranulation: piecemeal and anaphylactic degranulation. In anaphylactic degranulation, the cells undergo rapid morphologic changes and exocytosis of intracellular granules containing preformed mediators. In contrast, in piecemeal degranulation, cells secrete granule contents without exocytosis [6, 7]. Basophils express unique surface markers depending upon whether the cells undergo anaphylactic or piecemeal degranulation, which can then be measured by flow cytometry.

CD63, also known as lysosomal-associated membrane glycoprotein-3 (LAMP-3), is a 53-kDa member of the transmembrane-4 superfamily (tetraspanins). In 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 [4]. 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 [4, 8]. 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 [9].

Buhring et al. described an additional basophil activation marker, CD203c, a glycosylated type II transmembrane molecule that belongs to the family of ectonucleotide pyrophosphatsase/phosphodiesterase enzymes and is present on CD34+ progenitor cells, basophils, and mast cells. CD203c is constitutively expressed in low levels on the basophil surface membrane and is quickly up-regulated upon cell activation via allergen or more slowly via IL-3 [10-12]. This up-regulation differs from CD63 in terms of the inciting stimuli and early signaling events, which suggests that CD203c may be associated with piecemeal degranulation [13-15].

An alternate measure of basophil activation includes the degree of phosphorylation of intracellular molecules, such as p38 MAPK (mitogen-activated protein kinase) [16]. Furthermore, three new basophil activation markers (CD164, CD13, CD107a) have been identified through screening of monoclonal antibodies against resting and activated CD203c

basophils [15]. Only CD164 has been validated in a study of pollen allergy, but further correlative and mechanistic studies need to be performed [17].

Update on the performance of basophil activation tests

Despite the fact that the BAT has been validated for a wide-range of IgE-mediated conditions, there is still considerable variation in the performance of this test. The European Interest Group for the Evaluation of BAT in clinical routine (EuroBAT), a working group of the European Academy of Allergy and Clinical Immunology (EAACI), is attempting to establish standardized techniques. Below, we summarize some of the recent findings regarding the technical performance of the BAT.

Sampling and preservation of basophils

Until recently, it was unclear how quickly basophils needed to be processed for optimal expression of CD63 and CD203c. In 2009 and 2010, Sturm et al.(•) performed two studies, where they demonstrated that storing basophils for 24 and 48 h at 4 °C resulted in decreased CD63 and CD203c expression. Furthermore, the authors noticed a decrease in CD63 and CD203c expression after storage for just 4 h, at the lower concentrations of anti-IgE stimulation [18, 19]. It was thus recommended that basophils undergo processing within 3 h of sampling in order to optimize viability and functionality. In contrast, Sousa et al. found that CD63 expression was diminished only after storage for 48 h at 4° C, but this may be due to the small sample size (n = 3). Similar shifts were seen in CD63 expression between blood preserved in acid-citrate dextrose (ACD) or EDTA tubes [20].

Basophil specific markers

A common strategy to identify basophils in peripheral blood samples by flow cytometry is based on their surface expression of IgE. However, IgE surface levels vary considerably between individuals [21], and it has been demonstrated that up to 50 % of peripheral leukocytes expressing IgE are actually monocytes [22]. However, the search for new identification markers has resulted in many potential candidate markers: CCR3, CRTH2, CD203c, and CD123.

CCR3, or eotaxin CC chemokine receptor-3, is expressed on basophils, mast cells, and Th2 lymphocytes. This marker is the basis of the commercial Flow2-CAST assay, in contrast to Flow-CAST, which identifies basophils based on IgE. In a study on patients with confirmed beta-lactam allergy, Eberlein et al. found that the Flow2-CAST assay had similar specificity but slightly higher sensitivity (55 vs. 53 %) compared to the original Flow-CAST [23]. As CCR3 is also expressed on T cells, the addition of CD3 was recommended to eliminate contamination [24]. However, a different study found that CCR3 was had less inter-individual variability as a single marker for basophils relative to IgE and CD123, even without an additional T-cell marker [21].

CRTH2 is also expressed on basophils, eosinophils, and Th2 lymphocytes. Basophils can be further identified within this subset on the basis of side-scatter (to differentiate from eosinophils) and the secondary marker, CD3 (to differentiate from T cells) [25]. Boumiza et

al. validated this gating strategy in 18 patients with latex or dust mite [26], but to our knowledge, further studies with this technique have not been published.

Finally, CD123, a subunit of the IL-3 receptor, is highly expressed on basophils and plasmacytoid dendritic cells in the peripheral blood. Plasmacytoid dendritic cells can be further excluded on the basis of either an anti-HLA-DR antibody or anti-BDCA-2. In comparing approaches to identify basophils based on the markers CD123, CCR3, or IgE, CD123 was found to have similar expression levels to CCR3 and superior to that of IgE. However, the variability of CD123 expression was significantly larger than that of CCR3, which led the authors to conclude that CCR3 is a superior basophil identification marker [21].

Basophil Activation Markers: Comparative studies of CD63 v. CD203c

As mentioned above, most basophil activation tests are based on the activation markers CD63 and CD203c. A few recent studies have evaluated the differential expression of these two markers in various allergic conditions. In confirmed IgE-mediated amoxicillin allergy, the sensitivity for CD203c was found to be far superior to that of CD63 (60 vs. 20 %) [22]. In contrast, the same group demonstrated that CD63 expression was up-regulated more frequently than CD203c in patients with non-allergic NSAID hypersensitivity [27]. In patients with venom hypersensitivity, CD203c and CD63 were found to have similar kinetics, with maximum expression detected after 20 min of allergen stimulation. Furthermore, the addition of 300 pM of IL-3 for 10 min prior to stimulation was found to optimize CD63 expression but actually decreased CD203c expression to antigen stimulation in a dose-dependent manner. It was also demonstrated that neither CD63 nor CD203c expression was influenced by prior in vivo ingestion of the antihistamine desloratidine [18, 19]. This was further confirmed in vivo with CD63 expression in pollen allergic patients [28]. While CD63 and CD203c may represent different pathways of basophil activation and degranulation, they have both been validated as acceptable markers for the basophil activation test.

Update on the application of basophil activation tests

The basophil activation test has been shown to be sensitive and specific for the diagnosis of IgE-mediated hypersensitivity reactions to hymenoptera venom, pollens, foods, natural rubber latex allergy, and drugs. Many of these validation studies have been extensively reviewed elsewhere. Recent advances over the past 3 years in the application of the basophil activation test to allergic disease are summarized in Table 1.

Drug Allergy

Aspirin and NSAIDs—Aspirin and NSAID intolerance is a very heterogeneous disorder, manifested by urticaria, angioedema, sinusitis, asthma, rhinoconjunctivitis, and anaphylaxis. It is accepted that many of these reactions are not IgE-mediated but rather result from inhibition of cyclooxygenase-1 (COX-1) and thus unregulated synthesis of cysteinyl leukotrienes and mediator release from mast cells, basophils, and eosinophils [29]. This has made the study of basophil activation tests particularly problematic, in that subjects are often

included who do not have true IgE-mediated reactions to NSAIDs. Abuaf et al. demonstrated this clearly when they looked at 60 patients with NSAID hypersensitivity, characterized by angioedema and urticaria. In addition, 22 of these patients had hypotension, laryngeal edema, dyspnea, abdominal pain, vomiting, or diarrhea after NSAID intake. They found that the sensitivity among those patients without the visceral symptoms was only 21 %, but this increased to 64 % among those with more severe reactions [27]. Similarly, Korosec et al. demonstrated that the BAT among NSAID-intolerant patients may only have diagnostic value for those with anaphylactoid reactions rather than those with asthma/rhinitis symptoms [30]. It has been further shown by Gomez et al. that the timing of the basophil activation test impacts the sensitivity in the evaluation of NSAID allergy. In a study of 51 patients with allergy to pyrazolones, they demonstrated an overall sensitivity of 54.9 %, but this increased to 85.71 % among patients with positive skin tests. However, after 6 months, 60 % of those with previously positive BATs were now negative [31]. Thus, it appears that the BAT may be useful in the evaluation of severe NSAID hypersensitivity, but it has limited value in assessing non-IgE-mediated reactions or milder reactions.

Radiocontrast Media (RCM)—The incidence of hypersensitivity reactions to radiocontrast media has decreased with the introduction of nonionic formulations. However, even with these newer agents, 0.7–3.1 % of patients will experience a mild reaction and approximately 0.04 % will experience a severe immediate hypersensitivity reaction [32, 33]. It was previously thought that all of these reactions were the result of non-IgE-mediated mechanisms; however, recent studies have shown the benefit of skin prick testing in identifying a causative agent in many of these patients [34]. Thus, a few recent studies have evaluated the utility of using the basophil activation test in the diagnosis of RCM allergy. One case series described three patients who all experienced anaphylaxis coincident with the administration of gadolinium-derived agents. In each case, the causative agent was determined by a positive skin test and BAT [35]. Finally, in 2011, Pinnobhun et al. performed BATs to five different radiocontrast media agents at two different dilutions in 26 patients with a history of RCM allergy. They found that the BAT sensitivity ranged from 42.6 to 61.5 % and specificity ranged from 88.4 to 100 % [36], which is comparable to that of many other drugs that have been validated through BAT.

Fluoroquinolone Antibiotics—Hypersensitivity reactions to fluoroquinolone antibiotics are difficult to diagnose because of the false positive results of skin testing. Three recent studies have looked at the use of basophil activation tests to assist in the diagnosis of these reactions. In the first study, Ben Said et al. found that skin tests as well as BATs were positive in five of five patients with confirmed quinolone allergy [37]. Aranda et al. then looked at 38 patients with confirmed quinolone allergy and performed BATs to ciprofloxacin, levofloxacin, and moxifloxacin. They found this test to have a sensitivity of 41.7–45 %, but this increased to 79.5 % when ciprofloxacin BATs were included with moxifloxacin in those patients allergic to moxifloxacin [38]. In addition, Rouzaire et al. performed BATs in 34 patients who were being evaluated for quinolone hypersensitivity. They found that 50 % of their subjects had negative BATs. Given that information, they were able to successfully reintroduce quinolones in 15 of the 17 patients [39]. These studies

demonstrate that the BAT appears to be a useful confirmatory test in patients being evaluated for quinolone hypersensitivity.

Other Drugs—Case reports in which the basophil activation test was used to confirm the culprit drug involved in hypersensitivity reactions have recently been published for atropine [40], glatiramer acetate [41], methylprednisone [42], and antihistamines [43]. Not only was the culprit agent identified, but in some of these cases, alternate therapies were chosen because of their negative BATs.

Food Allergy

Wheat-dependent exercise induced anaphylaxis (WDEIA)—It was recently discovered that patients with WDEIA could be sensitized to two different wheat proteins: ω-5 gliadin [44] and hydrolyzed wheat protein (HWP) [45]. Chinuki et al. performed BATs in ten individuals with WDEIA, in which five were sensitized to ω-5 gliadin and five were sensitized to HWP. They found that basophils from subjects sensitized to ω-5 gliadin had increased CD203c in a concentration-dependent manner when exposed to purified ω-5 gliadin, but not HWP. Those who were sensitized to HWP similarly had positive BATs to HWP but did not react to ω-5 gliadin [46]. It is thus possible that the BAT may be used as an adjunctive test to diagnose subtypes of WDEIA.

Monitoring tolerance in cow's milk allergy—Currently, skin tests and specific IgE are used to predict whether an individual has outgrown their food allergy, but these are not always reliable. In a recent study of 112 children with cow's milk allergy presenting for oral food challenges (OFC), Rubio et al. found that the BAT was significantly higher in children who failed the OFCs compared to those who passed. The BAT was found to have a sensitivity of 91 % and a specificity of 90 %, which were higher than those for both skin tests and specific IgE [47]. Ford et al. similarly demonstrated that children who tolerated straight milk and baked milk had lower basophil reactivity to serial dilutions of milk protein than those who were unable to tolerate baked milk in an oral food challenge [48]. These findings suggest that BATs may be used in the diagnostic algorithm of when to safely perform an oral food challenge.

Differentiating sensitization from true food allergy—It is commonly accepted that the presence of IgE or a positive skin test to a food does not always indicate a true food allergy. In 2009, Ocmant et al. performed basophil activation tests on 63 children with documented peanut or egg allergy, 28 children who were asymptomatically sensitized to these foods, and 51 controls. They found that the basophils from the food-allergic children had significant higher levels of activation to in vitro allergen exposure than the other two groups. Furthermore, only 2 of the asymptomatically sensitized children had activated basophils [49].

Testing the allergenicity of foods—The basophil activation test can be used in vitro to measure the potential allergenicity of foods. Sebato et al. published two studies in which they used this technique to study thermally processed peanuts. In the first study, they performed BATs in ten patients with severe peanut allergy to five different thermally

processed peanut varieties. They found that the BATs varied considerably between patients and varieties, and these results did not correlate with the results predicted from IgE immunoblotting [50]. In their second study, they sought to determine whether the BAT could be used to detect trace amounts of peanut in food. They performed BATs on five peanut-allergic patients and five controls with peanut-spiked biscuits and chocolates, and they demonstrated that the peanut-allergic basophils responded to the peanut-spiked foods, whereas those of the controls did not [51].

In addition, Dolle et al. used the BAT to investigate the allergic potential of two tomato varieties: Reisetomate and Matina. They performed the BAT among 6 patients with confirmed tomato allergy and discovered that there was greater basophil reactivity to the Matina variety, which correlated to increased clinical reactivity, as measured by increased positivity on skin prick testing and oral food challenges, in 25 patients with tomato allergy [52]. These results, taken together, demonstrate that the BAT may in fact be a useful tool to detect the allergic potential of food.

Other foods—Over the past 3 years, case reports have been published in which the basophil activation test was used to confirm the culprit food involved in a hypersensitivity reaction. These foods have included sesame [53], ginseng [54], sulfite [55], and beef [56].

Venom Hypersensitivity

Identifying the culprit venom with inconclusive skin tests and serum IgE—Approximately 80–100 % of patients with venom hypersensitivity can undergo successful desensitization with venom immunotherapy [57], but this success relies on the correct identification of the culprit insect. This is often difficult, as venom skin tests and specific IgE can be discordant or inconclusive. In 2009, Ebo et al. found that 54 of 118 (45 %) patients presenting for venom hypersensitivity evaluation had inconclusive in vitro tests, with most of these patients having positive IgE to both wasp and honeybee venom. BATs were performed in all patients and the culprit venom was detected in 38 of those patients with inconclusive results [58]. Similarly, Korosec et al. examined 47 patients with a convincing history of insect venom allergy but negative specific IgE and SPT. They performed BATs and intradermal skin testing in 37 of these patients, and found significantly higher diagnostic sensitivity for the BAT compared to intradermal testing (76 vs. 46 %) [59]. In contrast, in a study looking at seven patients with systemic mastocytosis, a convincing history of venom hypersensitivity, and negative venom skin tests, the BAT was only positive to the negative control in one patient and thus did not provide any useful information [60]. The basophil activation test may be a helpful diagnostic tool for identifying the culprit venom in patients with inconclusive conventional in vitro tests, but further studies in those with systemic mastocytosis need to be performed.

Testing the allergenicity of cross-reactive carbohydrate determinants (CCDs)—It is common for patients being evaluated for venom hypersensitivity to have IgE against both honeybee and yellow jacket venom, which may be due to true co-sensitization or cross-reactivity such as to carbohydrate determinants found on both venoms. In 2010, Mertens et al. (••) sought to answer whether these IgE antibodies against CCDs had biologic activity

through the use of the basophil activation test. Skin prick testing, venom, and CCD-specific IgE measurements, and BATs to both native venom and CCD-depleted venom, were performed in 62 patients with hymenoptera venom hypersensitivity. In the patients who were only positive to one venom on skin testing, but had IgE to CCD, the BAT was positive to both native venoms but only positive in the CCD-depleted skin test venom in 67 % of patients. Conversely, in patients who were only positive to one venom on skin test, but did not have IgE to CCD, the BAT was only positive to the skin test venom in both native and CCD-depleted forms. The authors thus concluded that the CCDs do in fact have biologic activity, but they are likely clinically irrelevant because the skin tests were only positive to one venom [61]. Recently, Eberlein et al. demonstrated that performing the BAT with horseradish peroxidase, a marker for CCD sensitization, has a sensitivity of 92 % for basophil reactivity to CCDs [62], which may be helpful in distinguishing those patients who are truly sensitized to more than one venom.

Pollen Allergy

The basophil activation test has used to determine the allergenicity of individual pollens, but few studies have compared the basophil activation test to skin testing and specific IgE in the diagnosis of allergy to multiple inhalants. In 2012, Khan et al. compared the BAT to skin test and sIgE to nine allergens. They calculated a sensitivity of 57–84 % and specificity of only 73–81 %, and they found a large number of false positives with BAT among atopic patients. They thus concluded that the BAT is not sensitive enough to use for the routine diagnosis of individual pollen allergy, which they believe may have been due to non-specific IgE cross-linking in the performance of the CD63 basophil activation test [63]. In contrast, Ozdemir et al. found a sensitivity of 77–100 % and specificity of 100 % when performing the BAT with CD203c expression in grass-allergic patients, and they concluded that this is a reliable tool in the diagnosis of pollen allergy [64].

Monitoring immunotherapy responses

Many recent studies have looked at the basophil activation test as a means of monitoring response to immunotherapy. Erzen et al. (••) demonstrated that patients who had a negative sting challenge after completing venom immunotherapy had decreased BATs 1 year after VIT. In comparison, the one patient who did not pass the sting challenge in their study did not have a change in their BAT at the same time point [65]. Zitnik et al. similarly showed that children who underwent VIT to honeybee had a decrease in their baseline BAT to the lowest concentration of honeybee venom starting at just 6 months of VIT [66]. In contrast, Chicocka-Jarosz et al. examined the use of the BAT in monitoring rush VIT in children with honeybee allergy, and they did not see a difference in the baseline BAT after 40 days [67].

Similar findings were found in studies of SLIT. In patients with pollen allergy, Van Overtvelt et al. did not find a significant decrease in the BAT to various grass allergens after 2 or 4 months of SLIT [68]. In looking at SLIT for natural rubber latex allergy, the BAT to Hev b 6.01 and Hev b 6.02 decreased at 6 months but then returned to pre-treatment levels after 1 year of therapy [69]. Finally, in a Phase I study of timothy grass and dust mite dual-SLIT for pollen allergy, it was demonstrated that the BAT to those two allergens decreased after 24 months of SLIT compared to baseline values [70]. Given these findings, it is likely

that the BAT may be a useful marker for immunotherapy success; however, the changes may not be evident until approximately 6 months, and the duration of this effect demands further study.

Similar studies have also recently been performed for food allergy. It has been shown that basophils of patients treated with FAHF-2 were found to have a significant reduction in their CD63 expression after 6 months of treatment [71]. In contrast, basophils from patients with pollen food allergy syndrome who ate increasing amounts of apple over an 8-month period were not found to have any change in reactivity [72]. Further mechanistic studies on basophils in the induction of food tolerance need to be performed to further clarify these observations.

Conclusions

The basophil activation test is an in vitro assay in which the activation of basophils upon exposure to various stimuli is measured by flow cytometry. Though this technique has not yet been standardized, IgE, CCR3, and CD123 are commonly used to identify basophils in the peripheral blood samples, whereas CD63 and CD203c are used as markers of IgE receptor activation. The basophil activation test has been validated in many IgE-mediated conditions, including drug allergy, food allergy, venom hypersensitivity, and pollen allergy. Furthermore, in recent years, the application of this test has been expanded to include quinolone and NSAID drug allergy, differentiating sensitization from true allergy, determining the allergenic potential of CCD determinants, and monitoring the success of immunotherapy. The basophil activation test continues to be a useful in vitro tool for the study of allergic disease.

References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Lichtenstein LM, Norman PS, Winkenwerder WL. Clinical and in vitro studies on the role of immunotherapy in ragweed hay fever. Am J Med. 1968; 44(4):514–524. [PubMed: 4171086]
2. May CD. High spontaneous release of histamine in vito from leukocytes of persons hypersensitive to food. J Allergy Clin Immunol. 1976; 58(3):432–437. [PubMed: 61221]
3. Togias AG, Naclerio RM, Warner J, et al. Demonstration of inhibition of mediator release from human mast cells by azatadine base. in vivo and in vitro evaluation. JAMA. 1986; 255(2):225–229. [PubMed: 2416958]
4. 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. [PubMed: 1716273]
5. Schroeder JT. Basophils: Emerging roles in the pathogenesis of allergic disease. Immunol Rev. 2011; 242(1):144–160. [PubMed: 21682743]
6. Dvorak AM, Galli SJ, Schulman ES, Lichtenstein LM, Dvorak HF. Basophil and mast cell degranulation: Ultrastructural analysis of mechanisms of mediator release. Fed Proc. 1983; 42(8): 2510–2515. [PubMed: 6840300]
7. Dvorak AM, MacGlashan DW Jr, Morgan ES, Lichtenstein LM. Vesicular transport of histamine in stimulated human basophils. Blood. 1996; 88(11):4090–4101. [PubMed: 8943842]

8. Furuno T, Teshima R, Kitani S, Sawada J, Nakanishi M. Surface expression of CD63 antigen (AD1 antigen) in P815 mastocytoma cells by transfected IgE receptors. Biochem Biophys Res Commun. 1996; 219(3):740–744. [PubMed: 8645251]
9. MacGlashan D Jr. Expression of CD203c and CD63 in human basophils: Relationship to differential regulation of piecemeal and anaphylactic degranulation processes. Clin Exp Allergy. 2010; 40(9): 1365–1377. [PubMed: 20633031]
10. Buhring HJ, Simmons PJ, Pudney M, et al. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. Blood. 1999; 94(7):2343–2356. [PubMed: 10498606]
11. Buhring HJ, Seiffert M, Giesert C, et al. The basophil activation marker defined by antibody 97A6 is identical to the ectonucleotide pyrophosphatase/phosphodiesterase 3. Blood. 2001; 97(10): 3303–3305. [PubMed: 11342463]
12. Hauswirth AW, Sonneck K, Florian S, et al. 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(20):267–278. [PubMed: 17624239]
13. Monneret G, Boumiza R, Gravel S, et al. Effects of prostaglandin D(2) and 5-lipoxygenase products on the expression of CD203c and CD11b by basophils. J Pharmacol Exp Ther. 2005; 312(2):627–634. [PubMed: 15388786]
14. Macglashan D Jr. Marked differences in the signaling requirements for expression of CD203c and CD11b versus CD63 expression and histamine release in human basophils. Int Arch Allergy Immunol. 2012; 159(3):243–252. [PubMed: 22722613]
15. Hennersdorf F, Florian S, Jakob A, et al. 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(5):325–335. [PubMed: 15916720]
16. 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(11):1668–1675. [PubMed: 17868401]
17. Wolanczyk-Medrala A, Barg W, Liebhart J, et al. Validation of basophil CD164 upregulation for pollen allergy diagnosis. Arch Immunol Ther Exp (Warsz). 2010; 58(6):459–465. [PubMed: 20872290]
18. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: Technical issues and critical factors. Allergy. 2009; 64(9):1319–1326. [PubMed: 19243362]
•19. Sturm EM, Kranzelbinder B, Heinemann A, Groselj-Strele A, Aberer W, Sturm GJ. CD203c-based basophil activation test in allergy diagnosis: Characteristics and differences to CD63 upregulation. Cytometry B Clin Cytom. 2010; 78(5):308–318. This article similarly examined the effect of technical issues on the expression of CD203c. [PubMed: 20533392]
20. Sousa N, Martinez-Aranguren R, Fernandez-Benitez M, Ribeiro F, Sanz ML. Comparison of basophil activation test results in blood preserved in acid citrate dextrose and EDTA. J Investig Allergol Clin Immunol. 2010; 20(6):535–536.
21. Hausmann OV, Gentinetta T, Fux M, Ducrest S, Pichler WJ, Dahinden CA. Robust expression of CCR3 as a single basophil selection marker in flow cytometry. Allergy. 2011; 66(1):85–91. [PubMed: 20608915]
22. Abuaf N, Rostane H, Rajoely B, et al. Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. Clin Exp Allergy. 2008; 38(6):921–928. [PubMed: 18331364]
23. Eberlein B, Leon Suarez I, Darsow U, Rueff F, Behrendt H, Ring J. A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. Clin Exp Allergy. 2010; 40(3):411–418. [PubMed: 20082620]
24. Monneret G. CCR3 for basophil activation test: A necessary but insufficient step. Clin Exp Allergy. 2010; 40(6):953. author reply 954. [PubMed: 20557552]
25. Monneret G. Is this time for CRTH2/DP2 in a flow cytometric basophil activation test? Clin Exp Allergy. 2008; 38(7):1239–1240. [PubMed: 18477015]

26. 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. [PubMed: 15989690]
27. Abuaf N, Rostane H, Barbara J, et al. Comparison of CD63 upregulation induced by NSAIDs on basophils and monocytes in patients with NSAID hypersensitivity. J Allergy (Cairo). 2012; 2012:580873. [PubMed: 22187572]
28. Wolanczyk-Medrala A, Gogolewski G, Liebhart J, et al. A new variant of the basophil activation test for allergen-induced basophil CD63 upregulation. the effect of cetirizine. J Investig Allergol Clin Immunol. 2009; 19(6):465–473.
29. Mastalerz L, Setkowicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: Common eicosanoid alterations in urticaria and asthma. J Allergy Clin Immunol. 2004; 113(4):771–775. [PubMed: 15100686]
30. Korosec P, Mavsar N, Bajrovic N, Silar M, Mrhar A, Kosnik M. Basophil responsiveness and clinical picture of acetylsalicylic acid intolerance. Int Arch Allergy Immunol. 2011; 155(3):257–262. [PubMed: 21293144]
31. Gomez E, Blanca-Lopez N, Torres MJ, et al. Immunoglobulin E-mediated immediate allergic reactions to dipyrone: Value of basophil activation test in the identification of patients. Clin Exp Allergy. 2009; 39(8):1217–1224. [PubMed: 19400910]
32. Katayama H, Yamaguchi K, Kozuka T, Takashima T, Seez P, Matsuura K. Adverse reactions to ionic and nonionic contrast media. A report from the japanese committee on the safety of contrast media. Radiology. 1990; 175(3):621–628. [PubMed: 2343107]
33. Wolf GL, Arenson RL, Cross AP. A prospective trial of ionic vs nonionic contrast agents in routine clinical practice: Comparison of adverse effects. AJR Am J Roentgenol. 1989; 152(5):939–944. [PubMed: 2495706]
34. Brockow K, Romano A, Aberer W, et al. Skin testing in patients with hypersensitivity reactions to iodinated contrast media - a european multicenter study. Allergy. 2009; 64(2):234–241. [PubMed: 19178403]
35. Javaloyes G, Goikoetxea MJ, Sanz ML, Cabrera-Freitag P, Gastaminza G. Basophil activation test in the diagnosis of gadobutrol anaphylaxis. Ann Allergy Asthma Immunol. 2012; 108(4):286–287. [PubMed: 22469455]
36. Pinnobphun P, Buranapraditkun S, Kampitak T, Hirankarn N, Klaewsongkram J. The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media. Ann Allergy Asthma Immunol. 2011; 106(5):387–393. [PubMed: 21530870]
37. Ben Said B, Berard F, Bienvenu J, Nicolas JF, Rozieres A. Usefulness of basophil activation tests for the diagnosis of IgE-mediated allergy to quinolones. Allergy. 2010; 65(4):535–536. [PubMed: 19845576]
38. Aranda A, Mayorga C, Ariza A, et al. In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. Allergy. 2011; 66(2):247–254. [PubMed: 20722637]
39. Rouzaire P, Nosbaum A, Denis L, et al. Negativity of the basophil activation test in quinolone hypersensitivity: A breakthrough for provocation test decision-making. Int Arch Allergy Immunol. 2012; 157(3):299–302. [PubMed: 22041525]
40. Cabrera-Freitag P, Gastaminza G, Goikoetxea MJ, Lafuente A, de la Borbolla JM, Sanz ML. Immediate allergic reaction to atropine in ophthalmic solution confirmed by basophil activation test. Allergy. 2009; 64(9):1388–1389. [PubMed: 19392999]
41. Soriano Gomis V, Perez Sempere A, Gonzalez Delgado P, Sempere JM, Niveiro Hernandez E, Marco FM. Glatiramer acetate anaphylaxis: Detection of antibodies and basophil activation test. J Investig Allergol Clin Immunol. 2012; 22(1):65–66.
42. Ben Said B, Leray V, Nicolas JF, Rozieres A, Berard F. Methylprednisolone-induced anaphylaxis: Diagnosis by skin test and basophil activation test. Allergy. 2010; 65(4):531–532. [PubMed: 19839979]
43. Bobadilla-Gonzalez P, Perez-Rangel I, Camara-Hijon C, Garcia-Menaya JM, Sanchez-Vega S. Positive basophil activation test result in a patient with acute urticaria induced by cetirizine and desloratadine. Ann Allergy Asthma Immunol. 2011; 106(3):258–259. [PubMed: 21354029]

44. Matsuo H, Dahlstrom J, Tanaka A, et al. Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. Allergy. 2008; 63(2):233–236. [PubMed: 18186814]
45. Fukutomi Y, Itagaki Y, Taniguchi M, et al. Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol. 2011; 127(2):531–533. e1–3. [PubMed: 21094523]
46. Chinuki Y, Kaneko S, Dekio I, et al. CD203c expression-based basophil activation test for diagnosis of wheat-dependent exercise-induced anaphylaxis. J Allergy Clin Immunol. 2012; 129(5):1404–1406. [PubMed: 22464646]
47. Rubio A, Vivinus-Nebot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. Allergy. 2011; 66(1):92–100. [PubMed: 20608919]
48. Ford LS, Bloom KA, Nowak-Wegrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. J Allergy Clin Immunol. 2012
49. Ocmant A, Mulier S, Hanssens L, et al. Basophil activation tests for the diagnosis of food allergy in children. Clin Exp Allergy. 2009; 39(8):1234–1245. [PubMed: 19549026]
50. Sabato V, van Hengel AJ, De Knop KJ, et al. Basophil activation reveals divergent patient-specific responses to thermally processed peanuts. J Investig Allergol Clin Immunol. 2011; 21(7):527–531.
51. Sabato V, van Hengel AJ, De Knop KJ, et al. Human basophils: A unique biological instrument to detect the allergenicity of food. J Investig Allergol Clin Immunol. 2011; 21(3):179–184.
52. Dolle S, Lehmann K, Schwarz D, et al. Allergenic activity of different tomato cultivars in tomato allergic subjects. Clin Exp Allergy. 2011; 41(11):1643–1652. [PubMed: 21955222]
53. Raap U, Wieczorek D, Schenck F, Kapp A, Wedi B. The basophil activation test is a helpful diagnostic tool in anaphylaxis to sesame with false-negative specific IgE and negative skin test. Allergy. 2011; 66(11):1497–1499. [PubMed: 21752028]
54. Lee JY, Jin HJ, Park JW, Jung SK, Jang JY, Park HS. A case of korean ginseng-induced anaphylaxis confirmed by open oral challenge and basophil activation test. Allergy Asthma Immunol Res. 2012; 4(2):110–111. [PubMed: 22379608]
55. Garcia-Ortega P, Scorza E, Teniente A. Basophil activation test in the diagnosis of sulphite-induced immediate urticaria. Clin Exp Allergy. 2010; 40(4):688. author reply 689-90. [PubMed: 20447080]
56. Kim JH, An S, Kim JE, Choi GS, Ye YM, Park HS. Beef-induced anaphylaxis confirmed by the basophil activation test. Allergy Asthma Immunol Res. 2010; 2(3):206–208. [PubMed: 20592921]
57. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med. 1978; 299(4):157–161. [PubMed: 78446]
58. Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Hymenoptera venom allergy: Taking the sting out of difficult cases. J Investig Allergol Clin Immunol. 2007; 17(6):357–360.
59. Korosec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. Clin Exp Allergy. 2009; 39(11):1730–1737. [PubMed: 19689457]
60. Bonadonna P, Zanotti R, Melioli G, et al. The role of basophil activation test in special populations with mastocytosis and reactions to hymenoptera sting. Allergy. 2012; 67(7):962–965. [PubMed: 22676063]
••61. Mertens M, Amler S, Moerschbacher BM, Brehler R. Cross-reactive carbohydrate determinants strongly affect the results of the basophil activation test in hymenoptera-venom allergy. Clin Exp Allergy. 2010; 40(9):1333–1345. This paper used the basophil activation test to assess the biologic activity of cross-reactive carbohydrate determinants. [PubMed: 20545702]
62. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. J Allergy Clin Immunol. 2012; 130(1):155–161. [PubMed: 22421265]

63. Khan FM, Ueno-Yamanouchi A, Serushago B, et al. Basophil activation test compared to skin prick test and fluorescence enzyme immunoassay for aeroallergen-specific immunoglobulin-E. Allergy Asthma Clin Immunol. 2012; 8(1):1. [PubMed: 22264407]

64. Ozdemir SK, Guloglu D, Sin BA, Elhan AH, Ikinciogullari A, Misirligil Z. Reliability of basophil activation test using CD203c expression in diagnosis of pollen allergy. Am J Rhinol Allergy. 2011; 25(6):e225–31. [PubMed: 22185730]

••65. Erzen R, Kosnik M, Silar M, Korosec P. Basophil response and the induction of a tolerance in venom immunotherapy: A long-term sting challenge study. Allergy. 2012; 67(6):822–830. This paper monitored basophil response in 23 patients undergoing venom immunotherapy They found that basophil activation was decreased one year after completion of immunotherapy. [PubMed: 22469017]

66. Zitnik SE, Vesel T, Avcin T, Silar M, Kosnik M, Korosec P. Monitoring honeybee venom immunotherapy in children with the basophil activation test. Pediatr Allergy Immunol. 2012; 23(2):166–172. [PubMed: 22136583]

67. Cichocka-Jarosz E, Dorynska A, Pietrzyk JJ, Spiewak R. Laboratory markers of mast cell and basophil activation in monitoring rush immunotherapy in bee venom-allergic children. Immunotherapy. 2011; 3(8):1013–1017. [PubMed: 21843104]

68. Van Overtvelt L, Baron-Bodo V, Horiot S, et al. Changes in basophil activation during grass-pollen sublingual immunotherapy do not correlate with clinical efficacy. Allergy. 2011; 66(12): 1530–1537. [PubMed: 21883279]

69. Lasa Luaces EM, Tabar Purroy AI, Garcia Figueroa BE, et al. Component-resolved immunologic modifications, efficacy, and tolerance of latex sublingual immunotherapy in children. Ann Allergy Asthma Immunol. 2012; 108(5):367–372. [PubMed: 22541410]

70. Swamy RS, Reshamwala N, Hunter T, et al. Epigenetic modifications and improved regulatory T-cell function in subjects undergoing dual sublingual immunotherapy. J Allergy Clin Immunol. 2012; 130(1):215–24. e7. [PubMed: 22677046]

71. Patil SP, Wang J, Song Y, et al. Clinical safety of food allergy herbal formula-2 (FAHF-2) and inhibitory effect on basophils from patients with food allergy: Extended phase I study. J Allergy Clin Immunol. 2011; 128(6):1259–1265. e2. [PubMed: 21794906]

72. Kopac P, Rudin M, Gentinetta T, et al. Continuous apple consumption induces oral tolerance in birch-pollen-associated apple allergy. Allergy. 2012; 67(2):280–285. [PubMed: 22070352]

73. Bavbek S, Ikinciogullari A, Dursun AB, et al. Upregulation of CD63 or CD203c alone or in combination is not sensitive in the diagnosis of nonsteroidal anti-inflammatory drug intolerance. Int Arch Allergy Immunol. 2009; 150(3):261–270. [PubMed: 19494523]

74. De Week AL, Sanz ML, Gamboa PM, et al. Diagnosis of immediate-type beta-lactam allergy in vitro by flow-cytometric basophil activation test and sulfidoleukotriene production: A multicenter study. J Investig Allergol Clin Immunol. 2009; 19(2):91–109.

75. Kim MS, Cho YJ. Flow cytometry-assisted basophil activation test as a safe diagnostic tool for aspirin/NSAID hypersenstivity. Allergy Asthma Immunol Res. 2012; 4(3):137–142. [PubMed: 22548206]

76. Rodriguez-Trabado A, Camara-Hijon C, Ramos-Cantarino A, et al. Basophil activation test for the in vitro diagnosis of nonsteroidal anti-inflammatory drug hypersensitivity. Allergy Asthma Proc. 2008; 29(3):241–249. [PubMed: 18534081]

77. Peternelj A, Silar M, Bajrovic N, et al. Diagnostic value of the basophil activation test in evaluating hymenoptera venom sensitization. Wien Klin Wochenschr. 2009; 121(9-10):344–348. [PubMed: 19562299]

78. Frezzolini A, Cadoni S, De Pita O. Usefulness of the CD63 basophil activation test in detecting anisakis hypersensitivity in patients with chronic urticaria: Diagnosis and follow-up. Clin Exp Dermatol. 2010; 35(7):765–770. [PubMed: 19874355]

Table 1

Clinical application of the basophil activation test in allergic disease

Author (year)	Stimulus	Reference T test	Activation Mmarker	Sensitivity (%)	Specificity (%)	# No. of patients and controls	Ref.
Drug allergy							
Abuaf et al. (2012)	NSAIDs	ST + H	CD63	37–-64	90/90	85	27
Aranda et al. *et al* (2010)	Quinolones	H ± DPT (urticaria cases)	CD63	41.7–-79.2	88	73	38
Bavbek et al. *et al* (2009)[*a])	Lysine-ASA Diclofenac	H + DPT	CD63 /CD203c	16.7–-33.3 16.7–-22.2	79.2–-91.7 100	42	73
De Week et al. *et al* (2009)	β-lactams	H + ST + IgE	CD63	50	89-97	262	74
Eberlein et al. *et al* (2010)	β-lactams	H + ST + IgE	CD63:Flow -CAST . CD63:Flow 2-CAST	53 55	80 80	39	23
Gomez et al. *et al* (2010)	Metamizole	H + ST + DPT	CD63	54.9	85.71	107	31
Kim et al. *et al* (2012)	NSAID	H + DPT	CD63	61	91	29	75
Korosec et al. *et al* (2011)	ASA	H + ST ± DPT	CD63	79	70	59	30
Pinnobphun et al. *et al* (2011)	Radiocontrast media	H + ST	CD63	46–-61.5	88–-100	40	36
Rodriguez-Trobado et al. *et al* (2008)	NSAID	H	CD63	42.85	100	72	76
Food Aallergy							
Ocmant et al.*et al*(2009)	Peanut Egg	H + ST + IgE ± DBPCFC	CD63 /CD203c	86.7–-88.9 62.5-–88.9	94.1–-97.1 96.4–-100	142	49
Venom Aallergy							
Eberlein et al. *et al* (2012)	Honeybee and YJ	H+ ST + IgE	CD63	57.1	90	32	62
Korosec et al. *et al* (2009)	Honey bee Wasp	H + ST + IgE	CD63	75 %	92%	72	59
Peternelj et al. *et al* (2009)	Honeybee, Wasp	H + ST	CD63	90		204	77
Inhalant Aallergy							
Khan et al. *et al* (2012)	Inhalant allergens	H + ST + IgE	CD63	57–-84	73–-81	72	63
Ozdemir et al. *et al* (2011)	Grass pollen mix	H + ST + IgE	CD203c	87–-100	100	40	64
Wolznczyk-Medrala et al. *et al* (2009)	Dust mite, grass mix	H + ST	CD63	87.5–-94 75–-91.66	100%	41	28
Miscellaneous							
Frezzolini et al. *et al* (2009)	Anasakis	ST + IgE	CD63	67	100	99	78

ST, skin test; *H*, history; *Ref*, reference; *DPT*, drug provocation test; *ASA*, aspirin, *Ova*, ovalbumin; *YJ*, yellow jacket. *DBPCFC*= double-blind, placebo -control, food challenge.

[*a] The top row of sensitivity and specificity values (representing both CD63 and CD203c data) is for lysine-aspirin, whereas the bottom row is for diclofenac.