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52 **Keywords**

53 BAT, mast cell, basophil, granulocyte, CD63, allergy, chronic urticaria

54 **Abstract**

55 **Purpose of Review:** We review basophil testing by flow cytometry with an emphasis on
56 advantages and disadvantages.

57 **Recent Findings/Summary:** There are many tools available to assess the presence and
58 severity of allergic diseases in patients. For 50 years, peripheral blood basophils have
59 been used as tools to study these diseases. It is a very accessible cell that binds IgE
60 antibody and secretes the classical mediators responsible for the symptoms of allergic
61 reactions. In the last decade, an even more accessible methodology, using flow cytometry,
62 has been developed to enhance the ability to use basophils for both mechanistic and
63 clinical diagnostics. Basophil testing has been included in diagnostics for different forms of
64 allergies as well as to monitor disease status. A variety of studies have begun to establish
65 both precise methods and their clinical relevance for disease diagnosis but there remain
66 some important questions on how to take optimal advantage of the behaviors of basophils.

67 **Introduction**

68 Most diagnostic testing is preceded by mechanistic studies that explain the biology underlying
69 the activation measured in this test. When studying the mechanism of activation of basophils,
70 one has to remember that CD63, the cell surface protein that is being used extensively today
71 as a metric of basophil function in clinical studies, is a very focused marker for anaphylactic
72 degranulation, and may not reflect other methods of activation (e.g., secretion of other
73 biologically relevant mediators such as cytokines or changes in basophil phenotype).

74 **Biological considerations**

75 **Relationship of mast cells and basophil granulocytes**

76 Mast cells and basophil granulocytes differentiate along different pathways from a
 77 granulocyte progenitor cell with very different kinetics. Basophils are myeloid cells closely
 78 related to the other myeloid cell types [1]. Mast cells mature in tissue from CD34⁺CD133⁺
 79 stem cells [2], whereas basophils mature in bone marrow and enter blood as mature
 80 granulocytes. In man, there is no evidence for a common precursor beyond the common
 81 CD34⁺CD133⁺ myeloid precursor, and there is no evidence that basophils can differentiate
 82 into mast cells [3].

83 Based on reactivity to allergen activation, skin mast cells are replaced over a period of months
 84 [4–7], whereas blood basophils have a half-life of 24-36 hours [8–10]. In some patients (non-
 85 responders), blood basophils respond much less if at all, to allergen that stimulates mast cells
 86 from the same person [11].

87 Both mast cells and basophils express the tetrameric FcεRI receptor, and degranulate upon
 88 crosslinking by allergen and IgE (Figure 1). Degranulation results in release of histamine,
 89 proteoglycan located in the granule, synthesis of arachidonic acid metabolites at the cell
 90 surface and synthesis of Th2 type cytokines. FcεRI-mediated activation is a relatively fast
 91 event, requiring 10-60 minutes in basophils granulocytes [12,13] and 2-20 minutes in mast
 92 cells [14]. Both cell types can be desensitised by low doses of allergen [15].

93 **Anaphylactic degranulation**

94 Assessing basophil activation by examining *ex vivo* histamine release is probably the best-
 95 known functional diagnostic test for functional specific IgE required for allergic disease.
 96 However, the analytical requirements for measuring released histamine severely limit broad
 97 application of this test to clinical practice. In the last decade, the measurement of CD63
 98 expression by flow cytometric methods has begun to replace histamine release
 99 measurements. Up-regulation of CD63 expression accurately reflects the anaphylactic
 100 degranulation that causes allergic disease [16–23]. Other flow cytometric readouts for
 101 basophil activation such as the up-regulation of CD203c expression and other surface markers
 102 [24,25], or the diamine oxidase measurement of intracellular histamine [26,27] will give a
 103 similar response, but experience with these metrics is not as extensive or there are additional

104 issues. A major difference is that up-regulation of CD203c occurs in response to many non-
 105 degranulation stimuli (such as IL-3), in contrast to CD63 [17,19].

106 **Survival of basophils: the requirement for fresh blood**

107 Fresh blood is required for basophil activation testing and expanding this time frame is a
 108 major challenge for the community. It is being addressed by weighing the positive predictive
 109 value of the test, and by designing the test such that basophil activation including erythrocyte
 110 lysis is temporally separated from flow cytometric measurements. Current guidelines are to
 111 test blood that is less than 4 hours old although several studies suggest that qualitative results
 112 may be valid within a 24-hour window.

113 **What do we measure in basophil testing?**

114 When studying mechanisms of activation, it may be useful to separate extracellular events –
 115 the contribution of plasma or serum (immunoglobulins and their receptors [21,28–30] and
 116 other modulatory molecules like the histamine receptors [31,32]) from the mechanisms of
 117 activation within the basophil granulocyte or mast cell.

118 **Response to a complex environment?**

119 It is possible to manipulate the cell surface IgE and/or the environment of the isolated
 120 basophil granulocyte as reactivity depends on IgE affinity for allergen, FcεRI clustering on the
 121 cell membrane and on the integrated signal transduced into the cell from a number of
 122 receptor pathways. Commonly, only allergen concentration is varied to obtain reactivity,
 123 sensitivity or AUC (area-under-the-curve). The complexity of IgE composition (epitope
 124 specificity, affinity and concentration of individual clones) and their contribution to effector
 125 cell activation is subject to intense research [21,33–35] (Hjort et al 2016, in preparation).
 126 Here the basophil activation test serves as a useful workhorse to decipher contribution of
 127 epitopes and molecules to the allergic response [36].

128 Without repeated stimulation, IgE specific for drugs appears to be detectable in circulation
 129 with a half life of less than 18 months depending on the responsible drug [37–39]. This sets a
 130 limit to the time frame in which it is possible to use the basophil to detect production of IgE
 131 that results from an acute exposure (such as exposure to penicillin). In passive sensitisation,
 132 the serum of one subject can be used to sensitize basophils of another subject [40,41]. This
 133 allows testing of an allergic subject's IgE status with a functional readout that doesn't depend
 134 on immediate access to the serum-subject's basophils. There are technical issues that must be

considered when performing this type of experiment [41]. Blood or enriched basophils can also be treated to enhance their response, offering greater sensitivity [42–46]. Lastly, blood basophils can be activated in autologous plasma from a previous visit to illustrate a change in plasma inhibitory components resulting from an intervention such as allergen immunotherapy [21]. Allergen extracts or recombinant molecules used in basophil testing (as well as it is in skin testing and serology) remain an approximation of the sensitising environmental agent.

Signal transduction leading to degranulation

The signal transduction apparatus within the cell is complex and there is clinically significant biological variation in the activation cascade that also determines the behaviour of basophils and mast cells [47,48]. There is a natural biological variation in the IgE-mediated basophil response. Non-responders (5-20% of the subjects tested) show little or no response through the IgE receptor no matter what IgE-mediated stimulus is used. The serum of these patients may contain adequate circulating allergen-specific IgE, this IgE is bound at reasonable cell surface densities on the basophil, but since the basophil is unresponsive through the IgE-receptor, a basophil-activation test does not show a positive result. The condition is easily detected by also stimulating the cell with a pan-crosslinking reagent like anti-IgE antibody or anti-FcεRI antibody, but this does not resolve the question of how to use the test results. There are some possible technical approaches to this issue (such as expressing results as a fraction of the pan-stimulus IgE-mediated response [23]) that are not yet incorporated into routine basophil activation testing. One of the causes of non-responders is the differential expression of the tyrosine kinase *Syk* that associates with FcεRI during activation [48–50].

An aspect of any cell's response to extracellular stimuli is its ability to self-terminate the response. Desensitization (the general term used to describe the self-termination mechanisms) has properties that distinguish its pathways from those of activation [51,52]. Overexpression of the SH-inositol phosphatase SHIP2 may limit degranulation, and hence documentation of a relevant allergy [53]. Most notably, desensitization pathways are more readily engaged than the activation pathways [54] that lead to secretion so that it is possible to incrementally expose the cells to allergen to induce desensitization without significant secretion [15,55–59]. Alternatively, it is possible to block the activation pathways without affecting the desensitization pathways [51,60]. All forms of activation (secretion of mediators,

166 expression of cell surface markers, etc.) are blunted by desensitization protocols.

167

168 **Basophil testing in the diagnosis of allergy**

169 Basophil testing is a complex but useful and manageable element of allergy diagnosis (Table
 170 1). It should only be considered if a) no serological or skin test is available, b) considerable
 171 risk of systemic reactions after skin testing exists or c) (most frequently) results of serological
 172 and skin tests are discordant with the clinical history. Basophil testing should always be
 173 considered as an alternative to provocation as a carefully planned and performed basophil
 174 test may predict a severe response as outcome of a provocation, and may replace some
 175 provocations [61]. A negative basophil test should always be confirmed with a provocation
 176 test. As basophil testing is performed *ex vivo*, it can be done with recombinant allergens and
 177 novel drugs or other substances not produced for use in man [36,62–64].

178 **Chronic urticaria**

179 The mechanism underlying chronic spontaneous urticaria (CSU) is still incompletely
 180 understood [65] and there are no biomarkers. CSU serum can induce histamine release from
 181 normal basophils [66] and can contain autoantibodies [67] mainly to the IgE receptor [68].
 182 However, this ability to activate normal basophils is found in 40% of the patients, the
 183 remaining 60% are elusive. BAT may replace autologous serum skin test (ASST) in the
 184 diagnosis of CSU, but the correlation between basophil degranulation and skin test responses
 185 is not perfect [69,70]. BAT is a reliable, safe and accurate method where there is no need to
 186 inject serum into patients. CD63 upregulation is a sensitive and specific activation marker for
 187 CU [71–73].

188 **Immediate Drug Hypersensitivity**

189 The diagnostic work-up of immediate drugs hypersensitivity reactions (IDHR) aims at
 190 identifying the culprit medication and identifying safe alternative(s) for future treatment [74].
 191 Although *in vivo* tests are the most widely used by the clinicians [75], they present some
 192 limitations: drug provocation tests (DPT) and to a lesser degree skin tests [76] are not exempt
 193 of risk especially in patients with severe reactions i.e. anaphylaxis. For some drugs, skin tests
 194 do not have optimal sensitivity and may produce false positive results [77,78].
 195 BAT shows complementary results to those obtained in skin testing [79]. It is possible to
 196 obtain positive results in BAT in a percentage of patients with negative skin test and sIgE

determination [38,80]. Importantly, this test has demonstrated an excellent negative predictive value as the case of fluoroquinolones [81,82], where false positive skin tests may occur.

In contrast with available IgE assays, BAT can be performed with a wide range of drugs without need of synthesizing the drug-carrier conjugates [83].

Principal applications of BAT have included IDHR to betalactams [80,84,85] especially with the new ones, as clavulanic acid, where no other *in vitro* method can be used [86], muscle relaxant [87–89], fluoroquinolones [81,90,91], radiocontrast media [92] and to single-NSAID-induced urticaria/angioedema or anaphylaxis [93] where an IgE mechanism is involved [38,94,95]. It may be useful to confirm the involvement of FcεRI in the response [13,90]. BAT has also been used for diagnosis of many other individual drugs, where no other *in vivo/in vitro* tests are available [82,96]. There are some specific points that have to take into account when using BAT for evaluating IDHR:

- i) Drugs induce less basophil activation than protein allergens. In addition, significant higher concentrations of allergen are required. This implies that cytotoxicity has to be checked. It is more difficult to calculate a diagnostic threshold.
- ii) Drugs can be degraded under different conditions as temperature, pH and light, producing metabolites that can interfere with or prevent BAT results [91,97]. Like other allergens, drugs must be prepared fresh for each test and avoiding the factors that influence their degradation [82].
- iii) As for other tests used for evaluation of IDHR, BAT sensitivity declines with time passed between reaction and testing. Therefore, it is recommended to perform BAT within a year after of last reaction to the drug [38,98,99].

Food allergy

Basophils are involved in food-induced acute allergic reactions and anaphylaxis [16,20]; thus, basophil testing can be used to diagnose IgE-mediated food allergy [100,101]. The main advantages of BAT to protein food allergens compared to sensitisation tests (i.e. skin prick test and specific IgE) are its high specificity (reaching 100% in some studies) and its high positive predictive value, which allow to confirm the diagnosis of food allergy in the case of a positive BAT with a high degree of certainty [61,102–104]. BAT can be performed using crude

227 extracts [61,102,105] or single allergens [106–108]. Due to the technical and logistical aspects
 228 involved in its performance, BAT is often used as a second step in the diagnostic process, only
 229 in selected patients in whom the combination of the clinical history and the results of skin
 230 prick test and/or specific IgE could not confirm or exclude the diagnosis of food allergy [61].
 231 This approach can lead to a significant reduction in the number of oral food challenges, in
 232 particular positive challenges, where patients suffer acute allergic reactions of varying
 233 degrees of severity. Interestingly, basophil reactivity reflects severity and basophil sensitivity
 234 reflects threshold of allergic reactions to foods during challenges [22,23,109,110]. This may
 235 provide useful information for the management of patients.

236 **Respiratory allergies**

237 Measurements of sIgE or skin testing in combination with the clinical history are usually
 238 sufficient to diagnose respiratory allergies. Basophil sensitivity provides a quantitative
 239 measure that gives additional information of the patients' allergic condition e.g. during
 240 treatment. Measurements of basophil sensitivity correlates with the nasal provocation titer in
 241 allergic rhinitis [111] , the allergen specific bronchial provocation threshold in allergic asthma
 242 [18] and the asthma control test [112]. When using an allergen titration, the correlation of the
 243 outcome between basophil allergen sensitivity and bronchial allergen sensitivity was
 244 statistically significant. This indicates that basophil allergen threshold sensitivity (CD-sens or
 245 EC50) may quantitatively reflect clinical allergen sensitivity [18].

246 Occupational allergies are a frequently overlooked area in which basophil testing can play a
 247 major role [113–115]. Convincing documentation is required for sensitisation to a workplace
 248 allergen before compensation can be obtained, and before the patient considers a change of
 249 career to move away from a daily, or regular exposure to an offending allergen. The route of
 250 exposure is often through the airways, and a standardised approach for testing exists [82].

251 **Hymenoptera Allergy**

252 The clinical history in combination with measurements of sIgE or skin testing may be
 253 sufficient to diagnose insect venom allergies, but up to 60% of insect venom patients may
 254 react with more than one insect in first line tests [116]. This complicates the decision for
 255 immunotherapy with the correct allergen. Introduction of recombinant molecular allergens,
 256 especially for wasp venom allergens, can solve many of these cases. However, more cases are
 257 solved with BAT than with molecular serologic tests. The diagnostic sensitivity and specificity

258 of BAT with insect venom extracts referred to history is good (83% to 100%) [117–119].
259 Furthermore BAT allows the identification of about two third of patients with a clear history
260 of systemic reactions after Hymenoptera stings with negative venom-specific IgE and negative
261 skin test to bee and wasp venom [120,121].

262 In patients with a double positive basophil activation test, the venom which the patient is
263 markedly more sensitive (10-fold is arbitrarily set), might represent the clinically relevant
264 trigger allergen [122], but this not yet proven, because diagnostic sting challenges are not
265 recommended or performed.

266

267

268 **Basophil testing to monitor therapy**

269 Great expectations to basophil testing are that it may be able to supplement and predict the
 270 outcome of provocations, and eventually reduce the number of provocations, and that it thus
 271 can be used to repeatedly test patients for their allergy without exposing them to allergen.
 272 This is true for both allergen immunotherapy for protein allergens [21,24,61,123–126], as
 273 well as for treatment effect of anti-IgE or other similar treatments for chronic urticaria [127–
 274 129], food- [130] and respiratory allergies [111,131–133].

275 **Food allergy**

276 BAT can be used to monitor IgE-mediated food allergy over time, namely to assess
 277 spontaneous resolution and to evaluate the response to immunomodulatory treatments. For
 278 example, in patients with cow's milk allergy BAT can help identifying different clinical
 279 phenotypes and can determine when to perform oral food challenge to assess the possible
 280 development of tolerance [105,134]. In many oral or sublingual immunotherapy studies to
 281 foods, decreased allergen-induced basophil activation particularly at low concentrations of
 282 the allergen accompanied clinical improvement [124,135–137]. BAT can also be used to
 283 monitor response to Chinese herbal formula for food allergy [138].

284 BAT can have additional applications in food allergy different from diagnostics; for instance,
 285 to assess the allergenicity of foods, to detect low amounts of allergen in food matrices and
 286 other complex mixtures and to confirm the biological activity of food allergens [139–144].

287 **Hymenoptera Allergy**

288 Side effects during the build-up phase of venom immunotherapy are associated with a higher
 289 basophil sensitivity compared to well-tolerated immunotherapy [145]. Furthermore, the
 290 decrease in basophil sensitivity over years in patients submitted to venom immunotherapy is
 291 associated with the induction of tolerance [24,123,146]. Therefore the parameter basophil
 292 sensitivity seems to reflect more closely the clinical situation with regard to anaphylactic
 293 reactions [17] to insect stings than other known parameters.

294 **Respiratory allergies**

295 One major advantage with measurements of basophil sensitivity is that it is an objective and
 296 reproducible method [107,147,148] and can be used to monitor treatment efficacy. During the

297 last decade several articles described the advantage of using basophil sensitivity during
298 allergen-specific immunotherapy (AIT) to aeroallergens. It has been used to monitor patients
299 treated with AIT for birch [126,149] and timothy grass [15,21,125], and showed reduced
300 allergen sensitivity already during the up-dosing stage. Basophil sensitivity has successfully
301 been used to objectively identify patients who respond to this anti-IgE treatment [131] and to
302 assess treatment efficacy [111,131–133] without the need of performing more complex
303 bronchial allergen challenges.

304

305 **Presentation and Interpretation of basophil testing**

306 The precise clinical implications of a measurable basophil activation test are that the patient
307 is sensitised as basophils react to allergen through IgE. It is well understood that reactive
308 basophils are not always concordant with the clinical expression of allergic disease. However,
309 this problem is similar to allergic testing that relies on serology or skin testing. It is possible to
310 research this response in mechanistic studies, and elaborate on the diagnostic implications of
311 an *ex vivo* basophil response to allergen.

312 Diverse methods are used to evaluate the response in a basophil activation test, i.e., the
313 commercial assay and even research methods have yet to settle on the best practice for
314 evaluation of results. Reactivity is the basic metric for basophil testing; to determine reactivity
315 one sets a baseline at 1, 2, 5 or 10% of resting basophils, and obtain the fraction of all
316 basophils responding to allergen – typically more than the resting population. Reactivity is
317 required to calculate the stimulation index (where the fraction of cells stimulated with
318 allergen is expressed as a multiple of the fraction set arbitrarily as positive in the non-
319 stimulated sample), for determination of basophil sensitivity (where the allergen
320 concentration required to activate a given fraction of all cells that can be activated) and for
321 calculation of the area under the curve obtained with different allergen concentrations.
322 Median fluorescence intensity of CD63 on the individual basophil granulocyte may prove to be
323 an alternate measure for activation. CD63 MFI sensitively reflects desensitisation [15]. A
324 graphically compelling but statistically weak approach is to set the threshold for basophil
325 activation midway between the peaks of resting basophils in the negative control and
326 activated basophils in the positive control [150].

327 CD63 reactivity to 200 ng/ml of food allergen was the best predictor of clinical reactivity in a
328 food challenge (area of 0.904 under a ROC curve) and strongly correlated with symptom
329 severity in food allergen challenges [22]. Similarly, the severity of food allergen challenges
330 could be predicted by the relationship of reactivity at 100 ng/ml peanut allergen divided by
331 the reactivity to a constant response to an antibody activating basophils by crosslinking FcεRI
332 [23]. The mechanistic framework underpinning this approach has yet to be developed.

333

334 Activation by drugs and other small molecules may depend on non-covalent [151] or covalent
 335 modification of patient protein. Typically, much higher molar concentrations of drugs (2
 336 mg/ml penicillin correspond to 6 uMol/ml) are used than of protein allergen (100 ng/ml of
 337 Der p 2 corresponds to 10 pMol/ml). In spite of this million-fold difference in concentration,
 338 *ex vivo* activation of basophil granulocytes by a drug may be marginal and may not achieve
 339 complete anaphylactic degranulation. In order to optimise the use of basophil testing in the
 340 diagnosis of drug allergy, results are considered as positive when the reactivity is >5% over
 341 spontaneous activation observed for the negative control (set at 2%) [89,152], or the
 342 stimulation index, calculated as the ratio between the percentage of degranulated basophils
 343 with the drug and the spontaneous basophil activation (set at 2%), is >2 [38,80,84,91,153]. It
 344 is always better to establish a precise cut off with a ROC analysis for each drug [89]. Either
 345 reactivity or stimulation index, based on a stated threshold for resting basophils, may be
 346 sufficient for establishing the result of basophil testing.

347 Clinically, a Bayesian approach to diagnosis that considers the merits of history, first line tests
 348 and basophil tests sequentially to calculate the likelihood of sensitisation is a very strong
 349 approach that will provide allergy diagnoses of many patients without a provocation.

350 **Conclusion:**

351 BAT is a safe approach and of great interest in the evaluation of those allergens where specific
 352 IgE detection tests are not available; clinical research questions regarding application of BAT
 353 are listed in table 2. Basophil activation tests makes a variety of allergen molecules accessible
 354 as diagnostic reagents, as they can successfully be performed with drugs [38,80,84–94] as
 355 well as recombinant venom- [154], food- [106–108] and aeroallergens [33,155–157]. BAT is
 356 an important tool for research of allergen molecules inducing activation of basophils by IgE-
 357 bridging on these cells.

358 As read out of basophil activation reactivity with CD63 is the most widely used parameter, as it in
 359 food allergy also correlates with symptom severity. The sensitivity measurement of basophils
 360 demonstrates that basophils were exposed to the relevant allergen concentrations, and correlates
 361 closely with clinical sensitivity. Sensitivity is important in diagnosis, but also in monitoring of
 362 allergic disease.

363

364

365 **Table 1: Pros and Cons**366 **Pros**

- 367 - Basophil granulocytes and mast cells are related cells that respond similarly to FcεRI cross-
- 368 linking.
- 369 - CD63 upregulation on basophil granulocytes and mast cells corresponds to anaphylactic
- 370 degranulation
- 371 - Basophil activation is a quick functional test of response to allergen
- 372 - BAT is a flexible testing module that can address many difficult issues of allergy testing.
- 373 - It is possible to separate intrinsic and extrinsic factors affecting basophil activation
- 374 - Many drugs can be used as allergen in BAT, and alternative drugs can be found.
- 375 - Occupational allergens can easily be tested in BAT.
- 376 - BAT has a high positive predictive value in food allergy diagnosis, and can reduce the
- 377 number of food challenges required for diagnosis.
- 378 - Basophil sensitivity is a quantitative measure of a patient's allergy.
- 379 - BAT can identify primary sensitising allergens in insect venom allergy.
- 380 - BAT can be used to monitor progression of natural resolution or treatment of allergy, and
- 381 basophil testing may be used as companion diagnostic for AIT.

382 **Cons**

- 383 - Drug BAT (and other allergy tests) should be performed within a year of the most recent
- 384 allergic response that elicits the diagnostic visit.
- 385 - BAT should be done on fresh blood (preferably less than 4 hours old)
- 386 - Non-responders cannot be tested with a BAT.
- 387 - BAT is complex and requires an experienced laboratory and interpretation.
- 388 - The testing setup and interpretation of BAT is complex and lacks standardisation
- 389 - BAT and ASST do not correspond exactly in the diagnosis of CSU.

390

391 **Table 2. Research needs in Basophil activation**

- 392 - Mechanisms of non-protein allergic reactions and non-IgE mediated basophil activation need
- 393 to be elucidated.

- 394 - The diagnostic utility of BAT needs to be validated for specific protein allergens (food, insect
395 venom, respiratory) and in different populations.
- 396 - Changes in the basophil response during AIT and anti-IgE treatment in food allergy should
397 be investigated.
- 398 - The minimal difference in sensitivity between primary sensitising and cross-reacting
399 allergens needs to be defined.
- 400 - Basophil sensitivity tests could be considered as a supplement, and eventually possibly as
401 replacement for allergen challenge tests.
- 402 - The predictive value of basophil suppression for treatment outcome has to be established.
- 403 - BAT should be established as a biomarker and eventually as a companion diagnostic for
404 subcutaneous allergen immunotherapy

405

406 **Figure 1 Legend:** Simplified cartoon of early IgE-mediated signal transduction as it applies to
407 human basophils and mast cells.

408 **A** Although no major differences in signalling have been found between human basophils and
409 mast cells, there are behaviours that indicate that differences must exist. Studies of basophils
410 have demonstrated that syk expression levels are a unique regulator of secretion while this is
411 not the case for human mast cells. In addition, there are indications that some basophil
412 phenotypes are associated with SHIP expression (a negative regulator) with the difference
413 that SHIP functional studies have been done while syk functional studies have been shown to
414 correlate with its expression. Beyond the early signaling, studies have shown that the rise in
415 cytosolic calcium tightly correlates with downstream functioning and it controls most
416 functional endpoints with the interesting exception of the expression of CD203c.

417 **B** Evidence suggests that CD203c and CD11c reside on small vesicles that are also under the
418 control of a PKC-dependent pathway, which is interesting since IgE-mediated signaling in
419 human basophils generally doesn't appear to depend on PKC enzymes. There are two
420 pathways to secrete granule-associated histamine, one which includes fusion of the granules
421 with the plasma membrane and leading to both histamine release and CD63 expression and
422 one which probably doesn't lead to CD63 expression and may be also influenced by a PKC-like
423 process.

424

425

426 **References**

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