

Biomarkers for evaluation of mast cell and basophil activation

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Summary

Mast cells and basophils play a pathogenetic role in allergic, inflammatory, and autoimmune disorders. These cells have different development, anatomical location and life span but share many similarities in mechanisms of activation and type of mediators. Mediators secreted by mast cells and basophils correlate with clinical severity in asthma, chronic urticaria, anaphylaxis, and other diseases. Therefore, effective biomarkers to measure mast cell and basophil activation in vivo could potentially have high diagnostic and prognostic values. An ideal biomarker should be specific for mast cells or basophils, easily and reproducibly detectable in blood or biological fluids and should be metabolically stable. Markers of mast cell and basophil include molecules secreted by stimulated cells and surface molecules expressed upon activation. Some markers, such as histamine and lipid mediators are common to mast cells and basophils whereas others, such as tryptase and other proteases, are relatively specific for mast cells. The best surface markers of activation expressed on mast cells and basophils are CD63 and CD203. While these mediators and surface molecules have been associated to a variety of diseases, none of them fulfills requirements for an optimal biomarker and search for better indicators of mast cell/basophil activation in vivo is ongoing.

KEYWORDS

basophil, histamine, lipid mediators, markers, mast cells, proteases

1 | INTRODUCTION

Mast cells and basophils are distinct cells with clearly different developmental pathways, tissue locations and life span. They share, however, common activation mechanisms, a unique profile of mediators secreted upon activation and, likely, similar pathogenetic roles in several human diseases.

Both mast cells and basophils have basophilic granules in their cytoplasm and express the high-affinity immunoglobulin E (IgE)

receptor FcεRI on their cell surfaces. Furthermore, both cells are associated with T helper 2 (Th2) immune responses. A role for mast cells and basophils has been demonstrated in many allergic, inflammatory, and autoimmune diseases. Mast cells and basophils play crucial, albeit distinct roles in allergic rhinitis (AR), bronchial asthma, chronic idiopathic urticaria (CIU), parasite infections, delayed-type allergic reactions, and anaphylaxis. Basophils exhibit rapid and potent expression of interleukin (IL)-4 and IL-13, leading to cutaneous Th2 skewing. Being tissue resident cells, mast cells are often activated directly within tissues that are sites of inflammation. By contrast, basophils are blood-borne cells that must be recruited within tissue where they reach their final activation. Accumulation

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of basophils has been reported in numerous human diseases, including allergic diseases, such as atopic dermatitis (AD), asthma, and AR, as well as autoimmune diseases and cancer.

Understanding the role of these cells in human diseases has been challenging given the difficulties in isolating pure mast cells and basophils from human sources in quantities suitable for *ex vivo* investigation. Research was therefore focused to explore potential markers of mast cell and basophil activation *in vivo* for two main reasons: first, to define useful diagnostic and prognostic markers for diseases in which their role was already established, eg, allergic diseases and, second, to identify mast cell and basophils activation products in diseases in which their role was strongly suspected.

Markers of activation of mast cells and basophils can be distinguished in two main types: secretory mediators and membrane associated activation markers. Most of the mediators secreted by mast cells and basophils are preformed molecules, such as histamine and proteases, that are virtually produced only by these cells *in vivo*. For the purpose of this review we will consider first the biomarkers that are common to mast cells and basophils and then those that are specific for either one of these cells.

1.1 | Common biomarkers and mediators of mast cells and basophils

1.1.1 | Histamine

Histamine is a biogenic amine with inflammatory and immunological functions secreted by both mast cells and basophils upon immunological and non-immunological activation.¹ Mast cells and basophils should be considered the main source of histamine in humans and the possibility that other cells, such as neutrophils and platelets, may contribute to the biosynthesis of this mediator under special circumstances remains to be convincingly demonstrated. Once synthesized from the amino acid histidine, histamine is stored within secretory granules of both mast cells and basophils. When these cells are activated by either immunological or non-immunological stimuli histamine is secreted within seconds and exerts its biological effects by acting on four different receptors (H1 through H4).² Histamine is then rapidly metabolized by conversion into methyl histamine or by oxidative deamination into imidazole acetaldehyde, which are both excreted in the urine.³

Increased levels of histamine have been detected in the nasal lavage of patients with allergic rhinitis, in bronchoalveolar lavage from patients with allergic asthma and in plasma from patients with AD or CIU.^{4,5}

While histamine can be more easily detected in biological fluids, the usefulness of measuring histamine in serum or plasma as mast cell or basophil activation marker is limited by several issues. First, histamine is produced by other non-mast cell/non-basophil cells such as neurons, enterochromaffin-like cells in the stomach and by certain bacteria in the gastrointestinal tract and urine, and it is present in many fermented foods and beverages. Thus, histamine elevation *per se* does not unequivocally identify activated basophils or mast cells as the source. In addition, measurement of plasma level of histamine can be difficult due to its short half-life, which after onset of

insect sting-initiated anaphylaxis, will return to baseline levels within 15–30 minutes.⁶

There are several methods available to measure histamine, including ELISA and solid phases but technically, histamine assays are relatively laborious and expensive, or require specialized equipment such as histamine autoanalyzers. HistaFlow is an alternative technique to measure histamine or its metabolites using an enzyme-affinity-fluorochrome which recognizes intracellular histamine in order to capture data of the single cell level.⁷ It has been useful to monitor basophil responsiveness during immunotherapy considering that immunotherapy inhibits basophil histamine release, and it can be used as potentially surrogate biomarker for detecting immunotherapy efficacy.⁸

The measurement of levels of histamine metabolites in urine, preferably on urine collected over 24 hours, can be a non-invasive means to monitor global histamine turnover. Histamine metabolites in the urine may be measured by high-performance liquid chromatography (HPLC) and HPLC coupled to mass spectrometry. Increased levels of histamine metabolites in the urine have been associated with systemic mastocytosis and/or mast cell and basophil activation.⁹

1.1.2 | Lipid mediators

Lipid mediators are arachidonic acid-derived proinflammatory and vasoactive mediators which are *de novo* synthesized in activated mast cells and basophils. Prostaglandin D₂ (PGD₂) and cysteinyl leukotrienes (CysLTs) are the major lipid mediators, shortly released after mast cell activation.¹⁰ They are produced from catalytic conversion of arachidonic acid by the action of cytosolic phospholipase A2 on membrane phospholipids.¹¹ PGD₂ exerts its biological effect of increasing vascular permeability and bronchoconstriction by binding to two receptors (DP1 and DP2). PGD₂ is also produced by eosinophils, other immune cells such as Th2 cells and dendritic cells and by non-hematopoietic tissues such as brain, heart, lungs, and kidney. The production of PGD₂ by other sources limits its usefulness as biomarker of only mast cell activation. Increased serum levels of PGD₂ are detectable after inhalants allergenic exposition; levels of PGD₂ and of its metabolite 11 β -PGF2 α have been observed augmented in the urine of patients with mastocytosis and mast cell activation syndrome.^{12,13} LTC₄, LTD₄ and LTE₄ are collectively referred to as CysLTs and are released from mast cells, basophils, myeloid dendritic cells, eosinophils and macrophages.¹⁴

They derived from the precursor LTA₄, subsequently converted to LTC₄ which is then metabolized to LTD₄ and LTE₄. These mediators, by binding to at least three distinct receptors (CysLTR1, 2, and 3), induce smooth muscle contraction and airway constriction at concentrations that are up to 1000-fold lower than those of histamine required to achieve a similar effect.^{15,16} CysLTs also increase vascular permeability in humans. Remarkably, human basophils rapidly release LTC₄ within minutes upon immunological and non-immunological activation but they do not synthesize PGD₂^{17,18}; therefore its secretion from washed leukocytes has long been used to evaluate the allergic status, since basophils within these suspensions are the only source of LTC₄ following IgE/Fc ϵ RI-dependent activation.¹⁹ Urinary LTE₄ was found to be elevated

in systemic mastocytosis and to correlate with 24 hours urine N-methylhistamine and serum tryptase levels.²⁰

Platelet-activating factor (PAF) is a potent phospholipid-derived mediator released by several types of cells, including mast cells, monocytes, tissue macrophages, platelets, eosinophils, endothelial cells, and neutrophils, in response to various stimuli.²¹ PAF is a potent bronchoconstrictor, induces profound vasodilation and edema formation and is involved in platelet aggregation as well as activation of neutrophils, eosinophils and platelets.²² Interestingly, while human mast cells synthesize PAF they produced even larger quantities of a PAF analog, 1-acyl-PAF, when activated by immunological stimuli.²³ 1-Acyl-PAF reproduces the same biological effects of PAF but it is a 1000-fold less potent. PAF is rapidly metabolized into its inactive metabolite, lyso-PAF, by the enzyme PAF acetylhydrolase (PAF-AH). PAF exerts its effects at subnanomolar concentrations and it has a half-life of few minutes, depending on the time to inactivation by PAF-AH. This mediator can be found in many body fluids, including serum, saliva, and bronchial fluids. In patients with anaphylaxis serum PAF levels correlate with severity of clinical symptoms even better than serum tryptase or histamine levels.²¹ In addition to its role in anaphylaxis, PAF has also been involved as a mediator of AR. PAF has been demonstrated in nasal lavage fluid from patients with AR after allergen challenge leading to nasal hyperresponsiveness to histamine and to recruitment of inflammatory cells within the nasal mucosa. In animal models PAF-receptor antagonists are able to reduce nasal airway resistance and vascular permeability after allergen challenge.²⁴

1.2 | Specific biomarkers and mediators of mast cells

1.2.1 | Heparin

Heparin, like histamine, is stored within preformed granules of mast cells; therefore it is rapidly released upon cell activation and plays a role in vascular permeability and smooth muscle contraction.²⁵

In patients with mast cell proliferative disorders it has been proposed that heparin could be associated with collections of mast cells of maculopapular lesions of urticaria pigmentosa and with the hemorrhagic complications in the aggressive forms.^{26,27} Despite this, coagulation times remains normal in most patients.

In 40% of cases with mast cell activation diseases, basal endogenous heparin levels have been reported to be outside the normal range, although in the most cases, this does not happen.²⁸

1.2.2 | Tryptase

The major prestored enzyme of human mast cells secretory granules is the tryptase, much lesser amounts being expressed by basophils.^{29,30} There are four tryptase isoforms, but only two of them, alpha and beta tryptase, are considered as biologically important.³¹

Low quantity of immature monomeric form of tryptase, is constitutively secreted by resting mast cells and it reflects the baseline serum levels of tryptases (ranging from 1 to 11 ng/mL), whereas active tetrameric form of mature β -tryptase is only released by activated mast cells.³²

Taken together these observations indicate that, serum tryptase concentrations usually increase in case of degranulation of mast cells and measurement of is used to diagnose and monitor disorders characterized by mast-cell activation.

There is no consensus on the minimal increase in serum tryptase suggestive to an "MC activation-related" event.³³ Baseline serum levels of α/β -tryptases >20 ng/mL is considered as a minor diagnostic criterion for systemic mastocytosis while transient and rapid increase of serum tryptase level ($>2 + 1.2 \times$ baseline level), within 4 hours of anaphylactic event, is considered laboratory evidence for mast cell activation.^{31,34}

1.2.3 | Chymase

Chymase is a chymotrypsin-like serine endopeptidase stored in mast cell secretory granules, and usually released in conjunction with carboxypeptidase A3 and distinctly from tryptase.³⁵ Once secreted, chymase exerts several biologic effects: it can mediate the conversion from angiotensin-I to angiotensin-II, generate TGF- β and fibronectin from extracellular matrix, activate several molecules including endothelin-1 and 2, matrix metalloproteinases (MMPs) and cytokines (IL 1- β , IL-18), degrade lipoproteins leading to macrophage foam cell formation.³⁶ Serum chymase represents target of inhibitory action of many circulating molecules including α -1 antitrypsin, α -1 antichymotrypsin and α -2 macroglobulin that capture it, preventing its interaction with substrates.³⁷ An immunoassay to detect chymase in human serum is not currently available, therefore alternative methods have been proposed. It has been developed an α -2 macroglobulin capture assay which use a synthetic substrate to detect enzymatic activity in chymase-spiked serum with a threshold of approximately 30 pg/mL. This method has pointed out that chymase activity in serum is influenced by total body burden of mast cells, and it is much higher in systemic mastocytosis than in more localized cutaneous forms.³⁸

1.2.4 | Carboxypeptidase A

Carboxypeptidase A is a zinc-dependent metalloproteinase content in mast cells and released upon activation. Its gene CPA3 has found to be one of 10 genes overexpressed in the bone marrow mononuclear cells of adult patients with systemic mastocytosis.³⁹ Interestingly plasma levels of mast cell carboxypeptidase appears to be elevated in children with allergic diseases but not in adult with the same disease.⁴⁰ Moreover, serum level of carboxypeptidase increase during anaphylaxis; this increase does not always correlate with serum levels of tryptase: it seems to be longer and present also in absence of tryptase elevation.⁴¹

1.3 | Activation markers of basophils in human diseases

Basophils are known to be involved in many human diseases, including allergic diseases (eg, urticaria,⁴²) allergic contact dermatitis,⁴³ AD,⁴³ allergic drug reactions,⁴⁴ immediate hypersensitivity reactions (eg,

anaphylaxis,⁴⁴ asthma,⁴⁵ autoimmune diseases (eg, bullous pemphigoid,⁴³ lupus nephritis,⁴⁶ Crohn's disease,⁴⁴) transplant rejection,^{47,48} and malignant neoplasms.⁴⁹ Recent studies have identified functionally distinct basophil populations in human allergic disease states, such as food allergies, eosinophilic esophagitis, asthma, urticaria, AR, and AD. In some conditions, such as food allergies,⁵⁰ urticaria,^{51,52} and asthma,⁵³ inflammation can be effectively mediated predominantly by IgE. However, the results of studies on anti-IgE therapy effectiveness among patients with AD⁵⁴ and eosinophilic esophagitis⁵⁵ have been controversial. This suggests that IgE-independent processes might be critical for the pathogenesis of these diseases.

Recent studies have indicated that specific surface markers are activated in these diseases. Major surface markers expressed on human basophils are CD63, CD69, and CD203c, and they have been used to demonstrate the role of basophils in vivo and in vitro.^{56,57} Currently, CD203c and CD63 are considered the most useful markers of basophil activation and differentiation.^{58,59} CD203c and CD63 on the basophil cell surface are upregulated as a result of early FcεRI-mediated reactions and play roles in the late cytokine-mediated activation of basophils.⁶⁰ CD69 expression was only weakly observed in basophils freshly isolated from human peripheral blood, but IL-3 induced extremely high levels of expression.⁶¹ The functional role of each surface marker in basophil differentiation and maturation is not fully understood. Basophils can, however, be clinically used to diagnose or assess the disease states of some human allergic diseases. Reports that have shown the activation of basophils in human diseases are summarized in this section (Table 1).

Regarding the mechanisms underlying these diseases, recent studies have suggested the importance of innate cell networks, especially in various innate-type immune cells, including innate-type 2 lymphoid cells (ILC2) and basophils, that are involved in the production of Th2-type cytokines in AD.^{62,63} Interestingly, type 2 immune responses can be caused by the absence of Th2 cells. Interaction between basophils and ILC2 can promote the sensitization and chronic phases of type 2 inflammatory responses.⁶⁴ These innate immune cells may directly or indirectly contribute to adaptive Th2 cell responses.⁶⁵

2 | IMMEDIATE-TYPE HYPERSENSITIVITY

Immediate hypersensitivity reactions involve the IgE-mediated release of histamine and other mediators from mast cells and basophils.⁶⁶ Flow cytometry-based basophil tests have been clinically used to diagnose immediate-type hypersensitivities, including anaphylaxis, AR, and other allergic diseases, as well as to confirm the sensitization of patients to foods, drugs, and insects.^{57,67,68} The flow cytometric basophil activation test (BAT), based on the detection of allergen-induced CD63 expression, has been proven effective in the diagnosis of various IgE-mediated allergies.⁵⁹ Because of the increased cell surface levels of CD63 and/or CD203c in response to IgE-dependent stimuli, this protein is also a promising marker of activation that can be used in flow cytometry-based allergy diagnoses. These tests therefore appear suitable for detecting immediate-type hypersensitivities and for monitoring the success of hyposensitization protocols in patients with allergies.⁵⁸

TABLE 1 Activation markers of basophils in human diseases

Disease	Activation marker	Reference
Immediate-type allergy		
Anaphylaxis	CD63, CD203c	67
Drug hypersensitivity	CD63, CD203c	68
Food allergy	CD63, CD203c	86
Seasonal allergic rhinitis	CD63, CD203c	87,88
Asthma	CD203c	58
Urticaria	CD63, CD69	85
	CD63, CD203c	89
AD	Not identified yet	43

3 | ASTHMA

Allergic asthma is a pulmonary inflammatory disease characterized by the infiltration of eosinophils into lungs that is controlled by type 2 cytokines. Basophils have been found to strongly infiltrate the lung tissues of allergy patients.^{69,70} IgE is thought to activate basophils to release histamine and LTC₄, which promotes inflammation.⁵¹ Anti-IgE monoclonal antibody (omalizumab) is the first anti-IgE therapy to have demonstrated efficacy and be approved by the United States Food and Drug Administration (FDA) for the treatment of asthma.⁵³ Ono et al⁵⁸ showed that CD203c expression in basophils was upregulated in asthma patients and associated with asthma exacerbation, although no differences were observed in the expression levels of CD63 or CD69. In contrast, in patients with aspirin-exacerbated respiratory disease, basophil responses to aspirin challenge in vitro are poor indicators of clinical sensitivity.⁷¹ Using a murine lung inflammation model, Motomura et al⁷² indicated that basophil-derived IL-4 controls the function of ILC2 in lung inflammation. Thus, activated basophils might contribute to the pathology of asthma via control of the ILC2 function through IL-4.

4 | URTICARIA

IgE stimulation is known to activate IL-3-elicited basophils, and prior studies have consistently demonstrated that basophils play a crucial role in IgE-dependent chronic allergic inflammation in the skin.⁷³ Additionally, basophils from urticarial patients have been reported to express high levels of CD63 and CD203c on their surface, indicating that they are activated by IL-3.^{74,75} Prior studies have also demonstrated that basophils play a crucial role in IgE-dependent chronic allergic inflammation in the skin.⁷³ Recently, urticarial patients were successfully treated with omalizumab.^{52,76} Considering that omalizumab might modulate basophil functions,^{77,78} future studies are required to determine the precise contribution of basophils to urticaria. Although the role of IgE in promoting urticaria is widely acknowledged, both IgE-dependent and IgE-independent mechanisms are likely involved in urticaria pathophysiology. Further studies are required to investigate the roles of TSLP and TSLP-elicited basophils in the context of urticaria.

5 | ATOPIC DERMATITIS

AD is a type of skin inflammation that mainly results from Th2 cytokine responses. Despite numerous studies on AD pathophysiology, the mechanism underlying this disease remains incompletely understood. High serum IgE levels are often observed, and infiltration of basophils into the inflamed skin of AD patients is reported,⁷⁹ strongly indicating that basophils play important roles in human AD. IgE-mediated inflammation against specific antigens in AD patients appears after infancy when individuals have acquired allergies to food or environmental agents. Clinical studies of AD patients treated with omalizumab have shown that IgE blockades are only effective in certain AD patients, indicating that the AD mechanism cannot be explained by only IgE-mediated antigens specific for Th2 type inflammation.⁵⁴ Although the spontaneous upregulation of activation markers in basophils from AD patients has not been observed,⁴³ allergen-induced CD63 expression in activated basophils from AD patients has been reported.⁸⁰ Recently, basophils stimulated with leukotrienes were reported to orchestrate skin inflammation in AD patients and in a murine AD model based on ILC2.⁶³ The mechanism underlying this phenomenon was partially clarified; basophils are thought to secrete IL-4 and IL-4 to directly modulate ILC2 function.^{63,81} Both murine and human ILC2s are also activated by leukotrienes.^{82,83} In addition, IL-33 and TSLP are involved in inflammation controlling the production of chemokines and cytokines by basophils.⁸⁴ TSLP-elicited basophils and their IL-4 secretion enhance skin inflammation by ILC2. Thus, TSLP-elicited basophils may be a therapeutic target to treat AD patients.

6 | CONCLUDING REMARKS

Mast cells and basophils play important pathogenetic roles in several human diseases. In some of these, like allergic disorders, these cells are primarily involved in the development of the disease and in most clinical manifestations. In some cases, like in asthma, besides being important effector cells, mast cells, and basophils contribute to maintain the innate and adaptive immune responses and to promote secondary activation of other effector cells like eosinophils. In other diseases, such as the autoimmune and chronic inflammatory diseases, eg, chronic inflammatory bowel diseases, the role of mast cells and basophils is less defined. A fascinating, although still largely unknown, hypothesis is the role of mast cells and basophils in the development of cancer. Recent data support the concept that resident mast cells or locally recruited basophils may have either positive or negative impact on the development of tumors, depending on their state of activation. Together these observations underscore the need to identify useful biomarkers of mast cell and basophil activation in vivo. Ideally, a practical biomarker should satisfy several criteria: it should be specific for mast cells and/or basophils, it should be detectable in blood or other biological fluids and its level should be a true indicator of mast cell/basophil activation in vivo; it should be stable

and not influenced by metabolism or by contamination from other sources; finally, it should be easily and reproducibly measured at an acceptable cost. Given these characteristics, the ideal biomarker for mast cells and basophils has not been yet identified. Further experimental and clinical research is clearly needed to improve detection of already existing biomarkers and to identify novel molecules or surface markers that can be suitable to monitor mast cell/basophil activation in human diseases.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

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