MAST CELLS, THE EFFECTOR CELLS OF IGE-MEDIATED ALLERGY

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ABSTRACT

Background

Mast cells, are the primary responder cells of IgE-mediated allergy and while extensive research has been done to elucidate their precise structural and functional characteristics, mast cells are a type of tissue resident cell which makes their isolation and use for various research and diagnostic procedures in the field of allergy and immunology very complicated. Recently, attempts have been made to devise a method measuring mast cell degranulation through allergen stimulation for allergy diagnosis – Mast cell activation test (MAT). The aim of this article is to summarize existing knowledge on mast cells, the MAT, and the related Basophil activation test (BAT) on which the MAT is based on.

Methods

We analyzed data from 51 relevant articles.

Results

We made an extensive literature review of available data on the cytology, biochemistry and role of mast cells in the pathogenesis of allergic diseases—anaphylaxis, allergic rhinitis, and bronchial asthma, as well as available data on the MAT and how it compares to the BAT.

Conclusion

While much key information is available on mast cells, further research is required to expand on their function and role in allergy. Further research is needed to adequately compare MAT and BAT and create standardized protocols for the application of MAT in clinical practice and allergy research.

Key words: Mast cells, degranulation, IgE-mediated allergy, Basophil activation test, Mast cell activation test.

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Mast cells are a type of granulocytes that originate from the myeloid stem cell.

They are part of the immune and neuroimmune systems and play an important role in triggering the inflammatory cascade (1).

Mast cells play a key role in the development of IgE-mediated allergy and anaphylaxis, as they are the primary cell type responsible for releasing histamine in the body (2).

Additionally, they have an important protective role, participating in the development of immune tolerance, and in innate immunity against bacteria, toxins and parasites. They are also involved in angiogenesis and wound healing (3).

Phylogenetically, mast cells are found in primitive reptiles that lived 276 million years ago. Their study as cells of the human body began in 1863 with the discovery of granulated cells from Recklinghausen. Later, in 1878, in his doctoral dissertation, Paul Ehrlich described a new type of granular cells in connective tissue. He noted their "tendency to accumulate around preformed structures in connective tissue and mucous membranes," such as blood vessels, nerves, secretory ducts, sites of inflammation, and neoplastic foci. Initially, Ehrlich believed that these cells originated from connective tissue cells that had absorbed large amounts of nutrients, and therefore gave them the name "Mastzellen", meaning "well-fed cells" in German (4).

Mast cells are unique among hematopoietic cells because they mature in peripheral tissues rather than in lymphoid organs or the bloodstream. Their progenitors are released from the bone marrow and complete their development as tissue-resident cells (5).

Mast cell progenitors (MCPs) circulate from the bone marrow to the tissues, where their migration is

mediated by the interaction of integrins with vascular cell adhesion molecules (VCAM-1) and (MAdCAM-1), as well as chemokine receptors (e.g., CXCR2), which mediates their homing to the tissues where they mature (6).

Unlike basophils, mast cells exhibit different phenotypic and functional subtypes depending on their location, protease secretion, and reactivity.

Based on the types of proteases released upon degranulation, human mast cells are classified into two main categories: tryptase-positive mast cells (MCT), which secrete tryptases, and tryptase-chymase-positive mast cells (MCTC), which secrete both (7).

MCT are the primary type of mast cell found in the pulmonary alveoli, the mucosa of the small intestine, and the eyes. During mucosal inflammation, a selective increase in MCT is observed in the affected epithelial surface. MCTC are typically found in normal skin, blood vessels, submucosa, and synovium. Both types of mast cells are involved in local or systemic allergic reactions (8).

Microscopically, mature human mast cells range from 7 to 20 μm in diameter and appear as round or spindle-shaped cells with round or oval nuclei. They stain purple with aniline dyes, a property known as "metachromasia". They are most commonly identified by immunohistochemical analysis using monoclonal antibodies against mast cell-specific proteases.

The cell membrane features thin 1–2 μm microprotrusions (microplicae). Immature mast cells have a multilobed nucleus, while mature mast cells have a nucleus without nucleoli, with loosely condensed chromatin. The most remarkable characteristic of mature mast cells is their abundant cytoplasmic secretory granules, which constitute about half of their volume (9).

The granules contain numerous preformed mediators (Table 1.).

At an ultrastructural level, the secretory granules of human mast cells contain proteoglycans, of which Heparin is the most abundant. It stabilizes the β-tryptase tetramer. Neutral proteases, acid hydrolases, and histamine are bound to heparin by ionic bonds.

Depending on its size and location, each human mast cell contains 1-10 pg of histamine (10).

Histamine is synthesized in the Golgi apparatus by decarboxylation of the amino acid histidine by the enzyme histidine decarboxylase (HDC). After exocytosis at neutral pH, histamine dissociates from mast cell proteoglycans.

Once released into the extracellular environment, histamine is rapidly metabolized (half-life ≈1 min) through methylation or oxidation.

The initial product, N-methylhistamine (MHA), can be excreted through the kidneys, or deaminated by

Table 1. Types of preformed mediators and their biological properties in mast cell granules**.**

Mediator	Biological properties
Histamine	Bronchoconstriction; tissue edema; ↑ vascular permeability; ↑ mucus secretion; ↑ fibroblast proliferation; ↑ collagen synthesis; ↑ endothelial cell proliferation; differentiation and activation of dendritic cells.
Heparin	Anticoagulant; mediator binder; releases growth factors; activates fibroblasts; ↑ endothelial cell migration.
Tryptase	Breaks down inhalant allergens and cross-linked IgE; generates C3a and bradykinin; breaks down neuropeptides; activates TGF-β; increases basal heart rate and contractility of airway smooth muscle; ↑ fibroblast proliferation and collagen synthesis; ↑ ICAM-1 expression on epithelial cells and CXCL8 release; promotes histamine release from mast cells; attracts neutrophils.
Chymase	↑ mucus secretion; degradation of extracellular matrix; processing of type I procollagen; converts angiotensin I to angiotensin II; reduces T cell adhesion to airway smooth muscle; activates IL-1β; degrades IL-4; releases membrane-bound SCF.

the enzyme monoamine oxidase, to be excreted as N-methylimidazoleacetic acid (MIAA). Histamine can also be oxidized by diamine oxidase (histaminase) to imidazoleacetic acid (IAA) (11).

Histamine acts through four different G-protein coupled receptors (GPCRs) - from H1 to H4. Its binding to these receptors mediates effects that include: vasodilation and increased vascular permeability; spasm of bronchial and gastrointestinal smooth muscle; increased gastric acid secretion and itching (12).

The majority of effects associated with histamine in allergic reactions, such as pruritus, vascular effects and contraction of smooth muscle (bronchospasm), are mediated by the H1 receptor.

Histamine also exerts both stimulatory and inhibitory effects on immune cells: enhancement of antigen presentation by dendritic cells; suppression of TNF-α and IL-12 synthesis, and increased production of IL-10 by monocytes and dendritic cells; regulation of Th1, Th2 and Treg cell balance; chemoattractant activity for T cells and eosinophils (13).

Activation of H2 receptors primarily leads to increased gastric acid secretion and also plays a role in the allergic immune response – increased mucus production in the airways; vasodilation and constriction of bronchial smooth muscle (14).

H3 receptors are found mainly in the central and peripheral nervous system as presynaptic receptors controlling the release of histamine and other neurotransmitters. This is the only variety of histamine receptor mast cells don't possess. Yet, it has been implicated in neuro-inflammatory disease, with mast cells still playing a role (15,16).

H4 receptors are expressed by leukocytes, including mast cells, and mediate mast cell chemotaxis in vivo. H4 receptors influence the induction of dendritic cells in the Th2 response in a mouse model of asthma and may also play a role in the pathogenesis of itching (17).

In mast cell granules, neutral serine proteases are represented by two main families: tryptases (with trypsin-like activity) and chymases (with chymotrypsin-like activity). They contribute to the development of symptoms during the immediate-type allergic reaction (18).

Mast cell activation occurs primarily through three main receptors:

- **The high-affinity IgE receptor (FcεRI)**, which mediates the pathogenesis of clinical manifestations in IgE-mediated allergy.

IgE-dependent activation of mast cells leads to the release of preformed inflammatory mediators stored in their secretory granules (histamine, neutral proteases, preformed cytokines and proteoglycans – Table 1.). In addition, mast cells activated by FcεRI also secrete newly synthesized lipid mediators which are products of endogenous arachidonic acid metabolism, such as prostaglandin (PG) D2, leukotrienes (LT): LTB4 and LTC4, as well as the parent molecule of cysteinyl leukotrienes (cys-LTs). Furthermore, activated mast cells synthesize and secrete numerous proinflammatory cytokines.

The ultimate result of cell activation is plasma extravasation, tissue edema, bronchoconstriction, leukocyte recruitment, and persistent inflammation, with clinically recognizable symptoms of anaphylaxis, urticaria, angioedema, and dyspnea (19).

- The second receptor is the so-called **Mas-like G protein-coupled receptor X2 (MRGPRX2)**. It is primarily expressed by mast cells, which contain tryptase and chymase (MCTC), in the connective tissue of the skin,

MRGPRX2 is classified as an orphan receptor, meaning that its recognized ligands have not yet been fully identified (20). Studies conducted so far have shown that it responds to a wide range of molecules. For example, complement anaphylatoxins C3a and C5a, certain neuropeptides like substance P (SP), and antimicrobial peptides such as LL-37 can stimulate this receptor and induce mast cell activation independently of IgE receptor (FcεRI) cross-linking. In addition, it has been shown that some of the drugs that trigger anaphylaxis and pseudoallergic reactions are also agonists of the MRGPRX2 (21).

- **Toll-like receptors (TLRs)** are widely expressed on mast cells.

Mast cells respond to TLR activation by secreting cytokines, chemokines, and lipid mediators. Several studies have shown that activation via TLR2 can also trigger mast cell degranulation. Moreover, stimulation through TLR ligands can act synergistically with FcεRI signaling, enhancing the cellular response to allergens in vivo (22).

IgE-mediated activation is well-studied. It occurs

through cross-linking of two high-affinity IgE receptors (FcεRI) by allergens. This process triggers intracellular signaling pathways leading to degranulation, with the release of preformed mediators, activation of phospholipid metabolism, and transcription of specific genes, resulting in the production and secretion of cytokines, chemokines, and growth factors (23).

Mast cell degranulation occurs in several phases: Enlargement of granules; liquefaction of the crystalline structure; fusion of granules with each other and with the cell membrane and and finally, exocytosis and release of mediators into the extracellular space (24).

Recent studies on the mechanism of mast cell degranulation have revealed that FcεRI engagement is associated with activation of the AKT/PKC (protein kinase C) signaling pathway, leading to phosphorylation of the nuclear factor kappa-β kinase β-subunit (IKK-β) inhibitor. This initiates mast cell degranulation. The process is slow and occurs after fusion of the granules into large, irregularly shaped granules and the formation of membrane complexes comprised of synaptosomal-associated protein 23 and syntaxin-4 (SNAP23-STX4), which mediate exocytosis. Due to the large size of the granules, mediators are released slowly and thus can mediate immune responses at sites distant from the initial site of mast cell degranulation. Additionally, an inflammatory component contributes to the onset of allergic inflammation (25).

Interestingly, after nearly complete degranulation, human lung mast cells are able to survive and undergo regranulation for a period of 15 minutes (26).

A key aspect of IgE-mediated activation and degranulation of mast cells is the activation of phospholipid metabolism (arachidonic acid pathway) and the synthesis of newly formed mediators, such as prostaglandin (PG) D2, leukotrienes (LT): LTB4 and LTC4, whose biological effects play a central role in the initiation and maintenance of allergic inflammation (27).

Another less well-known mechanism of mast cell activation and degranulation is through IgE in its monomeric form. The binding of monomeric IgE to FcεRI in the absence of a specific antigen may lead to increased surface expression of FcεRI, which stabilizes the expression of the receptor on the cell surface and enhances the functional responses of the cells to activation through FcεRI aggregation. In this regard, it has been found that human mast cells, isolated from umbilical cord blood, activated with monomeric IgE, release chemokines (CCL1, CCL3) and growth factors (GM-CSF), but not histamine (28).

However, another study showed, that in similar condition human lung mast cells secreted histamine, leukotrienes (LTC4), and chemoattractants (CXCL8), which is a sign of complete degranulation (29).

In recent years, numerous studies have been conducted on the so-called non-immunological (direct) degranulation. It was found that this process primarily occurs upon engagement of the Mas-related G protein-coupled receptor X2 (MRGPRX2) by various ligands, including vasoactive intestinal polypeptide (VIP); endothelin 1; poisons; signaling molecule substance P (SP); anaphylatoxin complement fractions C3a and C5a; certain medications, etc.

This type of degranulation is direct, rapid with short-term release of small, spherical granules that are unstable and do not transport their cargo to the regional lymph nodes. Thus, direct degranulation is induced quickly and completes in less than five minutes. In this case, the main effector molecules are proteases in the granules. They have direct functions in the tissues - degradation of potentially harmful endogenous proteins, such as vasoactive intestinal polypeptide (VIP) and endothelin 1; and exogenous substances, such as poisons. They can also break down signaling molecules like substance P (SP) (30).

In such cases, rapid release of proteases may be sufficient to limit the effect of such substances and restore tissue homeostasis without causing unnecessary damage. There is no release of inflammatory mediators and no allergic inflammation develops (21,25).

The role of mast cells in the pathogenesis of allergic diseases is well known. They are key cells in the development of skin, food and inhalant allergies, which occur through IgE-mediated signaling. Most often, their biological effects are associated with the development of rapid allergic and anaphylactic reactions.

In anaphylaxis, systemic activation of mast cells is observed. The clinical manifestations

are mediated primarily by tryptase contained in mast cell granules. Alpha-tryptase (α-tryptase) is the most abundant enzyme in mast cells and its increased release is observed in mastocytosis. In IgE-mediated degranulation, β-tryptase is mainly released. Measuring β-tryptase levels is considered the best marker for systemic activation of mast cells in anaphylaxis (31).

Allergic rhinitis can be defined as an IgE-mediated, mast cell-dependent disease. It is characterized by continuous mast cell activation in the nasal mucosa, and the biological effects following mast cell degranulation may explain much of the symptomatology and pathology of allergic rhinitis. Evidence supporting this includes an increased number of mast cells in the epithelium; elevated expression of Th2 cytokines in mast cells; and increased expression of IL-4, which is affected by the application of topical corticosteroids (32).

The atopic phenotype of bronchial asthma results from the interaction between allergens, specific IgE and hyperreactive mast cells.

The early phase of the asthmatic reaction is due to mediators released by human pulmonary mast cells: histamine - during the first 2 minutes of the reaction onset, and later - prostaglandins and leukotrienes. These mediators lead to bronchoconstriction, mucosal edema, and mucus secretion

In the late phase of the asthmatic reaction, the following changes are observed: Increased concentrations of histamine, PGD2, LTC4, but in different proportions compared to the early asthmatic reaction; Decreased levels of tryptase and recruitment and activation of inflammatory cells (33,34).

Accumulated knowledge shows that mast cells have unique and diverse functions; they play a protective and regulatory role, and are also involved in the development of allergic inflammation.

The wide range of potent effector mechanisms of mast cells determines their broad distribution in the organism and their evolutionary conservatism.

The application of therapies targeting their key mediators will continue to be a major part of the treatment of allergic diseases, and the repertoire of such treatments is likely to expand (35).

Since mast cells play a key role in the development of IgE-mediated allergy, the most commonly used diagnostic methods are based on demonstrating their degranulation. Thus, the main in vivo tests such as skin tests and the various provocation tests for demonstrating allergen-specific sensitization are based on the functional determination of mast cell degranulation in different tissues after exposure to a specific allergen.

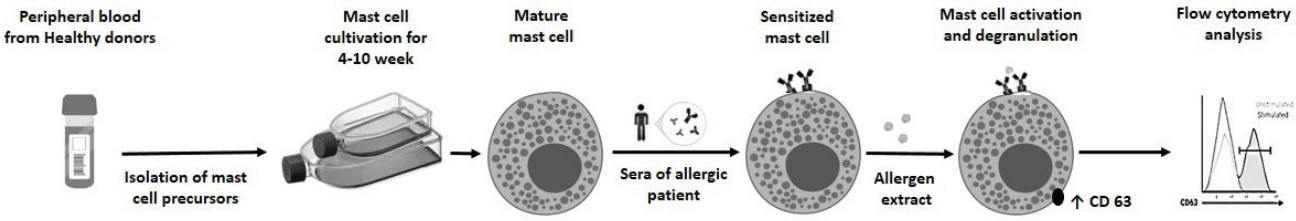
Numerous attempts have been made to develop in vitro methods for detecting mast cell degranulation in response to various allergens. Modern in vitro methods are based on measuring released mediators, such as tryptase (36) or histamine (37) or using the meso scale discovery (MSD) ELISA-based assay platform (38).

Mast cells are usually difficult to detect by flow cytometry and therefore their quantification is not of essential clinical significance. Considering the low mast cell content in peripheral blood, in order to ensure adequate analytical sensitivity, it is recommended to collect a large number of events (~500,000). The significant granularity of mast cells and their autofluorescence can further complicate assay design (39,40).

With the discovery of lysosomal-associated membrane protein (LAMP)-3, or CD63, as a marker of basophil degranulation, a flow cytometric basophil activation test (BAT) was developed in 1991. This test analyzes basophil degranulation in response to specific stimulation with a particular allergen (41).

CD63 is a glycoprotein that, in resting basophils, is located on cytoplasmic granules. During allergen-stimulated basophil degranulation, CD63 increases its expression on the cell membrane and can be determined by flow cytometry as a marker of the immediate-type allergic reaction (42). Currently, BAT is widely used for the diagnosis of IgE-mediated allergy, and various protocols have been developed to enable the identification of basophils and the quantitative assessment of their degranulation (43).

In recent years, several researchers have developed a new alternative in vitro test based on BAT designed to evaluate the direct degranulation of mast cells, known as the Mast cell Activation Test (MAT) (44,45). An important feature of the test is that, since mast cells are primarily tissue-resident cells, the test is performed on a cell culture of human mast cells



(hMCs) - Fig 1.

After passive sensitization with patient's serum, the cells are activated directly with the relevant allergen and their degranulation is measured by flow cytometry. In cases of sensitization, patients demonstrate allergen-specific and dose-dependent degranulation, which is measured by the expression of surface activation markers, such as CD63 (46).

Initial comparisons between BAT and MAT showed that despite the more complicated methodology of MATq, it is much more sensitive. MAT shows markedly higher levels of surface expression of the activation marker CD63 after allergen stimulation, even at concentrations up to 2 log lower than those used in BAT (Table 2).

Moreover, in BAT, 10-15% of non-responders are observed, whose basophils do not respond to IgE-mediated activation, making the test inapplicable for these patients.

The BAT methodology requires the use of fresh blood, ideally processed within 12 hours of its collection. In MAT, patient sera are used, which can be frozen and used repeatedly in the course of routine laboratory testing (47).

One of the greatest advantages of MAT is the possibility of studying non-immune (direct) degranulation, which will lead to a more precise diagnosis of drug allergy.

The disadvantages of MAT include its high cost and the time required to generate a cell line from mature mast cells, which ranges from 4 to 10 weeks depending on the protocol (47).

MAT, being a novel testing tool, is still not routinely used in allergy diagnostics, unlike BAT, whose application in this field spans close to two decades, during which, a lot of practical data have been obtained. For example, in the area of drug allergy testing, BAT can accurately predict negative result values, as well as obtain positive results, even after other testing has produced a negative (48,49). In food allergy diagnostics, specificity, as well as sensitivity are very high, and testing could be carried out by using either crude allergen extracts or single molecules, limiting the potential number of oral provocations these patients could undergo in the course of the diagnostic algorithm (50).

Still, BAT is a supplemental tool in allergy diagnostics, providing further information and an additional

Table 2. Comparison between BAT and MAT.

Characteristics	BAT	MAT
Non-responders (%)	10%–15%	No
Optimal time to perform the test after sample collection	12 h.	Does not depend on time
Possibility of repeatedly examination	No	Yes
Passive sensitization required	No	Yes
Stable response to FcεRI	Yes	Yes
Possibility to test MRGPRX2	No	Yes
Releasing of mediators	++	+++
Donor-dependent variability	Yes	Yes
Standardized protocols	Yes	No
Cost of the test	+	+++
Routine use in clinical practice	Yes	No

layer of security for practitioners when diagnosing allergic disease in patients at high risk for anaphylactic reactions. It is still not recommended to be used as a single diagnostic tool due to various limitations, such as differences in cut-off values and lack of standardized interpretation protocols for certain allergens (51). MAT, in a similar way, could expand the scope of allergy testing but more data is needed on its potential applications and relevant shortcomings in clinical practice.

For now, it is difficult to make a definitive analysis of the superiority or weaknesses of the two methods, since the reproducibility of the tests is still not good, mainly due to the lack of standardization of the protocols according to which BAT and MAT are performed.

One thing is clear - both methods are gaining increasing importance in the diagnosis of IgE-mediated allergy and their use is beginning to be regularly recommended in guidelines and program documents.

However, for these methods to be incorporated into clinical practice as routine diagnostic tools for allergies, further studies are needed regarding their standardization and validation.

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