

Mast Cells and Anaphylaxis

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Abstract For half a century, it has been known that the mast cell is the cell responsible for the majority of anaphylactic events. Its mediators, taken as a whole, are capable of producing all of the clinical manifestations of these events. With the discovery of immunoglobulin E (IgE), it was originally felt that the vast majority of anaphylactic episodes were due to antigen coupling with two cell-bound IgE molecules. More recently it has been learned that many episodes are produced by direct activation of mast cells, not involving antigen binding to IgE, and that monomeric IgE under certain conditions can also cause degranulation. Of note—in regard to antigen independent degranulation—are recent reports that the human G-protein-coupled receptor, MRGPRX2, may be the receptor for many drugs and cationic proteins capable of producing direct mast cell degranulation and anaphylactic events.

Keywords Anaphylaxis · Mast cell · Basophil · Immunoglobulin E · Mast cell secretagogues · MRGPRX2 · Tryptase · Mast cell mediators

Introduction

Today, it is common knowledge that the mast cell and anaphylaxis are intimately related. One can hardly speak of one without the mention of the other. But their relationship was originally far from obvious, and the sequence of scientific discoveries that elucidated the relationship between the two followed a circuitous path. It is interesting to note that in Robert A. Cooke's classic text "Allergy in Theory and Practice", published in 1946, there is no mention of the mast cell although anaphylaxis was a well-recognized entity discussed in detail in this text [1].

Mention of the mast cell came first. It was discovered by Paul Ehrlich in 1877 [2]; however, Ehrlich did not discern the function of the mast cell. Anaphylaxis came later, in 1901, through the work of Charles Richet and Paul Portier who were attempting to induce prophylaxis to the venom of the sea anemone and accidentally caused a phenomenon that they characterized as the opposite ("ana", Greek for "opposite") of the desirable goal, and so coined the term [3]. However, a link between the mast cell and anaphylaxis was not established until 1952 when J. F. Riley and G. B. West found that the mast cell was a major source of histamine [4]. It was just a short step thereafter, nonetheless over 50 years after Richet and Portier's seminal work, to link the mast cell, with its abundance of histamine and other mediators, to anaphylactic events in animal models [5].

In this article, we plan to explore the now well established role of the mast cell in the production of anaphylactic events.

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The Mast Cell

Origin, Maturation and Function of the Mast Cell

Because of the role of the mast cell in the production of allergic events, it is easy to lose sight of its participation in homeostasis and protection. But the mast cell is also a “good” cell and is now considered an immune cell with multiple functions. These activities are involved with homeostasis and tissue repair, angiogenesis, innate immunity, adaptive immunity, and immune tolerance [6]. The contents of mast cells (Table 1) are known to be important biological mediators which are released in response to various threats to survival. They are thus purveyors of “helpful inflammation” as well as healing.

All human mast cells are derived from a common pluripotent hematopoietic progenitor originating in the bone marrow [7•]. This forbearer cell enters the systemic circulation bearing the cell-surface marker, CD34. Thus, even though mature tissue mast cells express different neutral granule proteases, dependent upon their final tissue of residence after leaving the circulation, their circulating progenitor is of a single cell type [7•].

The major growth cell factor for human mast cells is stem cell factor (SCF). SCF is the ligand for CD117, a tyrosine-kinase transmembrane receptor located on the surface of all mast cells and other cells as well. SCF (also known as KIT-ligand because CD117 is encoded by the c-Kit gene) induces dimerization of CD117. This results in enhanced survival, growth, cell migration, and effector functions of mast cells. The binding of stem cell factor induces mast cell progenitors to migrate from the bone marrow to the systemic circulation and hence to the tissues. This process is probably aided and abetted by other mast cell chemo-attractants as well. And in these tissues they assume long-term residence. They settle in areas of the body which face the external environment. Thus, they are found in the highest quantities on mucosal surfaces and in connective tissue. Therefore, the tissues with the highest quantity are the skin, lung, gastrointestinal tract, and uterus. When they enter these tissues, they acquire new phenotypes, a process induced by their local microenvironment [8••]. These micro-environs foster phenotypic changes which effect function, contents, structure and the response to endogenous stimuli and drugs.

In human beings, mast cells exist in two subtypes based on the phenotypic changes occurring in the respective tissue of residence. The subtypes are referred to as mucosal (MC_T) and connective tissue (MC_{TC}) mast cells. This nomenclature is based on the fact that MC_{TC} cells contain tryptase and chymase as well as carboxypeptidase and cathepsin, whereas MC_T cells characteristically contain only one of these, tryptase. MC_T cells in general reside in mucosal tissue whereas MC_{TC} cells settle in connective tissue as well as smooth muscle. Both cell types contain heparin. They both

manufacture interleukins and other cytokines [9–13]. In general, MC_{TC} cells produce high amounts of IL-4 and IL-13. Lower levels are produced by MC_T cells which make higher amounts of IL-5 and IL-6. However, there is a greater heterogeneity than expressed by these classically described phenotypes with some cells; for example, containing carboxypeptidase and chymase but no tryptase [14, 15].

These phenotypes also differ in their shape as well as in their response to endogenous and exogenous stimuli provoking degranulation. Both can be activated by IgE-antigen union whereas only MC_{TC} cells respond to complement split products (C5_a) and substance P, whereas platelet activating factor only stimulates MC_T cells. Opiate receptors are found only on MC_{TC} cells. Each of the two phenotypes also differs in their response to agents designed to prevent degranulation. For example, sodium cromoglycate appears to only prevent degranulation of MC_T cells.

Degranulation of the Mast Cell

The definitive conclusion that IgE and antigen union results in degranulation of mast cells awaited the discovery and purification of IgE in 1966 and the ability to culture mast cells as well as the identification of the structure of the high affinity IgE receptor which occurred in 1989 [16•]. Originally, it was thought that the vast majority of anaphylactic reactions were related to IgE-antigen binding. However, it is now clearly known that there are many other existing pathways that can result in mast cell degranulation and thus produce events clinically identical to those caused by the union of antigen and IgE (Fig. 1). For example, oversulfated chondroitin sulfate contamination of heparin resulted in events very similar to those produced by mast cell degranulation, but appear rather to be related to direct activation of the contact system and/or complement with perhaps no involvement of mast cells whatsoever [17, 18]. However, it should be noted that activation of the mast cell can clearly result in the recruitment and activation of the complement system as well as the contact system, and can induce intravascular coagulation as well. Thus other inflammatory pathways can further escalate the clinical manifestations occurring during anaphylactic events [19–21].

Furthermore, a single drug can produce anaphylactic events through more than one pathway. An example of this is heparin which causes anaphylactic incidents through activation of the contact system as well as via an IgE-mediated pathway [22•, 23•].

Because of the multiple mechanisms underlying mast cell degranulation, knowledge of receptors on the mast cell is of importance. Triggers for mast cell degranulation (Fig. 1) other than IgE-antigen binding include neuropeptides such as substance p, cytokines, many drugs, some contents of insect venom, physical factors such as heat and cold, polycationic molecules such as 48/80, stem cell factor, and other agents. Of

Table 1 Mast cell mediators and their pathophysiologic activities

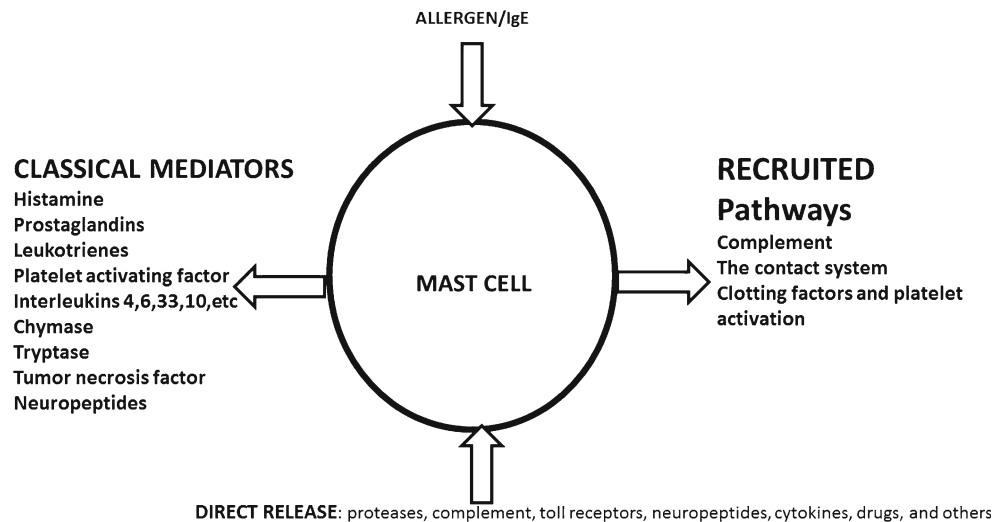
Mediator	Pathophysiologic activities	Clinical manifestations	Comment
Histamine	Contraction of smooth muscle, vasodilatation, increased vascular permeability, mucus secretion, and activation of nociceptive neuronal receptors	Wheezing, flushing, urticaria, pruritus, rhinorrhea, abdominal pain, diarrhea, and angioedema	Works through both H1 and H2 receptors. Although accounting for a large portion of the clinical manifestations, blockade of its activity does not control all manifestations completely
Prostaglandins	Smooth muscle spasm, affects platelet adherence, and vasodilatation	Wheezing, diarrhea, cramping abdominal pain, and hypotension	
Leukotrienes	Smooth muscle contraction, increased vasopermeability	Probable main effect is wheezing with bronchoconstriction, possible hypotension	
Platelet activating factor	Activation of platelets, vasodilatation	May contribute to intravascular coagulation with platelet activation, probable hypotension, and shock	Levels have been directly correlated with severity of events and fatalities, also levels of platelet activating hydrolase, the enzyme responsible for its degradation, have been inversely correlated with severity and fatalities as well
Interleukins	Not well defined	Not well defined	In spite of lack of knowledge of exact effects, levels of some interleukins have been associated with severity of episodes
Chymase	Possibly recruits complement by cleaving C3, may have a beneficial effect by inactivation of neuropeptides, can recruit contact system	Not well defined	Levels have been associated with direct activation of contact system and thus indirectly associated with severity of events
Tryptase	Not well defined	Not well defined	Used as biomarker to diagnose events
Tumor necrosis factor	Not well defined	Not well defined	Levels have been associated with severity of events
Neuropeptides	Not well defined	Not well defined	Can magnify events by further degranulating mast cells
Recruited Pathways	Activation of coagulation pathway has been associated with decreased Factor V, decreased Factor VIII, and decreased fibrinogen. Activation of complement cascade has been demonstrated by decreased levels of complement components 3 and 4 as well as the formation of C3a. Activation of the contact system has been demonstrated by formation of kallikrein C1 inhibitor complexes as well as elevated levels of kinins.	Disseminated intravascular coagulation and hypotension	Levels of activation have been shown to correlate with severity of episodes

particular importance in this regard is the recent discovery of the cell surface mouse receptor, MRGPR2, the orthologue of the human G-protein-coupled receptor MRGPRX2 [24•, 25•]. McNeil, et al. [24•], found that mice connective tissue mast cells possess MRGPRB2 and that this moiety serves as the mast cell receptor for neurokinins such as substance P, basic secretagogues such as 40/80, and a number of peptidergic drugs that have been shown to produce anaphylactic events. These include fluoroquinolones, the bradykinin receptor antagonist icatibant, and several neuromuscular blocking drugs known for their propensity to induce histamine release. Of note is that this mouse receptor was present only on MC_{TC}, connective tissue mast cells. The loss of the receptor in

knockout mice did not alter reactions to IgE-mediated release, but did prevent histamine release to basic secretagogues. Downstream signaling from the receptor appears to involve activation of the phospholipase C-gamma pathway ending in the release of both preformed and granule-stored mediators, and the de novo synthesis of eicosanoids [24•, 25•]. Thus a single mast cell receptor can be responsible for mast cell degranulation due to multiple agents, many of which are drugs known to produce anaphylactic episodes. The clinical implication of course is that blockade of this receptor might be useful in preventing such episodes.

Another mechanism involved in the activation and degranulation of mast cells is perhaps less well-known, but still

Fig. 1 Triggers for mast cell degranulation



intriguing. This mechanism involves activation of the mast cell via monomeric immunoglobulin E-dependent activity. As mentioned previously, classical mast cell degranulation is caused by the union of two dimeric IgE molecules by antigens with multiple epitopes causing the subsequent dimerization of the high affinity IgE receptor [26]. More recently, it has been demonstrated that mast cell mediator release can be initiated by monomeric IgE [27]. This release appears to be enhanced by the presence of SCF. The release also seems to be dependent on free IgE. It is thought that monomeric-induced IgE mediator release also works through aggregation of the high affinity IgE receptor, but the exact mechanism has not been elucidated. Also, the clinical significance of this phenomenon remains unknown, but it is interesting to speculate that, since both monomeric IgE as well as stem cell factor are found in the lung, monomeric release may be important in asthma. This is even of greater interest since monomeric IgE is found in the lungs of “intrinsic” or nonallergic asthmatics as well as the allergic variety [28].

Control and Prevention of Mast Cell Degranulation

It naturally follows that one of the major goals of understanding the factors that result in the pathologic degranulation of mast cells is to assist in developing agents that can prevent the pathologic release of mediators.

The best understood mechanism accounting for mast cell degranulation is that which participates in IgE-antigen-dependent activation and thus efforts have been concentrated on inhibiting different steps of this process. As mentioned, the union of two dimeric molecules of IgE affixed to the high affinity receptor results in the dimerization of this receptor. Downstream signaling activates a series of tyrosine kinases beginning with Lyn which phosphorylates tyrosine residues in the intracellular portion of the IgE receptor molecule. Shortly thereafter, a second protein tyrosine kinase, Syk, is

recruited and further phosphorylates this chain. This begins a series of activities that produce activation of inositol triphosphate (IP₃). The production of IP₃ results in intracellular calcium mobilization and the influx of extracellular calcium into the cell. Subsequently granules are extruded from the cell, and the de novo synthesis of mediators such as leukotriene occurs. Theoretically interruption at any point of this cascade could prevent the pathophysiologic degranulation of these cells.

Attempts have been made to utilize spleen tyrosine-kinase (Syk) inhibitors in the treatment of rhinitis [29] and other tyrosine kinase inhibitors such as imatinib, dasatinib, and midostaurin in the treatment of mastocytosis and mast cell activation disorders [30••].

Probably the first agent employed to prevent mast cell degranulation was cromolyn sodium. It was the first “mast cell stabilizer.” It was followed shortly thereafter by nedocromil. These drugs appeared to stabilize mucosal (MC_T) but not connective tissue (MC_{TC}) cells. The action of cromolyn and nedocromil appears to be mediated through a G-protein-coupled receptor (G-protein-coupled receptor 35) [31]. Both drugs have fallen out of favor because of the need for frequent administration and the perception of lack of potency. However, the oral form of cromolyn is still used to treat mast cell activating syndromes in certain circumstances.

Conceptually, treatment to prevent degranulation might be successful by stimulating mast cell receptors that contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The binding of these receptors to their ligand recruits phosphatases that are capable of suppressing cell activation by dephosphorylating signaling molecules. Examples of such receptors are CD72, Siglec-8, and CD300a [32].

Ketotifen, a drug which has both antihistamine and mast cell stabilizing properties, has also been used to treat mast cell activating disorders, specifically in patients who have experienced episodes of idiopathic anaphylaxis [33].

Application of our Knowledge of the Mast Cell to the Patient Experiencing Anaphylactic Episodes

The realization that abnormalities of mast cells can be responsible for anaphylaxis [34••], in particular for episodes previously thought of as idiopathic, has profoundly altered our approach to patients. Specifically, it has lowered our threshold for the performance of bone marrow biopsies in patients without a known cause of their episodes and intensified a search for mutations in Kit. The seminal article establishing a relationship between abnormal mast cells and idiopathic anaphylaxis was published in *Blood* in 2007 [35].

In this article, patients with a diagnosis of idiopathic anaphylaxis were evaluated. From a cohort of 72 consecutive patients, the authors reported 12 patients with recurrent anaphylaxis who on bone marrow biopsy showed some findings suggesting mastocytosis. But the biopsies did not meet the criteria established by the World Health Organization cited as necessary to establish a diagnosis of this disorder. However, some of the patients did demonstrate one or more minor criteria for mastocytosis, such as exhibiting the 816D>V (D816V) c-KIT mast cell activating mutation. Only 2 of the 12 patients had baseline serum tryptase concentrations over 20 ng/mL and the traditional cutoff value of 20 ng/mL used to establish an elevated level of serum tryptase may be too high. Since that publication, a number of other studies have confirmed the presence of D816V c-KIT mutations in patients with idiopathic anaphylaxis and even in patients where an allergen was identified. These investigations have fostered a change in the classification of anaphylactic events [36].

This proposed classification suggests that disorders of mast cells, some of which predispose to anaphylaxis, be classified into three distinct categories:

1. Mastocytosis and mast cell activating syndromes (MCAS).
2. IgE-mediated anaphylactic events.
3. Idiopathic anaphylactic episodes.

Mast cell activating syndrome (MCAS) resembles mastocytosis and can cause anaphylaxis, but individuals with this disorder lack sufficient bone marrow findings to make a diagnosis of mastocytosis according to the criteria established by the World Health Organization [37]. Such patients have some of the bone marrow findings seen in mastocytosis and also can have gain in function mutations in KIT.

The importance of the establishment that mastocytosis and MCAS can be the cause of idiopathic anaphylaxis lies in the fact that mast cell activating disorders can, on occasion, be controlled with tyrosine-kinase inhibitors. Baseline elevations in serum tryptase, plasma histamine, 24-hour urinary histamine metabolites, or prostaglandin D₂ suggest these

conditions. Mastocytosis and MCAS can be present in patients with lower levels of serum tryptase. A study of patients who had hymenoptera anaphylaxis found a level of 11.7 ng/mL was a marker for underlying mastocytosis [38].

A screening test performed on peripheral blood to detect the 816 V mutation found in the majority of cases of mastocytosis can now be performed [39]. Using an analysis with high sensitivity has been suggested especially in patients with serum tryptase <20 ng/mL. Still the most definitive way to make a diagnosis of mastocytosis and MCAS is to do a bone marrow biopsy. Thus one is faced with the decision as to whether or not to do a bone marrow biopsy in patients in whom no cause for anaphylaxis has been determined. When to do so remains a cause of debate but suggested algorithms for the management of such patients are available in the literature. However, there is growing importance regarding making such a diagnosis since as noted some mast cell activating syndromes and some cases of mastocytosis which are 816 V-negative can be treated with available tyrosine-kinase inhibitors [40].

Another clinically significant result of our basic research into mast cell biology is the application of the measurement of mast cell contents to assist in the diagnosis of anaphylaxis and its causes with specific reference to mastocytosis. Of particular importance in this regard is the measurement of serum tryptase. Tryptase is of particular importance because levels do not peak until approximately 60 to 90 min after the onset of symptoms in most patients, and occasionally levels can remain elevated for 5 h and sometimes even longer in protracted cases [41, 42]. Thus tryptase is more useful than plasma histamine which begins to rise within 5 to 10 min after symptoms, but it remains elevated for only 30 to 60 min. Nonetheless, urinary histamine metabolites can also be useful and are elevated for a longer period after the onset of symptoms.

It should be noted that disparities between histamine and tryptase levels can occur. In an emergency department evaluation of patients presenting with acute allergic reactions, plasma histamine concentration was elevated in 42 of 97 adult patients, but serum tryptase was elevated in only 20. Histamine levels correlated better with clinical signs than tryptase. Patients with elevated plasma histamine levels were more likely to have urticaria, more extensive erythema, wheezing, and abnormal abdominal findings [43]. Thus, both mediators can be of use in establishing a diagnosis of anaphylaxis. More recently, measurement of urinary prostaglandin D₂ levels have been shown to correlate with anaphylactic events, and occasionally do so when there is no elevation of serum tryptase [44, 45]. All three of these mediators can be measured commercially.

Serum tryptase determinations are also useful in another context. Elevations of baseline serum tryptase levels are a marker for mastocytosis. Recently, the upper limits of normal

for baseline serum tryptase have been revised from a level of 20 ng/mL to a level of 11.4 ng/mL based on studies of anaphylactic reactions to hymenoptera stings [46]. Analyzing reactions to stings, it was demonstrated that a serum tryptase level of greater than 11.4 was a significant risk factor for anaphylaxis. In evaluating patients who had stings with levels above 11.4 it was shown that such patients were likely to have systemic mastocytosis.

Conclusions

The vast majority of anaphylactic events, but not all, are due to systemic mast cell degranulation. This degranulation process is in many instances IgE-mediated, but in a significant number of episodes direct mast cell degranulation independent of IgE occurs. Of note in this regard are recent reports that the human G-protein-coupled receptor, MRGPRX2, may be the receptor for many drugs and cationic proteins capable of producing direct mast cell degranulation and anaphylactic events.

The basic knowledge of the events involved in mast cell degranulation has permitted us to understand mast cell pathology that results in a predisposition for anaphylactic events in disorders such as mastocytosis and MCAS. There have been advances in controlling these conditions using tyrosine-kinase inhibitors which have improved the outcomes in these disorders.

Compliance with Ethical Standards

Conflict of Interest Dr. Lieberman reports that he is a speaker for Mylan and Teva. Dr. Garvey declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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