

CHAPTER 1

MAST CELL BIOLOGY: Introduction and Overview

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Abstract: In recent years, the field of mast cell biology has expanded well beyond the boundaries of atopic disorders and anaphylaxis, on which it has been historically focused. The biochemical and signaling events responsible for the development and regulation of mast cells has been increasingly studied, aided in large part by novel breakthroughs in laboratory techniques used to study these cells. The result of these studies has been a more comprehensive definition of mast cells that includes added insights to their overall biology as well as the various disease states that can now be traced to defects in mast cells. This introductory chapter outlines and highlights the various topics of mast cell biology that will be discussed in further detail in subsequent chapters.

INTRODUCTION

Mast cells are cells of hematopoietic origin which have gained notoriety over the years for their role as central players in atopic disorders and anaphylaxis. Indeed, it has been in this context that much of the research in this field has been conducted. It is only recently that their role in other aspects of health and disease has been fully appreciated. The manifestations of mast cell-driven disease are considered to be a consequence of an inappropriate activation of mast cell immune responses which have evolved to protect the body against a host of pathogens and perhaps toxins. The biochemical processes regulating mast cell development and mast cell activation have been extensively investigated and comprehensively reviewed in recent years. Hence, rather than reviewing these topics at length, in this work we have opted to focus on the emerging concepts in mast cell biology

Mast Cell Biology: Contemporary and Emerging Topics, edited by Alasdair M. Gilfillan and Dean D. Metcalfe. ©2011 Landes Bioscience and Springer Science+Business Media.

with regards not only to mast cell development and activation, but also on the newly defined roles of mast cells in health and disease. To accomplish this goal, we have solicited contributions from recognized experts in the field of mast cell biology who are focusing on these topics. The scope of this effort cannot be all encompassing and accordingly, not all recent contributions to the field of mast cell biology can be covered. The lack of citation of specific studies thus does not imply that they are of lesser merit or impact.

To set the stage for the more in depth discussions that follow, we begin by presenting a brief overview of mast cell biology in general, in which we indicate those topics that will be elaborated upon in subsequent chapters.

MAST CELL GROWTH, DEVELOPMENT AND SURVIVAL

Mast cells, at least in the human, develop from CD34⁺/CD117⁺ pluripotent progenitor cells originating in the bone marrow.¹ The progression of these cells to fully mature mast cells is dependent on KIT activation which occurs as a consequence of stem cell factor (SCF)-induced KIT dimerization and auto-phosphorylation. Hence, *Kit*^{W/W^{-v} and *Kit*^{W-sh/W-sh} mice in which surface expression of KIT, or KIT catalytic activity, is defective, have substantially reduced mast cell numbers.² Nevertheless, whereas human mast cells in culture require SCF for growth, mouse mast cell growth and expansion from bone marrow progenitors can be maintained by IL-3 in the absence of SCF.¹}

In both the mouse and human, committed bone marrow mast cell progenitors are released into the bloodstream from where they subsequently migrate into the peripheral tissues, during which time they mature and become terminally differentiated under the influence of cytokines within the surrounding milieu.³ As discussed by Jenny Hallgren and Michael F Gurish in Chapter 2,⁴ the migration of mast cell progenitors appears to be controlled in a tissue specific manner. They describe, for example, that basal trafficking of mast cell progenitors into the intestine, a process regulated by dendritic cells expressing the transcriptional regulatory protein, T-bet, requires that $\alpha 4\beta 7$ integrin and the chemokine receptor, CXCR2, be expressed on the surface of the mast cell progenitors and that MAdCAM-1 and VCAM-1 be expressed on the intestinal endothelium. In contrast, the marked recruitment of committed mast cell progenitors to the lung, observed with the onset of pulmonary allergic inflammation, requires the expression of the $\alpha 4\beta 7$ and $\alpha 4\beta 1$ integrins on the mast cell progenitors and associated expression of VCAM-1 on the endothelium, as regulated by CXCR2. Full recruitment however requires activation of CCR2 pathways following binding of CCL2. Such migration of mast cell progenitors from the vasculature into the peripheral tissues is increased under inflammatory conditions, thus providing an explanation for the increased mast cell burden observed at sites of inflammation.⁴

Terminally differentiated, tissue-resident mast cells are long lived, a feature, at least in the human, that is dependent upon the continued presence of SCF. Accordingly, inhibition of KIT catalytic activity by tyrosine kinase inhibitors induces human mast cell apoptosis.⁵ As discussed by Maria Ekoff and Gunnar Nilsson in Chapter 4,⁶ the Bcl-2 family of proteins are key regulators of such mast cell homeostasis through balancing mast cell survival and apoptosis. They further explain that apoptosis is regulated by both extrinsic pathways and intrinsic pathways which respond to stress from SCF deprivation, DNA damage and other intracellular stimuli and that these pathways involve caspase activation. Extrinsic apoptotic signals are transmitted through death receptors belonging

to the TNF receptor family, such as Fas/CD95R and TRAIL, which have been identified on both murine and human mast cells. However, whereas Fas/CD95R activation has been found to induce apoptosis in murine mast cells, in human mast cells, apoptosis follows TRAIL-R crosslinking.⁶

Ekoff and Nilsson⁶ moreover discuss how SCF regulates mast cell survival through inactivation of FOXO3a and subsequent down-regulation of the pro-apoptotic BH3-only protein, Bim, by its ubiquitination and proteasomal degradation following its phosphorylation. They additionally note that studies using Bcl-2 family gene-deficient mice and cells have revealed that the BH3-only protein, Puma, is also critical for the induction of mast cell apoptosis following cytokine deprivation. Under such cytokine deprived-conditions, in wild type cells, both Bim and Puma are upregulated with resulting mast cell apoptosis. The role of the downstream effector proteins Bax and Bak is demonstrated by the fact that their absence abolishes most apoptotic responses induced by BH3-only proteins and by the increased mast cell numbers found in tissues of mice lacking Bax or Bak.⁶

MAST CELL MEDIATORS AND DISEASE

The ability of mast cells to function in both health and disease is dependent on not only the relative abundance of mast cells in tissues, but also on the extent and nature of mediators released. These latter features are respectively dictated by the threshold and magnitude of mast cell activation and by the selective activation of specific signaling pathways controlling the release of individual classes of inflammatory mediators.

Mast cells have the capacity to release a multitude of pro-inflammatory mediators.³ The immediate response upon mast cell activation to an appropriate stimulus is degranulation; a process characterized by the extrusion of cytoplasmic granule contents into the extracellular space by the process of exocytosis. Although the membrane-proximal signaling processes that allow mast cells to degranulate have been extensively studied and described,^{7,8} it is only recently that the detailed mechanics of degranulation and the molecular players regulating this process have been investigated. In Chapter 7, Ulrich Blank⁹ discusses recent work on defining the roles of specific proteins which participate in the regulation of membrane fusion during exocytosis and the connection of the fusion machinery with early signaling events initiated upon mast cell activation.

Mast cell granules are rich in bioactive amines, proteoglycans and proteases.³ Clinically, the most important bioactive amine present in the granules is histamine. The role of histamine in human disease is well established. For example, histamine, along with PGD₂ and LTC₄, induces bronchoconstriction, mucus secretion and mucosal edema and thus contributes to the symptoms of asthma.^{10,11} As described by Elena Moiseeva and Peter Bradding in Chapter 13,¹² the relative rate of mediator release from human lung mast cells *in vitro* is histamine > PGD₂ > LTC₄ with one-half maximal release occurring at 2, 5 and 10 minutes respectively and this appears to parallel *in vivo* release in the human lung. Serotonin (5-hydroxytryptamine) is also relatively abundant in mouse mast cell granules, but it only represents a minor component of the human mast cell granules¹³ and to date has not been shown to be a relevant mediator in human mast cell-driven disease.

Mast cell granules are particularly rich in proteases. These proteases are the major proteins present in mast cells; representing up to 50% of the total protein content of the granule.¹⁴ The major protease present in human mast cells is β -tryptase.¹⁵ Chymase is also

present in substantial amounts in particular sub-populations of mast cells and the relative abundance of tryptase and chymase in human mast cells has been employed to define particular mast cell phenotypes.¹⁶ These granule-associated proteases are complexed to proteoglycans. One example is heparin which helps to stabilize the proteases and influence function.¹⁴ As discussed by George Caughey in Chapter 12,¹⁷ mast cell proteases have both detrimental and beneficial actions. In this respect, although proteases have been implicated in inflammatory process including those associated with allergic inflammation, they may be protective and even anti-inflammatory in some settings.

Activation of mast cells results in the rapid generation of lipid-derived inflammatory mediators.¹⁸ Both membrane-associated phospholipids and sphingolipids provide substrate sources for lipid derived pro-inflammatory mediators. Through the action of cytosolic phospholipase (cPL)A₂, membrane phospholipids, particularly arachidonyl-containing phosphatidylcholine and, to a lesser extent, phosphatidylethanolamine, are hydrolyzed at the sn₂ position to yield free arachidonic acid which can be subsequently metabolized by the respective actions of cyclo-oxygenase and 5-lipoxygenase to produce the eicosanoids, prostaglandin (PG)D₂ and leukotriene (LT)C₄.¹⁹ PLA₂ activation also induces deacylation of the membrane-associated phospholipid, alkylacylglycerophosphorylcholine to form lyso-platelet activating factor (lyso-PAF) which is subsequently reacylated by the action of acetylCoA:lyso-PAF acetyltransferase to produce PAF which has been implicated in systemic anaphylaxis.²⁰ As discussed by Ana Olivera and Juan Rivera in Chapter 8,²¹ the sphingolipid-derived mediator sphingosine 1-phosphate, generated by mast cells following activation, is capable of inducing biological responses in mast cells and surrounding tissues. It, thus, may play a role in the regulation of diverse biological systems, including the immune system. Such responses can be mediated by both receptor-mediated and receptor-independent pathways.²²

Following these initial events, which happen within minutes of mast cell activation, there occurs an enhancement of gene expression leading to the generation of an array of cytokines including GM-CSF, TNF α , IL-3, IL-4, IL-5, IL-6, IL-10 and IL-13, chemokines including CCL2, CCL3, CCL5 and CXCL8 and growth factors such as SCF, FGF, VEGF and angiogenin.²³⁻²⁹ As discussed by Christopher Shelburne and Soman Abraham in Chapter 10,³⁰ by Mindy Tsai, Michele Grimbaldston, and Stephen Galli in Chapter 11,² by Elena Moiseeva and Peter Bradding in Chapter 13,¹² and by Domenico Ribatti and Enrico Crivellato in Chapter 14,³¹ these mediators can have a profound influence on surrounding tissues and can result in a wide array of cellular responses, including induction of immune and inflammatory responses, cellular hyperplasia, angiogenesis and tumorigenesis.

MAST CELL ACTIVATION

Mast cells express a multiplicity of cell surface receptors which have the capacity to impact mast cell responses through the regulation of proliferation, migration and activation. However, the two major receptors responsible for regulating mast cell functions are KIT and the high affinity receptor for IgE, Fc ϵ RI.^{32,33}

Allergen-induced mast cell activation occurs as a consequence of Fc ϵ RI aggregation through antigen-dependent cross-linking of antigen-specific IgE molecules bound to the Fc ϵ RI.³³ However, as described by Jun-ichi Kashiwakura, Iris M. Otani and Toshiaki Kawakami in Chapter 3,³⁴ recent studies have provided evidence that occupancy of Fc ϵ RI with IgE, in the absence of antigen, will provoke mast cell responses. These responses,

which are attributed to “monomeric” IgE, include induction of chemokine production, mast cell chemotaxis and the prevention of apoptosis.³⁵ Regardless, it is clear that FcεRI aggregation is required to elicit the necessary signaling events for the full extent and range of antigen-induced inflammatory mediator release.³⁶

There is an increasing appreciation that such mast cell activation can be up-regulated or down-regulated through other receptors expressed on mast cells and that many of these receptors can by themselves activate mast cells.^{37,38} However, the role of these receptors in regulating mast cell function in a physiological setting remains unclear. As described elsewhere in this overview, KIT is an essential growth and anti-apoptotic factor for the generation of mast cells from progenitors and for the subsequent survival of mature, tissue-resident mast cells. SCF-dependent KIT activation, however, also enhances antigen-mediated mast cell degranulation and cytokine production.^{39,40} Similarly, signaling events initiated by KIT and FcεRI synergistically interact to markedly enhance mast cell chemotaxis.⁴¹ These responses are also observed with a number of GPCR agonists including PGE₂ and adenosine.⁴¹

The demonstration that a wide array of bacterial products, viral products, parasite products, toxins, Toll-Like Receptor (TLR) agonists and host defense products also induce activation of mast cells has led to the realization that mast cells are an important component of innate immunity as outlined by Christopher Shelburne and Soman Abraham in Chapter 10.³⁰ The consensus of data, however, suggests that unlike antigen-, KIT- and GPCR-mediated responses, those elicited through TLRs interacting with viral, bacterial and parasitic products, are likely restricted to induction of cytokine and chemokine production. As with KIT and GPCRs, such responses are synergistically enhanced when mast cells are co-activated through the TLRs and FcεRI.⁴² This differential regulation of specific categories of mediators by alternative classes of mediators directly reflects their selective utilization of specific adaptor molecules for relaying signals. In this respect, unlike the transduction pathways induced by FcεRI, GPCRs and KIT, signals relayed by the major adaptor molecule for the TLRs, MyD88, do not lead to enhanced intracellular calcium levels, a necessary signal for mast cell degranulation.⁴² It is of interest that another MyD88-linked receptor, the IL-33 receptor, enhances cytokine production in mouse and human mast cells,⁴³ while having minimal effect on degranulation.⁴⁴

MAST CELL SIGNALING

Identification and characterization of the molecular components of the signaling pathways that regulate mast cell responses has been an active area of research over the past two decades and much is now known regarding these processes. These events have been extensively reviewed in a series of articles to which the readers are referred to for further details.^{7,8,33,37,45} Here we only present a brief overview of this topic, primarily focusing on FcεRI.

Signaling initiated by FcεRI aggregation leads to the intracellular assembly of a membrane-associated receptor-signaling complex. FcεRI comprises an IgE-binding α subunit, a tetramembrane-spanning β subunit and homodimeric disulphide-linked γ chain tandem subunit.³³ Both the β and γ chains contribute to the transfer of signals across the membrane. However, whereas the γ chain is essential for transducing signals required for mast cell activation, the role of the β chain appears to be to amplify the signals induced by the γ chains.³³

Although it is clear that the subsequent receptor-proximal signaling events involve a series of protein phosphorylation events and protein-protein interactions,⁷ the precise locations where interactions take place in the context of the three dimensional structure of the cell and the real time kinetics of these processes, are relatively unknown. However, as described by Bridget Wilson, Janet Oliver and Diane Lidke in Chapter 6,⁴⁶ a concept that has emerged during the past decade centers on the localization of early signal transduction events to specialized membrane microdomains. As described, electron microscopy and other approaches have revealed that, following aggregation, FcεRI and specific signaling molecules localize to discrete membrane “patches” which may represent morphological evidence of lipid rafts. These rafts, otherwise termed glycolipid-enriched domains, have been described based on their physical properties and chemical composition.⁴⁷ However, these studies also reveal that these regions are not uniform and that not all signaling molecules localize to the same domains.⁴⁸ It thus remains to be determined how the signaling molecules restricted to the discretely separate membrane domains interact. Nevertheless, biochemical approaches have provided evidence that, following antigen challenge, aggregated FcεRI coalesce with the putative lipid rafts, where they interact with key signaling molecules to initiate the cascades required for downstream mediator release.^{49,50}

The initial key receptor-signaling protein interactions required for mediator release follow phosphorylation of immunoglobulin receptor activation motifs (ITAMs) contained within the β and γ chain cytosolic domains as mediated by the Src family tyrosine kinase Lyn; and subsequent recruitment, phosphorylation and activation of Syk tyrosine kinase. There still remains an enigma, however, regarding the role of Lyn in these initial steps. Although it is assumed that Lyn is responsible for the initial phosphorylation of the FcεRI β and γ chain ITAMs and thus would be essential for downstream mediator release, data from *lyn*^{-/-} mice and mast cells derived from the bone marrow of these mice, suggest that Lyn is dispensable for mast cell activation. Indeed, depending on the genetic background of the mice, hyperactivation of mast cells following antigen challenge has been observed in the absence of Lyn.⁵¹ This may be a reflection of redundancy in the roles of individual Src kinases in the initial stages of mast cell activation, as other Src kinases including Fyn,⁵² Fgr⁵³ and Hck⁵⁴ have been documented to also contribute to mast cell activation.

Following these initial signaling events, a bifurcation in the pathways takes place allowing the activation of two major signaling enzymes; PLCγ and phosphoinositide 3-kinase (PI3K). However, intercommunication between these pathways likely occurs. These events are coordinated by specific protein-protein interactions and subsequent assembly of a macromolecular signaling complex through particular binding motifs contained within transmembrane and cytosolic adaptor molecules. PLCγ is recruited into the signaling complex through its direct binding to the transmembrane adaptor molecule LAT following its phosphorylation by Syk; an interaction stabilized through secondary indirect binding via the cytosolic adaptor molecules Gads and SLP76,⁵⁵ whereas PI3K is recruited to the receptor-signaling complex via the Fyn- and/or Syk-dependent phosphorylation of Gab2.^{52,56,57} There is also evidence to suggest that PLCγ₁ additionally binds indirectly to the LAT-related transmembrane adaptor LAT2/NTAL/LAB.⁵⁸ KIT also utilizes PLCγ for downstream signaling. However, in contrast to the FcεRI, KIT contains a recognized PLCγ-binding motif in its cytosolic domain. As a consequence, following KIT ligation and phosphorylation, KIT directly binds and activates PLCγ₁.⁵⁹ Although the GPCRs that influence mast cell mediator release do not activate PLCγ they do activate the

functionally related PLC β through GPCR $\beta\gamma$ subunits. TLRs, however, activate neither PLC γ nor PLC β , thus explaining their lack of effect on mast cell degranulation.

Through the hydrolysis of phosphoinositide 4,5, bisphosphate (PIP₂) and the consequential production of inositol trisphosphate (IP₃) and diacylglycerol, PLC activation leads respectively to an increase in cytosolic calcium levels and activation of protein kinase C (PKC).⁶⁰ IP₃ induces elevated cytosolic calcium concentrations by receptor-mediated liberation of calcium from the endoplasmic reticulum (ER).⁶¹ The emptying of the ER stores of calcium in this manner triggers a secondary, more pronounced, calcium signal through store operated calcium entry (SOCE) from extracellular sources. As described by Hong-Tao Ma and Michael Beaven in Chapter 5,⁶² recent studies have begun to identify the molecular players and interactions that regulate this latter process. In this respect, the sensor that detects the emptying of calcium from the ER has been identified as stromal interacting molecule-1 (STIM1)^{63,64} and the corresponding calcium channel on the cell membrane allowing SOCE as ORAI.^{65,66} Other calcium channels, termed transient receptor potential canonical (TRPC) channels, also likely contribute to SOCE. However, the precise manner in which TRPC channels interact with ORAI and STIM has yet to be determined. The calcium signal is eventually terminated upon re-uptake of calcium and replenishment of the calcium stores within the ER via an ATP-dependent sarco/ER Ca²⁺ ATPase (SERCA) pump; and removal of excess cytosolic calcium across the cell membrane by TRPMV4-mediated depolarization of the cell membrane, through Na⁺/Ca²⁺ exchange, or through ATP-dependent plasma membrane Ca²⁺ ATPase (PMCA) pump.⁶⁷

PI3K phosphorylates PIP₂ at the 3' position, thereby generating phosphoinositide 3,4,5 trisphosphate (PIP₃).⁶⁸ This provides membrane docking sites for PH domain-containing signaling proteins, for example, PLC γ , Btk, PDK1 and AKT.⁶⁸ PI3K is a family of heterodimeric complexes comprising a catalytic and an adaptor subunit. Both KIT and the Fc ϵ RI signal through the PI3K δ family member,⁶⁹ whereas GPCRs signal through the PI3K γ family member.⁷⁰ PI3K is indispensable for KIT mediated mast cell responses and for Fc ϵ RI-mediated cytokine production. This likely reflects the control of multiple downstream signaling cascades by PI3K, including those regulated by the MAP kinases, ERK1/2, p36 and JNK, mTORC1 and mTORC2, and GSK3 β , which contribute to the regulation of gene transcription and or chemotactic responses.⁷⁰ PI3K also contributes to mast cell degranulation and the enhancement of this response by KIT. However, a fraction of Fc ϵ RI-mediated degranulation is refractory to the influence of PI3K.⁷¹ This has led to the suggestion that PI3K contributes to degranulation through the recruitment of Btk and subsequent amplification/maintenance of PLC γ -mediated calcium signals required for degranulation.^{72,37}

The status of mast cell activation at any point in time is in reality a balance between the signaling pathways discussed above and those that down-regulate these processes. As discussed by Laila Karra and Francesca Levi-Schaffer in Chapter 9,⁷³ this fine balance is essential to avoid inappropriate or exaggerated mast cell-mediated responses. A variety of receptors are expressed on mast cells that possess the capacity to downregulate and thus buffer, receptor-induced mast cell activation.⁷⁴ These receptors are characterized by a conserved immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytosolic domains which, when phosphorylated, recruits the protein phosphatase SHP-1 and the lipid phosphatases SHIP1 and SHIP2. Whereas SHP-1 reverses protein-protein interactions mediated by tyrosine kinases, SHIP1 and 2 dephosphorylate PIP₃, thus reversing the association of essential PH domain-containing signaling proteins with the membrane-associated signaling complex.⁷⁴ As discussed,⁷³ disruption of the balance

between these inhibitory pathways and those that lead to up-regulation of mast cell responses, could result in inappropriate activation of mast cells leading to disease conditions.

CONCLUSION

The understanding of mast cell biology has dramatically increased over the past two decades, largely due to three major developments. The first of these was the discovery of growth factors in both mouse and man that allowed the culture of mast cells from marrow and peripheral blood. The second major breakthrough was the realization that mast cells could be cultured from the marrow of mice with either known genetic defects or mice where specific gene expression was directly manipulated. A corollary was the development of approaches where mast cells could be used to replete the mast cell compartment in mice deficient in mast cells. This latter approach allowed the dissection of the role of mast cells in disease states, at least in the mouse. Finally, the application of genomics and proteomics applied to the study of human mast cells has allowed the association of mast cell defects to human disease states. One major example of this was the association of mutations in KIT with human mastocytosis.

The work summarized in this chapter, which reflects the contributions of the individual chapter authors, well illustrates how mast cell biology has advanced and how these discoveries have facilitated the recognition and acceptance of mast cells as critical to both innate and acquired human immune responses.

ACKNOWLEDGEMENT

Research in the authors' laboratory is supported by the Intramural Research Program of NIAID, National Institutes of Health.

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