

The mast cell and allergic diseases: role in pathogenesis and implications for therapy

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Summary

Mast cells have long been recognized for their role in the genesis of allergic inflammation; and more recently for their participation in innate and acquired immune responses. Mast cells reside within tissues including the skin and mucosal membranes, which interface with the external environment; as well as being found within vascularized tissues next to nerves, blood vessels and glandular structures. Mast cells have the capability of reacting both within minutes and over hours to specific stimuli, with local and systemic effects. Mast cells express the high affinity IgE receptor (FcεRI) and upon aggregation of FcεRI by allergen-specific IgE, mast cells release and generate biologically active preformed and newly synthesized mediators which are involved in many aspects of allergic inflammation. While mast cells have been well documented to be essential for acute allergic reactions, more recently the importance of mast cells in reacting through pattern recognition receptors in innate immune responses has become recognized. Moreover, as our molecular understanding of the mast cell has evolved, novel targets for modulation have been identified with promising therapeutic potential.

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Introduction

Mast cells arise from pluripotential stem cells, mature in tissue, and have the ability to generate inflammation following exposure to a variety of receptor-mediated signals initiated by both innate and acquired immune response mechanisms. Mast cells are easily identified by the presence of prominent granules within their cytoplasm. These mast cells are heterogeneous in morphology and staining characteristics (Fig. 1). Tissue mast cells can be activated in wound healing, fibrosis, cardiovascular disease and autoimmunity in addition to allergic inflammation.

Mast cell development and differentiation

Human mast cells arise from CD34⁺ pluripotent stem cells in the bone marrow, circulate in the blood as precursors, then home to tissues where they mature under the influence of stem cell factor (SCF) and local cytokines and other factors. SCF is produced mainly, but not exclusively, by stromal cells. SCF is released as a soluble mediator, but is also expressed on the cell surface of stromal cells [1]. Kit (CD117), expressed on haematopoietic stem cells and progenitor cells, is the tyrosine kinase

transmembrane receptor for SCF that is involved in differentiation of both myeloid and lymphoid lineages. While Kit is down-regulated on other bone marrow-derived cells during their differentiation, Kit remains highly expressed on mast cells and is critical for many mast cell functions such as survival, differentiation, chemotaxis, and enhancement of signaling events during mast cell activation [2, 3]. The importance of Kit is demonstrated by the mast cell deficiency observed in mouse strains with Kit mutations and deletions (*W/W^y* and *B6.Cg-Kit^{W-sh}*). Further, the removal of SCF results in mast cell apoptosis [4–6]. In addition to SCF, mast cell growth and differentiation are influenced by several other cytokines, including IL-3, IL-4, IL-6, IL-9, IL-10 and nerve growth factor (NGF), and in the gastrointestinal mucosa, PGE₂. Mast cells are long lived and are reported to proliferate in association with IgE-dependent activation and in the presence of IL-4 [7]. A secondary increase in mast cell numbers is associated with many inflammatory diseases including rheumatoid arthritis, scleroderma, with certain infectious diseases and in association with clonal disorders and chronic disease states such as lymphoma, leukaemia, osteoporosis, chronic liver disease and chronic renal disease.

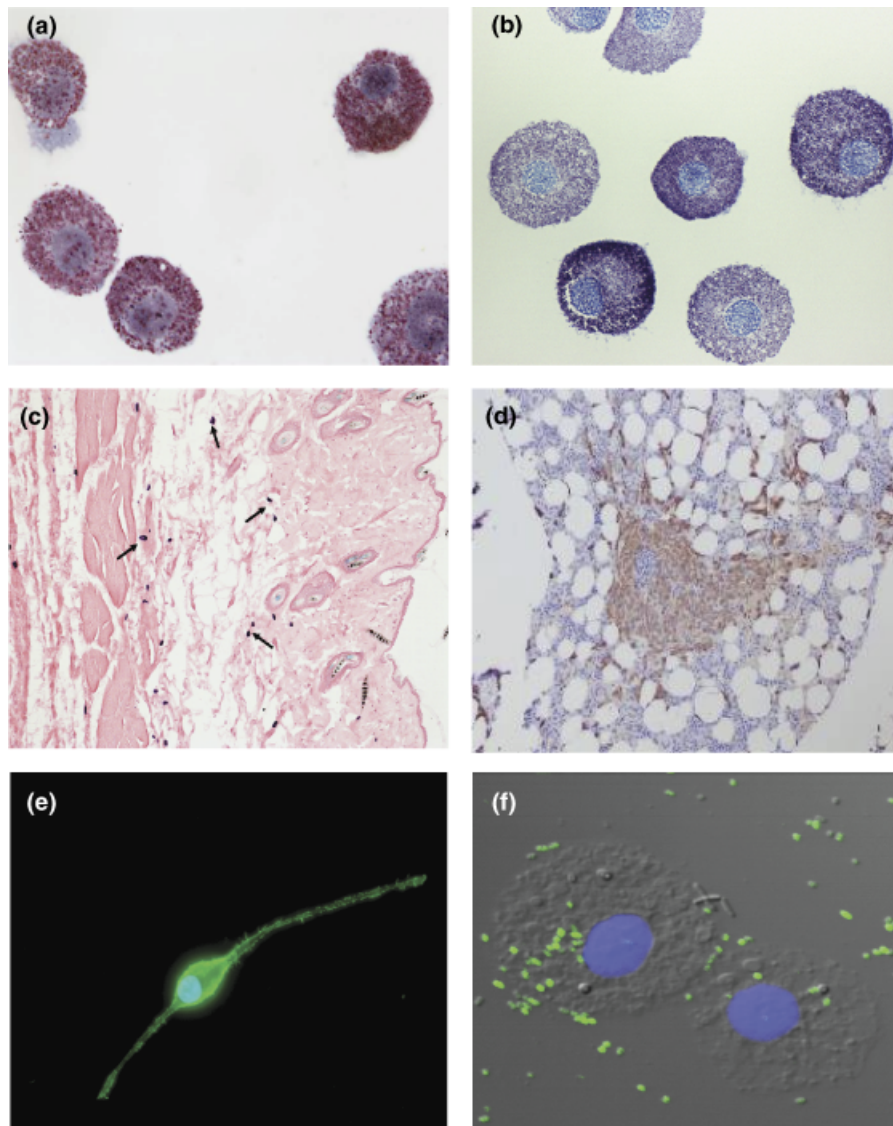


Fig. 1. Images of mast cells. (a) Murine bone marrow-derived mast cells stained with Wright-Geimsa. (b) Human CD34⁺-derived mast cells stained with toluidine blue. (c) Toluidine blue stained mast cells in skin of C57Bl/6 mice. (d) Tryptase stained mast cell aggregate in the bone marrow of a mastocytosis patient. (e) HuMC stained with phalloidin for F-actin following treatment with 5-HT. (f) Phagocytosed fluorescent *Escherichia coli* particles in BMMC.

Mast cell activation and mediator release

Upon activation of mast cells via crosslinking of the high affinity IgE receptor (FcεRI) or non-IgE-mediated activation through complement receptors or toll-like receptor (TLR) activation, mast cells can release a variable spectrum of pro-inflammatory mediators. These include preformed mediators such as histamine, serotonin and proteases; newly synthesized mediators including leukotrienes and prostaglandins; and cytokines and chemokines (Table 1). In addition to IgE-mediated activation, human mast cells exposed to IFN-γ can be activated following IgG-mediated aggregation of FcγRI to release similar mediators [8–10]. Additional IgE-independent mast cell triggers have been described. These include SCF, complement factors (C3a and

C5a), neuropeptides (substance P), adenosine, TLR and scavenger receptors [11, 12].

The level and pattern of mediator release is influenced by cytokines, growth factors and microenvironmental conditions. For example, IL-4 enhances FcεRI-mediated reactions from human mast cells [13]. In addition to enhancing activation of mast cells, several modulatory cytokines produced by regulatory T cells such as IL-10 and TGF-β can decrease FcεRI-mediated reactions [14, 15].

Role of mast cells in inflammation

Mast cell activation and mediator release can independently, as well as in concert with other immune cells,

Table 1. Major mast cell-derived mediators

Class	Mediators	Physiological effects
Preformed mediators	Histamine, serotonin, heparin, neutral proteases (tryptase and chymase, carboxypeptidase, cathepsin G), major basic protein, acid hydrolases, peroxidase, phospholipases	Vasodilation Vasoconstriction Angiogenesis Mitogenesis Pain Protein processing/degradation Lipid/proteoglycan hydrolysis Arachidonic acid generation Tissue damage Inflammation
Lipid mediators	LTB ₄ , LTC ₄ , PGE ₂ , PGD ₂ , PAF	Leucocyte chemotaxis Vasoconstriction Bronchoconstriction Platelet activation Vasodilation
Cytokines	TNF- α , TGF- β , IFN- α , IFN- β , IFN- γ , IL-1 α , IL-1 β , IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-18, IL-25, SCF, MIF	Inflammation Leucocyte migration/proliferation
Chemokines	CXCL8, CCL3, CCL2, CCL7, CCL13, CCL5, CCL11, CCL19	Chemoattraction and tissue infiltration of leucocytes
Growth factors	CSF, GM-CSF, bFGF, VEGF, NGF, LIF	Growth of various cell types Vasodilation Neovascularization Angiogenesis

SCF, stem cell factor; GM-CSF, granulocyte macrophage-colony stimulating factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; NGF, nerve growth factor.

induce much of the pathology observed in allergic inflammatory conditions. Mast cell mediators such as histamine, leukotrienes and prostaglandins contribute to eosinophil recruitment, increase vascular permeability and smooth muscle contraction. Proteases can activate fibroblasts thereby promoting collagen deposition and fibrosis [16]. Mast cell-derived cytokines have numerous effects on other cells of the immune system as well as endothelial cells. For example, mast cell-derived cytokines can cause B cells to class switch to synthesize IgE, induce basophil histamine release, recruit neutrophils and eosinophils, and promote the development of T cells into a T helper 2 (Th2) phenotype [17–19].

Mast cell products may both induce an immediate reaction and contribute to a late-phase reaction. The immediate phase reaction occurs within minutes of Fc ϵ RI crosslinking and its consequences are referred to as an immediate hypersensitivity reaction. Pre-formed granule-associated and newly generated mediators released during this phase include histamine, proteases and lipid-derived mediators (Table 1). Late-phase reactions peak 6–12 h following antigen challenge and are associated with cytokine and chemokine production and release in part from eosinophils, neutrophils and basophils that have entered the inflammatory site following the immediate reaction (Fig. 2). Mast cells are also involved in chronic

allergic inflammation where symptoms relapse and remit over time, of which asthma is a classical example.

The mast cell in allergic diseases

Asthma

Asthma is complex inflammatory disorder associated with alterations in airway smooth muscle reactivity and remodelling, excessive production of mucus, increased collagen deposition, hyper-responsiveness with bronchoconstriction and the cellular infiltration of lymphocytes, eosinophils and neutrophils. In many instances, asthma has an allergic component characterized by allergic sensitivity to allergens and increased serum levels of antigen-specific IgE and total IgE. The role of the mast cell in asthma is of renewed interest due to reports that mast cell numbers are increased within the airway smooth muscle bundles of asthmatic patients [20–25]. This has led to a re-evaluation of the mast cell as a crucial effector cell in the pathogenesis of asthma, especially asthma with an allergic basis.

Mast cells numbers, but not T cells or eosinophils, are increased within the airway smooth muscle (ASM) of asthmatic patients [20, 21, 24, 25]. The location of mast cells within the ASM is believed to facilitate hyper-

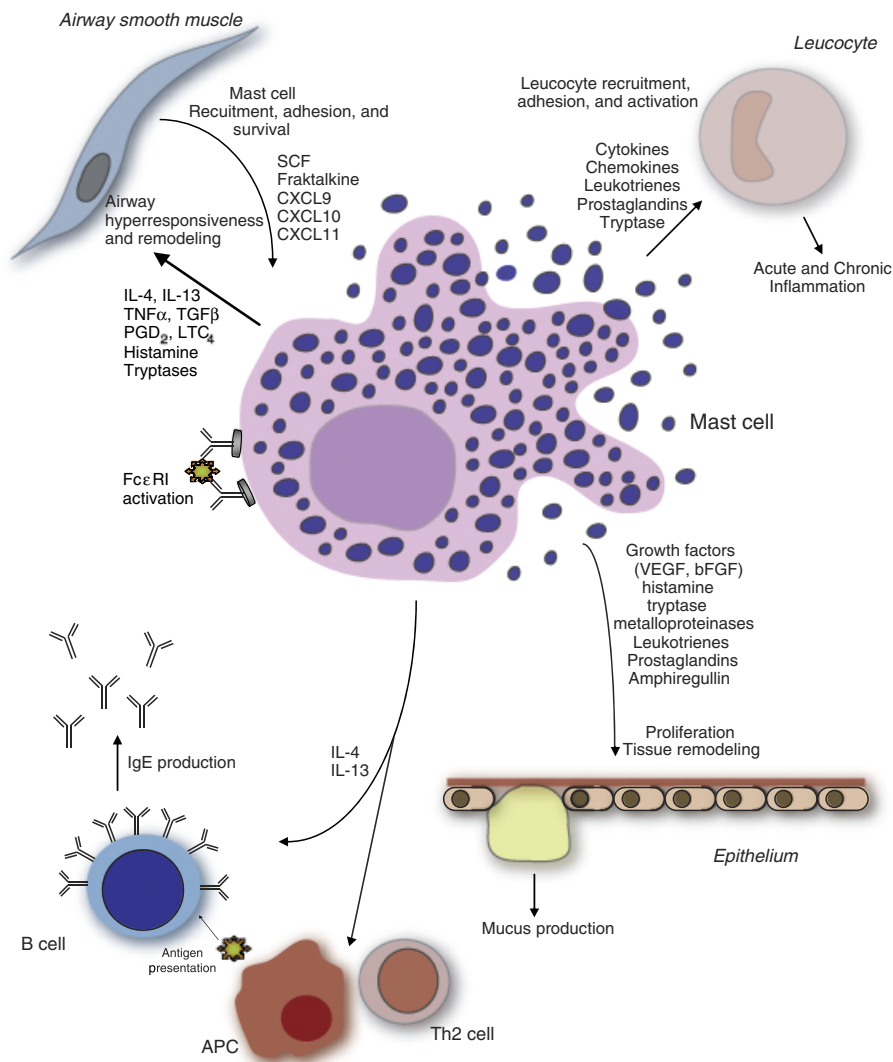


Fig. 2. Role of mast cells and mast cell products in mediating effects on airway smooth muscle, leucocytes and epithelium. In asthmatics, airway smooth muscle cells can lead to recruitment, adhesion and survival of mast cells by production of stem cell factor (SCF) and fraktalkine as examples. Mast cells contribute to airway hyper-responsiveness and remodelling through production of lipid mediators, cytokines, histamine and tryptase, which influence airway smooth muscle cells. Additionally, activated mast cells and their products have effects on proliferation and remodelling of epithelium. Production of growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), as well as proteases, histamine, metalloproteinases, and lipid mediators, all have effects on the epithelium, including the promotion of excess mucus production. Mast cell-derived mediators produced following Fc ϵ RI crosslinking with allergen lead to leucocyte recruitment, adhesion, and activation. Mast cell-derived IL-4, IL-9 and IL-13 lead to T helper 2 (Th2) differentiation, which promotes production of allergen-specific IgE by B cells.

responsiveness through localized mediator release and/or direct cell-to-cell contact. ASM cells can recruit and retain mast cells through the release of chemokines and growth factors for receptors expressed on human lung mast cells. Human lung mast cells express CXCR3 and ASM cells produce the CXCR3 ligands CXCL9, CXCL10 and CXCL11, thereby possibly contributing to mast cell recruitment [26, 27]. ASM cells also produce SCF, which itself induces mast cell recruitment, differentiation and survival [28]. Recently, an additional chemokine, fraktalkine (CX $_3$ CL1), which is increased in asthmatic lung, has been shown to be produced by ASM cells, thereby possibly contributing

to mast cell recruitment [25]. Recently, human ASM cells have been reported to express CCR7, while mast cells located within airways of asthmatic patients highly express the ligand for CCR7, CCL19 [29]. Importantly, mast cell-derived CCL19 is reported to induce ASM cell migration and wound repair [29].

Human lung mast cells have also been shown to adhere to ASM cells, while T cells and eosinophils do not adhere under the same conditions [24]. ASM cells express adhesion molecules that may thus aid in recruitment, retention and cross-talk of mast cells. Of recent interest is the role of tumor suppressor in lung cancer-1 (TSLC-1) (now known as

cell adhesion molecule-1 [CADM-1]) in mediating the adherence of mast cells to ASM. Inhibition of CADM1 can partially reduce adhesion of human lung mast cells to ASM cells [30]. In addition, vascular CADM-1 (VCAM-1) and intracellular adhesion molecule (ICAM) are expressed on ASM and may be involved in retention of mast cells [31].

Many mast cell mediators such as histamine, tryptase, IL-4, IL-13, PGD₂ and LTC₄, will induce contraction of ASM cells. For example, tryptase causes bronchial hyper-responsiveness in dogs and in isolated human bronchi stimulates cytokine production from ASM [32–34].

The complement anaphylatoxins C3a and C5a are also elevated in bronchoalveolar lavage fluid of patients with allergic asthma following allergen challenge [35]. A recent study demonstrated that ASM cells enhance C3a-mediated mast cell degranulation through an SCF-independent mechanism [36]. Mechanisms involved are not completely understood and similar effects have not been reported on human lung mast cells, but presumably involve C3a activation and release of mast cell products that enhance ASM contraction [37]. In line with this observation, degranulated mast cells are found in the ASM of asthmatic patients and there may be a correlation between the number of degranulated mast cells and the severity of asthma as a greater number of degranulated mast cells are found in fatal cases of asthma [21, 38].

In addition to preformed mediators, the release of cytokines by mast cells is an important mechanism in the activation of ASM. Mast cells found within the ASM of asthmatics express IL-4 and IL-13, both of which have been studied extensively as major mediators in asthma by acting through IL-4 receptor α [39, 40].

Mast cells also infiltrate the bronchial epithelium in asthmatics [41–43]. Infiltration of the bronchi potentially allows the mast cell increased access to allergens. This would facilitate the inflammatory response through antigen presentation, Th2 differentiation and IgE production.

Increased mucus production is a common feature of asthma, and mediators released from mast cells stimulate mucus gland secretion [44]. In fact, the number of mast cells located near mucosal glands correlates with the degree of mucus production [38, 45]. Further, an increase in mast cells as identified by tryptase staining occurs near mucosal gland stroma in non-fatal asthma, while an increase in degranulated mast cells near mucosal glands is observed in fatal and non-fatal asthma [45]. Of recent interest is the role of amphiregulin that is produced by mast cells following Fc ϵ RI aggregation which leads to an increase in mucin gene expression in epithelial cells [46, 47].

SCF and CD117 (Kit) significantly increases in the epithelium and subepithelium of the bronchi in asthmatic patients providing more suggestive evidence that mast cells contribute to the pathogenesis of asthma [48, 49]. In

addition, SCF deficient mice have decreased allergen-induced airway hyper-responsiveness and eosinophil infiltration as compared with wild-type mice [50]. Further evidence for the importance of SCF and mast cells in asthma is provided by the consequences of intratracheal instillation of SCF in mice. This results in airway hyper-responsiveness only in wild-type mice, but not in mast cell-deficient mice [50].

In addition to the effects mast cells directly exert on the pathogenesis of asthma, they also have the ability to contribute to the initiation of Th2 responses by the production of IL-4 and IL-13 [18]. Recently, thymic stromal lymphopoietin (TSLP), released by epithelial cells in response to physical injury and/or inflammatory cytokines, has been reported as a possible initiator of asthmatic responses through the potent stimulation of mast cells, to produce high levels of Th2 cytokines [51]. Mast cells are also known to present antigen, further providing a mechanism supporting Th2 differentiation and T cell activation [52–55]. In addition to the role of Th2-differentiated T cells, CD4⁺CD25⁺ regulatory T cells are being intensively studied in the regulation of asthmatic responses [56]. Of future interest will be to determine the ability of mast cells to alter CD4⁺CD25⁺ regulatory T cell responses in asthma, because a recent report has demonstrated that mast cells are essential intermediaries in regulatory T cell tolerance [57].

An additional mast cell-activation pathway that may be important in the pathogenesis of asthma is via monomeric IgE. It has been reported that monomeric IgE, in the absence of allergen, can induce Ca²⁺ flux, degranulation, arachidonic acid metabolism, chemokine and cytokine production as well as increased cell survival [58–60]. These observations may become important as a correlation between serum levels of IgE, bronchial hyper-responsiveness and asthma has been reported [61, 62].

In addition to the data on the role of mast cells in human asthma, many studies have attempted to elucidate the role of mast cells in asthma through the use of murine models. A variety of responses and pathways have been described in murine models of asthma with varying results.

Murine models of AHR have been described that are both mast cell dependent and independent. For example, sensitized and challenged mast cell-deficient mice in one study have been reported to fully develop a Th2 response, airway inflammation and AHR that is similar to wild-type mice [63]. In contrast, another mouse model of chronic asthma in mast cell-deficient mice has reported that AHR and inflammation is more mild in the absence of mast cells following sensitization and repeated allergen exposure compared with wild-type mice [64]. Supporting this latter finding, Fc ϵ RI-deficient mice failed to develop allergic inflammation, IL-13 production and AHR following sensitization and allergen challenge [65]. Further, allergic inflammation, IL-13 and AHR was restored in

these mice by reconstitution of FcεRI bearing cells [65]. However, the role of mast cell-derived IL-13 in mediating AHR is controversial, as reconstitution of FcεRI cells not able to express IL-13 could also restore inflammation and AHR in these mice [65].

The importance of murine models of asthma has been in defining mechanisms and the role of mast cells in asthma. Mast cells contribute to murine AHR by production of inflammatory mediators that can directly induce AHR, or by production of chemokines that recruit other inflammatory cells including eosinophils and effector T cells. The variable role of mast cells in these murine models of allergic asthma may in part be attributed to the mode of sensitization that can determine T cell vs. mast cell-dependent AHR responses (i.e. the use of alum can drive a mast cell-independent response) [64]. While murine airway hyper-responsiveness is not asthma, murine models have had a large impact on defining the immunological mechanisms and understanding of asthma.

Thus, mast cells in asthmatics release a variety of mediators which may induce and/or sustain chronic inflammation, alter ASM reactivity, increase mucus production and lead to Th2 polarization. In addition to mediator release, mast cells are also being recognized for their ability by direct cell-to-cell contact to alter ASM, mucus production and T cell activation.

Allergic rhinitis

Allergic rhinitis (AR) is the most common allergic disease in the United States. It affects up to an estimated 40% of children and 25% of adults. The pathophysiology of AR shares many similarities to allergic asthma and the two diseases are often considered manifestations of 'one airway, one disease' [66].

Mast cells constitutively reside in the nasal mucosa and do not normally venture into the superficial airway epithelium. Mast cells within the subepithelium phenotypically are both tryptase (MC_T) positive and tryptase/chymase (MC_{TC}) positive. With allergen exposure, mast cell migration to, and proliferation within, the epithelium occurs [67]. However, these epithelial mast cells predominantly express only tryptase (MC_T) and are selectively increased in AR [68–70]. Although SCF is a strong mast cell chemoattractant and elevated in the nasal lavage fluid of seasonal AR, relatively low levels of SCF are present in the nasal epithelium as compared with the lamina propria. CCL5 may instead have a more prominent role in this epithelial migration. CCL5 is found in significantly greater levels in the epithelial compartment and appears *in vitro* to be a more potent chemoattractant for human mast cells than SCF or CCL11 [71]. A low SCF environment is known to decrease mast cell chymase expression, which may explain the selective accumulation of MC_T in the nasal epithelium of AR [72].

Mast cell degranulation is evidenced by elevated tryptase, histamine, LTB₄, LTC₄ and PGD₂ levels in the nasal lavage fluid of individuals with AR following nasal allergen provocation [73–76]. These mediators contribute to the sneezing, pruritus, rhinorrhea and nasal congestion characteristic of the early-phase symptoms of AR. Histamine is a principal mediator inducing vasodilation, increased vascular permeability and increased glandular secretion. In addition, histamine acts on the sensory nerve endings of the trigeminal nerve to cause sneezing. A strong Th2 cytokine expression profile (TNF-α, IL-4, IL-5, IL-6 and IL-13) follows mast cell activation and is believed central to the late-phase reaction. Mast cells induce eosinophilic infiltration through the release of platelet activating factor (PAF) and LTB₄; and the up-regulation VCAM-1 expression on endothelial cells. Eosinophil survival is promoted through mast cell release of granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-5. Additionally, histamine up-regulates CCL5 and GM-CSF, while IL-4, IL-13 and TNF-α up-regulate CCL11 and CCL17, further contributing to the late-phase eosinophilic/T cell infiltration. Clinically, this is displayed as an increase in nasal mucosal thickening with decreased nasal airway resistance [71, 77].

Of direct relevance is the pathophysiology behind the nasal hyper-responsiveness found in AR. There is evidence that this hyper-responsiveness is the result of exaggerated neural reactivity, with NGF being involved [78–80]. Baseline levels of NGF in the nasal lavage fluids from individuals with AR are abnormally elevated and may be amplified through nasal allergen provocation [79]. NGF is produced not only by neurons and nerve-associated cells, but also by selected immune cells, including mast cells [81–83]. In addition, *in vitro* studies have demonstrated the ability of NGF to increase expression of FcεRI and Kit on mast cells cultured from human umbilical cord blood, suggesting NGF may be an additional growth factor that impacts on human mast cell development [84].

Atopic dermatitis

Mast cells are increased in a variety of chronic inflammatory skin disorders, including atopic dermatitis (AD) [85]. Biopsies of AD lesions demonstrate an increase in mast cell numbers as compared with uninvolved sites [86]. The precise contribution of this mast cell presence to the pathophysiology of AD is not, however, understood. Mast cells reside in the papillary dermis and undergo migration through the basal lamina into the epidermis of AD lesions. Within the epidermis, mast cells may influence keratinocyte activation and stimulation of endothelial growth with neoangiogenesis [87].

Although histamine has an established role in other atopic diseases, the effect of histamine in AD is

questionable, given that levels are not increased compared with control subjects. Moreover, antihistamines provide minimal clinical efficacy in AD. Tryptase and activation of proteinase-activated receptor-2 (PAR-2) may contribute to the pruritus seen in AD, as tryptase is reported to be increased up to fourfold in AD patients and PAR-2 expression is markedly enhanced on primary afferent nerve fibers in skin biopsies from patients with AD [88]. Chymase may play a role in eliciting and maintaining chronic inflammation in AD by weakening the skin barrier, in turn allowing an enhanced permeability to allergens and microbes [89]. An association between a promoter polymorphism (rs1800875) of the mast cell chymase gene (CMA-1) and AD has been reported [90].

Significantly elevated levels of total IgE are found in about 80% of patients with AD. Beyond traditional signaling through the FcεRI receptor on mast cells, a novel IgE-independent mast cell activation pathway has been proposed for AD involving CD30. Mast cells were shown to be the predominant CD30 ligand-positive cell in AD lesions and activation through CD30 induced a *de novo* synthesis and secretion of CXCL8, CCL3 and CCL4, via the extracellular-signal regulated kinase (ERK)/(Mitogen-activated protein kinase) MAPK pathway [91].

As in AR, mast cell-nerve interactions may also play a role in promoting inflammation in AD. Contacts between mast cells and nerves are increased in both lesional and non-lesional samples of AD when compared with normal controls [92]. Inflammation appears to be mediated by neuropeptides such as substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and NGF [84, 93–95].

Anaphylaxis

Anaphylaxis is an acute, severe, systemic reaction to a foreign stimulus that is often thought to be associated with mast cell activation. The strongest evidence of a role for mast cells in anaphylaxis comes from assessments of serum tryptase levels during anaphylaxis [96, 97]. Serum levels of tryptase, which predominantly arise from mast cell degranulation, peaks 1–2 h following the onset of IgE-mediated anaphylaxis [98]. Classical IgE-dependent anaphylaxis occurs upon exposure to specific antigens including venoms, latex, and pharmaceutical agents. In addition to IgE-mediated mast cell activation, anaphylaxis may be elicited by certain agents or stimuli that activate mast cells independent of IgE. IgG and complement receptors expressed on mast cells may contribute to these IgE-independent events [3, 8, 9, 99].

Although anaphylaxis is considered a systemic event, the presence and activation of mast cells in specific organs may play a critical role in the severity. Within the heart, mast cells are located between myocardial fibers, around blood vessels and in the arterial intima. Activation of

these critically positioned mast cells may directly contribute to cardiopulmonary failure. Cardiac mast cells *in vitro* release many of the classic mast cell mediators of anaphylaxis including PAF [100, 101]. PAF is thought to be a critical factor in the development of anaphylactic shock through its ability to induce hypotension and cardiac dysfunction [102]. PAF-induced anaphylactic shock in mice appears directly dependent on phosphoinositide-3 kinase (PI3K) and endothelial nitric oxide synthase (eNOS)-derived nitric oxide (NO) which functions as a potent vasodilator [103].

The overall number of mast cells may also be relevant in anaphylaxis. It is known that individuals with recurrent anaphylaxis tend to have more dermal mast cells than those without anaphylaxis. Mastocytosis, a disease characterized by the pathologic accumulation of mast cells in tissues, is often associated with spontaneous episodes of hypotension and has served as a unique disease model. Activating mutations in the tyrosine kinase Kit, such as D816V, are strongly associated with mastocytosis [104]. Identification of this mutation suggests that additional yet unidentified genetic polymorphisms or mutations may potentially account for an increase in mast cell numbers, which may predispose individuals to recurrent anaphylaxis.

Another historically interesting area of research has been the possible activation of mast cells by high doses of γ -radiation as could be encountered in the environment under some circumstances. Early data suggested that mast cells might contribute to the acute radiation syndrome that occurs immediately following high-dose exposure to γ -radiation possibly by the release of histamine [105–107]. However, a recent report has demonstrated that mast cells are highly resistant to the effects of γ -irradiation and do not degranulate in response to γ -irradiation alone and surprisingly retain their ability to respond to FcεRI-mediated signals as well as TLR-mediated signals [108].

Despite convincing evidence for mast cell-dependent anaphylaxis, it is important to note that there are instances of anaphylaxis not associated with tryptase elevation [97]. This observation challenges the accuracy of the conclusion that mast cells are central to all forms of anaphylaxis. Indeed, mast cell-deficient mice have shown to be prone to fatal IgE-dependent anaphylaxis [109]. Subsequent mouse models suggest that an alternative pathway may proceed primarily through the IgG, FcγRIII, macrophage and PAF pathways [110]. Certainly, further investigation is required to delineate the pathophysiology of these anaphylactic events.

Allergic eye disease

Ocular allergy occurs in >50% of the allergic population [111, 112]. The location of mast cells in close proximity to

the external environment in the mucosa of the eye allows for exposure of these cells to allergen, thereby facilitating crosslinking of membrane-bound IgE, which leads to degranulation and release of inflammatory mediators. Although there are several types of ocular allergy, seasonal and perennial allergic conjunctivitis represent the majority of allergy cases [113].

In normal individuals, mast cells are abundant in the conjunctival stroma with an estimated 50 million cells residing at this environmental interface [114]. In symptomatic allergic patients, an increase in mast cells with evidence of degranulation is seen in conjunctival biopsies [115]. In addition to the increase in mast cells within the conjunctiva, the number of mast cells expressing IL-4 message is increased threefold in seasonal allergic conjunctivitis [116]. Further, use of a mast cell stabilizer (nedocromil sodium) reduces the amount of histamine and PGD₂ by more than 70% after challenge, thereby supporting a major role for mast cells in allergic conjunctivitis [117].

In addition to common mast cell mediators such as histamine and cytokines, chemokines released from activated mast cells mediate late-phase reactions by recruitment of additional inflammatory cells. Mast cells residing within the conjunctiva express CCR3 and the use of a CCR3 antagonist in a mouse model of allergic conjunctivitis ablated both the early and late-phase reactions [118]. In this model, the CCR3 antagonist lead to mast cell stabilization and inhibition of immediate hypersensitivity, but also impaired neutrophil and eosinophil influx during the late-phase response [118].

Mast cell therapeutics

Mast cell therapeutics may be broadly classified into those directed at cell membrane targets (membrane receptors), to intracellular targets (cell signaling, gene expression) or to extracellular targets (released mediators) (Table 2) (Fig. 3). Often treatment selection is tailored to include one or more of these agents depending on the individual patient and the specific allergic disease.

Cell membrane targets

Chromones are believed to target and 'stabilize' the mast cell membrane, but their precise mechanism of action is not understood. *In vitro* studies with activated mast cells suggest that chromones disrupt the Ca²⁺ influx, chloride ion transport, and exocytotic processes required for proper degranulation [119, 120]. Ca²⁺ mobilization is also required for arachidonic acid synthesis and the subsequent production of lipid mediators [121, 122]. Unfortunately, chromones are generally regarded as weak mast cell 'stabilizers' and display rapid tachyphylaxis resulting in their poor clinical efficacy.

Both short and long acting agonists of the β₂ adrenergic receptor have shown effective *in vitro* inhibition of histamine and cysteinyl-leukotriene release by mast cells [123, 124]. This inhibitory effect is mediated by a sustained increase in cAMP [125]. Although inhaled albuterol inhibits the increase in plasma histamine induced by allergen exposure in asthmatic patients [126, 127], substantial *in vivo* evidence for the modulation of mast cells by β₂ agonists is limited.

The humanized monoclonal antibody, omalizumab, is directed to the receptor binding domain of circulating IgE and blocks its attachment to FcεRI on inflammatory cells. This indirectly results in the down-regulation of FcεRI expression on the cell membrane [128]. Subsequently, higher concentrations of allergens are required to induce skin prick test reactivity [129]. It is hypothesized that FcεRI numbers in some instances decrease to such low levels that despite antigen excess, adequate receptor crosslinking is unachievable [119].

Additional mast cell membrane targets under investigation include chemokine receptors, particularly CCR3. Although regarded as a principal mediator of eosinophil chemotaxis, CCR3 antagonists have demonstrated the ability to provide mast cell stabilization and to inhibit both the early and late-phase of allergic inflammation in the mouse conjunctiva [118, 130, 131]. CCR3 antagonists are at various stages of development [132].

Ion channels are essential for many cellular processes including mast cell function and also represent attractive targets. Recently, it has been shown that Ca²⁺ influx through Ca²⁺ release-activated Ca²⁺ channels activate cytosolic phospholipase A₂, leukotriene C₄ secretion and expression of c-fos through ERK-dependent and -independent pathways in mast cells [133]. Opening of the Ca²⁺ activated K⁺ channel, KCa3.1, on mast cells enhances mediator release while blockade attenuates degranulation [134, 135]. Although the role of KCa3.1 in mast cell degranulation is modest, its function in human lung mast cell migration appears critical [136]. Modulation of these ion channels may have a role in the treatment of allergy as adenosine was recently shown to close KCa3.1 in human lung mast cells and inhibit their migration via the adenosine A_{2A} receptor [137].

CD63 is a tetraspanin present on the surface of mast cells that interacts with β1 integrins and modulates adhesion. Anti-CD63 monoclonal antibodies have shown the ability to decrease FcεRI-induced degranulation via impairment of the Gab2-PI3K pathway, suggesting a potential therapeutic application [138].

Intracellular targets

Glucocorticoids (GCs) exert global modulating effects on the immune system and are effective in the treatment of allergic diseases. Upon engagement of GC receptors in the

Table 2. Mast cell and mast cell product-directed therapeutics*

Mast cell target	Therapeutic class	Mechanism of action	Stage of development
Cell membrane	Chromones	Potential disruption of Ca ²⁺ influx, chloride ion transport and exocytic processes	Clinical use
	β ₂ agonists	Increase cytosolic cAMP levels through binding of β ₂ receptors	Clinical use
	Omalizumab	Monoclonal antibody to free IgE resulting in decreased FcεRI membrane expression	Clinical use
	CCR3 antagonists	Block chemotaxis and degranulation	Clinical trials
	Ca ²⁺ and K ⁺ channel antagonists	Disruption of ion influx with attenuation of degranulation and chemotaxis	Pre-clinical
	Anti-CD63 antibody	Monoclonal antibody to CD63 which interferes with cellular adhesion to β1 integrins and blocks FcεRI-induced degranulation via impairment of Gab2-PI3k pathway	Pre-clinical
Intracellular	Glucocorticoids	Regulate transcription of numerous inflammatory genes	Clinical use
	Syk kinase inhibitors	Block IgE-FcεRI-mediated downstream signaling (phosphorylation)	Clinical trials
	MAPK inhibitors	Block phosphorylation of multiple intracellular proteins (including transcription factors) that are involved in cellular proliferation, differentiation, survival and chronic inflammation	Clinical trials
Extracellular	PDE4 inhibitors	Block hydrolysis of cAMP to 5'AMP	Clinical trials
	5-LO inhibitor	Blocks the conversion of arachidonic acid to LTA ₄ which subsequently prevents CysLT formation	Clinical use
	Tryptase inhibitors	Block the protease activity of tryptase	Pre-clinical
	CysLTR1 antagonists	Block the binding to and effects of CysLT on target cells	Clinical use
	H ₁₋₄ receptor antagonists	Block the binding to and effects of histamine on target cells	H ₁ /H ₂ : Clinical use H ₃ : Clinical trials H ₄ : Pre-clinical
	PAR-2 antagonists	Block PAR-2 receptor signaling following activation by proteases (e.g., tryptase)	Pre-clinical
	DP and CRTH-2 receptor antagonists	Block the binding to and effects of PGD ₂ on target cells	Pre-clinical

*Representative strategies in each target class are presented.

MAPK, mitogen-activated protein kinases; PI3K, phosphoinositide-3 kinase; PDE, phosphodiesterase; 5-LO, 5-lipoxygenase; PAR-2, proteinase-activated receptor 2; CRTH-2, chemoattractant receptor homologue on T helper type 2 cells; DP, D prostanoid.

cytoplasm of inflammatory cells, GC traffic to the nucleus and regulate gene expression. Although mononuclear cell cytokine production is a main target, antigen-induced expression of IL-4, IL-5, IL-6, IL-13, TNF-α and GM-CSF by mast cells is also inhibited [139–143]. In addition, tissue SCF production required for the survival of local mast cells is down-regulated [144, 145]. Beyond cytokine modulation, recent evidence indicates that GC inhibit mast cell degranulation through suppression of intermediate signaling processes. Up-regulation of inhibitory factors such as Src-like adaptor protein (SLAP), downstream of tyrosine kinase-1 (Dok-1) and MAPK phosphatase-1 (MKP-1) are reported [146, 147]. GC-mediated down-regulation of IgE-dependent FcεRI expression in mast cells has also been demonstrated [148].

As our understanding of mast cell signaling has evolved, potential new intracellular targets have been identified. A Syk tyrosine kinase inhibitor, R112, which disrupts mast cell IgE-FcεRI signaling has displayed promising results in recent clinical trials and may represent a new class of allergy therapeutics [149, 150]. MAPKs

are also well-recognized mediators of mast cell activation, survival, differentiation and cytokine production. Clinical development of small molecule inhibitors of the p38 MAPK are presently ongoing [151–153]. Phosphodiesterase 4 (PDE4) is the major cyclic-AMP metabolizing enzyme in immune and inflammatory cells, including mast cells. Multiple PDE4 inhibitors are under clinical evaluation for the treatment of asthma, AD and AR [154].

Extracellular targets

Cysteinyl leukotrienes (CysLTs) are potent lipid mediators produced by activated mast cells via the 5-lipoxygenase (5-LO) pathway. Utilizing Cys-LT receptors, these mediators exert a diverse range of inflammatory effects including bronchoconstriction, smooth muscle proliferation, airway remodelling, fibrosis, and effector cell recruitment and activation [155]. Two known Cys-LT receptors, CysLT1 and CysLT2, have been characterized on a number of inflammatory cells, including mast cells [156, 157]. 5-LO inhibitors and CysLT1 receptor antagonists are

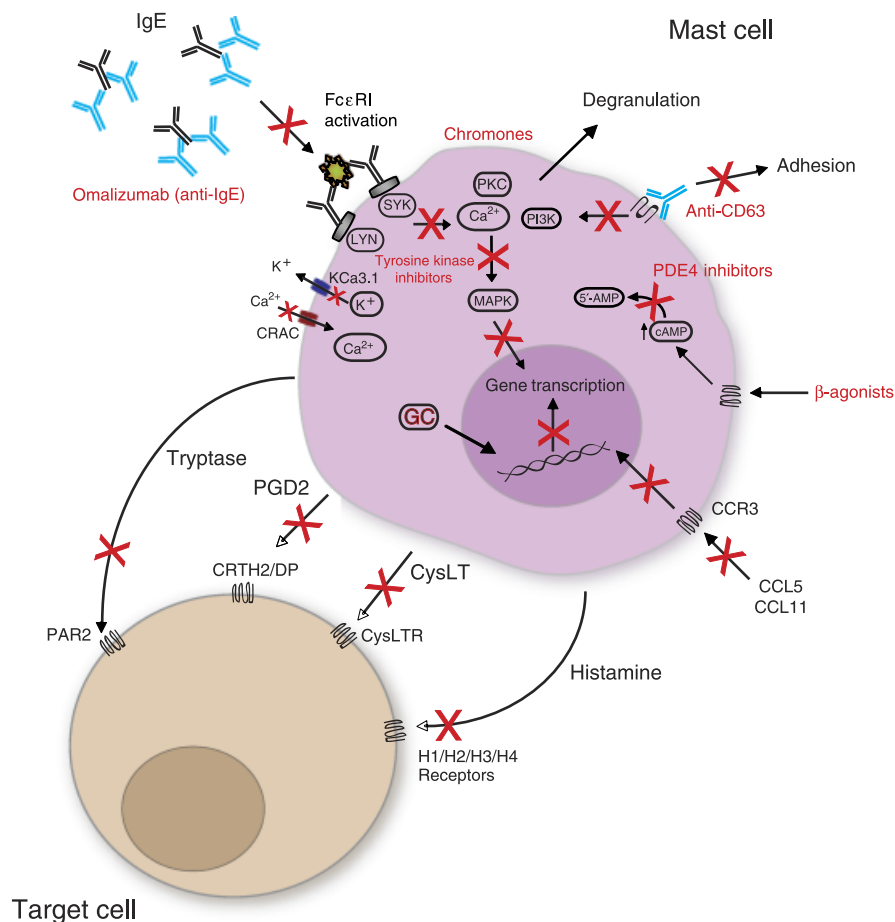


Fig. 3. This figure depicts potential sites for therapeutic mast cell modulation. These sites may be broadly grouped into cell membrane, intracellular or extracellular targets. Cell membrane inhibitors include omalizumab (down-regulation of FcεRI), cromones, β₂ agonists, CCR3 inhibitors, anti-CD63 blocking antibodies, KCa3.1 K⁺ channel and Ca²⁺ release-activated Ca²⁺ (CRAC) channel blockers. Glucocorticoids, phosphodiesterase (PDE) inhibitors and tyrosine kinase inhibitors exert their inhibitory effects within the cell, targeting either cell signaling or gene expression. Extracellular targets include mast cell mediators or their receptors released following activation. These therapeutics include tryptase inhibitors, proteinase-activated receptor 2 (PAR-2) antagonists, 5-lipoxygenase inhibitors, CysLTR1 inhibitors, and D prostanoid (DP)/chemoattractant receptor homologue on T helper type 2 cells (CRTH) antagonists or histamine receptor blockers. Also see Table 2.

currently available for clinical use. The CysLT2 receptor may represent a promising new therapeutic target and future antagonists are anticipated.

Histamine released by activated mast cells induces vasodilation, vascular permeability, and smooth muscle contraction in tissues via histamine receptors. To date four types of histamine receptors have been identified and antagonists, specifically to the H₁ receptor, are central to the management of allergy. Blocking of H₁ receptors results in decreased levels of nuclear factor-κB, a transcription factor important in the regulation of inflammatory cytokines and adhesion protein expression [158]. The migration, accumulation and activation of inflammatory cells such as eosinophils, neutrophils and basophils are also down-regulated. Recent findings support a pro-inflammatory role for the histamine activated H₄ receptor and antagonists are currently in development [159].

Tryptase is the most abundant protease stored in the human mast cell. Upon degranulation, tryptase cleaves and in turn activates PARs found on numerous cells. PAR-2 activation specifically induces airway inflammation and tissue remodelling in asthma [160]. Indeed, PAR-2 deficient mice demonstrated reduced airway inflammation following antigen challenge [161]. These characteristics make both tryptase and PAR-2 apparent targets for pharmacological intervention. While multiple tryptase inhibitors are in preclinical development, PAR-2 antagonists are currently unavailable [162, 163].

PGD₂ is the major cyclooxygenase metabolite produced by mast cells. PGD₂ is also the natural ligand for both the D prostanoid (DP) receptor and chemoattractant receptor homologue on T helper type 2 cells (CRTH-2). Several DP receptor antagonists have been identified with one reaching phase IIa clinical trials for the treatment of asthma.

Despite promising preclinical data, no statistically significant clinical benefit was observed and further development has been terminated (www.shionogi.co.jp/ir_en/news/detail/e_061027.pdf) [164]. CRTH-2 has recently received much attention for its proinflammatory role in allergic disorders, prompting an intense pursuit of CRTH-2 antagonists [165]. Although numerous antagonists have been reported in the literature, results from animal model studies have yet to appear [166, 167].

Conclusions

The mast cell clearly has a central role in the pathogenesis of allergic diseases, and more recently has been shown to be involved in innate immunity in response to bacterial and parasitic infections [168]. As our understanding of the various roles of mast cells in disease pathogenesis evolves, novel therapeutic targets will continue to be identified. It is beyond the scope of this review to address all the therapeutic approaches that are currently in development. It is anticipated that advances in gene therapy, vaccines and drug delivery will provide additional therapeutics to modulate mast cell function [169].

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