

Mast cells signal their importance in health and disease



Ana Olivera, PhD,^a Michael A. Beaven, PhD,^b and Dean D. Metcalfe, MD^a *Bethesda, Md*

FcεRI is the primary receptor in mast cells that mediates allergic reactions by inducing rapid release of mediators, an adaptive immune response that might have evolved as a host defense against parasites and venoms. Yet it is apparent that mast cells are also activated through non-IgE receptors, the significance of which is just beginning to be understood. This includes the Mas-related G protein-coupled receptor X2, which might contribute to reactions to diverse antimicrobials and polybasic compounds, and the adhesion G protein-coupled receptor E2, variants of which are associated with familial vibratory urticaria and are activated by mechanical vibration. Similarly, mast cells have long been recognized as the main repository for histamine, heparin, and proteases. Recent evidence also points to new functions, modes of delivery, and mechanisms of action of mast cell proteases that add new dimensions to the roles of mast cells in human biology. In addition, exposure of mast cells to environmental cues can quantitatively and qualitatively modulate their responses and thus their effect on allergic inflammation. Illustrating this paradigm, we summarize a number of recent studies implicating the injury/tissue damage cytokine IL-33 as a modulator of allergen-induced mast cell responses. We also discuss the discovery of markers associated with transformed mast cells and new potential directions in suppressing mast cell activity. (J Allergy Clin Immunol 2018;142:381-93.)

Key words: mast cells, degranulation, Mas-related G protein-coupled receptor X2, IL-33, adhesion G protein-coupled receptor E2, tryptase, allergy, mastocytosis

Mast cells, which are best known for the release and generation of mediators of allergic reactions, are critical sentinel cells in innate immunity, a role for which they are ideally suited because of their prevalence in tissues exposed to the environment, such as

Abbreviations used

ADGRE2:	Adhesion G protein-coupled receptor E2
CPA3:	Carboxypeptidase 3
CTMC:	Connective tissue mast cell
GPCR:	G protein-coupled receptor
MC _T :	Human mast cell subtype expressing tryptase
MC _{TC} :	Human mast cell subtype expressing tryptase and chymase
MMC:	Mucosal mast cell
MRGPRX2:	Mas-related G protein-coupled receptor X2
PD-1:	Programmed cell death protein 1
PD-L1:	Programmed cell death ligand 1
SCF:	Stem cell factor
sST2:	Soluble form of ST2
STAT3:	Signal transducer and activator of transcription 3
ST2L:	ST2 long-form, the IL-33 receptor
TLR:	Toll-like receptor
7TM:	Seven transmembrane
TPSAB1:	Human α-tryptase
TPSB2:	Human β-tryptases
Treg:	Regulatory T
VU:	Vibratory urticaria

the skin, airways, and gastrointestinal tract.¹⁻³ Mast cells express an array of receptors that allow these cells to recognize and respond to a wide spectrum of infectious pathogens and endogenous molecules produced by damaged or inflamed tissues. Thus, in addition to the high-affinity receptor of IgE, FcεRI, and other immunoglobulin Fc receptors, mast cells express the microbial pattern recognition receptors Toll-like receptor (TLRs) and NOD-like receptor⁴ and the more recently described Mas-related G protein-coupled receptor X2 (MRGPRX2 [human subjects]), which recognizes cationic neuropeptides, antimicrobial peptides, and insect venom peptides.⁵ Similarly, mast cells recognize molecules associated with tissue inflammation or damage through receptors such as complement receptors and the IL-33 receptor, also called ST2 long-form (ST2L). Recent findings suggest that they also respond to mechanical stimulation through adhesion G protein-coupled receptors (GPCRs).⁶

In response to these and other stimuli, mast cells variably release and generate histamine and lipid mediators; cytokines; chemokines; growth factors, including nerve growth factor, GM-CSF, platelet-derived growth factor, TGF-β, and vascular endothelial growth factor; and proteases to initiate innate immune responses and contribute to adaptive immune responses. Mast cells have also been implicated in tissue remodeling and angiogenesis through release of growth factors and mast cell-specific tryptases and chymases, thus conferring both reparative and defensive capabilities to mast cells.⁷⁻⁹ Activation of mast cells is also important for proper defense against certain

From ^athe Mast Cell Biology Section, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, and ^bthe Laboratory of Molecular Immunology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda.

Supported by the Division of Intramural Research Programs within the National Institute of Allergy and Infectious Diseases and National Heart, Lung, and Blood Institute, National Institutes of Health.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication October 27, 2017; revised January 11, 2018; accepted for publication January 24, 2018.

Available online February 15, 2018.

Corresponding author: Ana Olivera, PhD, Mast Cell Biology Section, Laboratory of Allergic Diseases (NIAID, NIH), Building 10, Rm 11N240, 10 Center Dr, Bethesda, MD 20892. E-mail: ana.olivera@nih.gov.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaci.2018.01.034>

helminth infections and other parasites and, as will be discussed later, against arthropod and animal venoms.¹⁰ However, mast cells are potentially lethal cells on widespread activation, as occurs in fatal anaphylaxis, and are able to cause tissue damage with sustained mast cell activation, as in chronic infections. Both the beneficial and detrimental roles of mast cells in fungal, viral, and bacterial infections are discussed in detail in recent reviews.^{1,11-13} Other roles attributed to mast cell dysfunction include promotion or, in a few instances, suppression of tumor growth^{14,15}; autoimmune diseases, such as multiple sclerosis^{16,17}; and atherosclerosis.⁸

Mast cells are among the first recognizable immune cells in evolution, and phylogenetic studies provide insights into how some of the functional capabilities of mast cells have evolved, as reviewed by Crivellato et al.¹⁸ Metachromatic staining of cells with the cardinal characteristics of mast cells first appeared more than 500 million years ago in urochordates as granulated hemocytes and “test cells” with properties indicative of a role in defense and tissue repair and in various fish species, including primitive jawless fish.^{19,20} After transition to vertebrate species and emergence of the immunoglobulin-based recombinase-activating gene network, mast cells appear to have acquired adaptive immune functions with the expression of immunoglobulin receptors.¹⁸ FcεRI is a relatively late acquisition, with the appearance of genes encoding both IgE and FcεRI being evident in marsupials and mammals,²¹ although the γ subunit of FcεRI and a functional “IgE-like” receptor are detectable in intestinal mast cells of zebrafish.²² Other mast cell–related receptors are present in zebrafish mast cells, including KIT, the receptor for the primary mast cell growth factor, stem cell factor (SCF), and the TLR adaptor protein myeloid differentiation response gene–88, which provides the capacity for recognition of a broad range of microbes and parasites.²³ Of interest, the ancestral mast cells in urochordates^{19,20} and those in zebrafish²² also respond to compound 48/80, a cationic polymer produced by condensation of N-methyl-p-methoxyphenethylamine with formaldehyde, through the recently described MRGPRX2 receptor. Compound 48/80 was first described as a “histamine liberator” that activated mammalian mast cells and induced anaphylaxis through undetermined mechanisms. The conservation of mast cells and their receptors during evolution and the ability of mast cells to respond to environmental cues and produce a myriad of physiologically relevant substances supports the conclusion that mast cells contribute to an organism’s ability to maintain a state of well-being not only as local sentinels of danger but also as regulators of tissue homeostasis.

In this review we will discuss recent developments in the understanding of antibody-independent receptors in mast cells that have resulted in a new appreciation of mast cell function and dysfunction. We will also briefly summarize the latest insights and updates on mast cell diversity, roles of mast cell–derived products, and development of humanized models to study mast cell–related disorders.

MAST CELL DIVERSITY

Mast cells are of hematopoietic lineage, but unlike other myeloid cells, they enter the circulation as progenitor rather than mature cells. At this stage, progenitor cells in mice and human subjects can express both FcεRI and KIT (CD117). In human

subjects immediate mast cell precursors from blood were recently defined as Lin[−]CD34^{hi}CD117^{int/hi}FcεRI⁺ cells,²⁴ and in mice immediate mast cell precursors from blood were recently defined as Lin[−]CD117^{hi}ST2^{hi} integrin β7^{hi} cells, in which the presence of FcεRI expression is not required but considered as a marker of greater maturity.²⁵ These mast cell progenitor populations in blood are extremely rare, representing 0.0053% of blood cells in healthy subjects²⁴ and 0.0045% of mononuclear cells in adult BALB/c mice.²⁵ The β7 integrin marker is characteristic of mouse mast cell progenitors^{25,26}; however, although it is present in human mast cell progenitors, it is less defining.²⁴ Entry of these precursors into tissues appears constitutive and might be enhanced by inflammation and tissue damage.

It has been recognized that mast cells in human and mouse tissues are heterogeneous in nature. In human tissues progenitor cells differentiate into 2 principle subtypes when defined by the neutral proteases expressed within intercellular granules, with skin mast cells commonly expressing tryptase and chymase (MC_{TC}) and mast cells in other organs, such as the lung, having more mast cells expressing tryptase alone (human mast cell subtype expressing tryptase [MC_T]).

In rodents, mature mast cells have been classified into 2 subclasses based on staining characteristics, complex patterns of protease expression, and tissue location: connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs). In general, mouse CTMCs correspond to MC_{TC}s and mouse MMCs to human MC_Ts. More recently, murine mast cells have been thought of as constitutive (or innate) and mucosal (or adaptive) mast cells based not only on their tissue location and granularity but also on their developmental patterns.^{26,27} Thus innate mast cells are present constitutively in connective tissue, have high granularity and expression of FcεRI and KIT, and are generally unaffected by T-cell deficiency. Adaptive mast cells are found primarily in intraepithelial tissues, are induced by T cell–dependent inflammation, and express more β7 integrin than innate mast cells. It has been suggested that although innate or constitutive mast cells might be more important for acute mast cell–related responses or to maintain tissue function, adaptive mast cells would add to the airway (and perhaps gastrointestinal) inflammation observed after an immunologic event and contribute to remodeling and secondary airways hyperreactivity.²⁶ In addition, it is likely that the differences among mast cell subtypes reflect functional specialization because Mrgpr family members, for example, are expressed in CTMCs and not MMCs.⁵ Functional specialization of human mast cell populations related to MRGPRX2 responses⁵ and other receptors, such as complement receptor C5a,²⁸ have also been described. However, how these functional subpopulations relate to mouse mast cell populations in rodents is a subject of current research.

Transcriptomic analysis within constitutive mast cells from various anatomic locations have indicated that although mast cells share a transcriptional signature substantially distinct from all other immune cells, including basophils, they do show heterogeneity among themselves, even in the absence of inflammation, supporting a role for mast cells in maintaining tissue integrity.^{27,29} This is consistent with a long-standing view of phenotypic plasticity of mast cells depending on assorted physiopathological environmental cues in the surrounding tissue. One common factor that appears important for mast cell maturation and survival in tissues is SCF. A recent study in mice indicates that a major source for SCF in the skin are

keratinocytes and that keratinocyte-produced SCF can be required for the recruitment and maturation of mast cells in the dermis. These studies also draw attention to the importance of the skin microbiome in induction of SCF production by keratinocytes, which can be reproduced by the TLR2 ligand lipoteichoic acid, which is produced by the skin microbiome.³⁰

FcεRI AND EMERGING RECEPTORS IN MAST CELL ACTIVATION AND FUNCTION IN ALLERGY

Aggregation of FcεRI through multivalent binding of allergen to IgE bound to the high-affinity IgE receptor activates a broad spectrum of responses promoting allergic inflammation. These include rapid release of granule-associated preformed mediators, such as histamine, sulfated proteoglycans (including heparin and chondroitin sulfates), and mast cell-specific proteases. This is accompanied by rapid production of lipid-derived inflammatory mediators, including prostaglandin D₂, leukotriene C₄, and platelet-activating factor, and subsequently by transcriptionally derived cytokines and chemokines that can promote or suppress inflammation and regulate tissue remodeling, as reviewed by Gilfillan and Beaven³¹ and Galli and Tsai.³² The immediate effects or “immediate hypersensitivity reaction” are due to this rapid release of inflammatory mediators. If localized to the skin, this results in a wheal-and-flare reaction or contraction of airway smooth muscle, mucus secretion, and increased vascular permeability in the airways. If systemic, the result can include severe hypotension caused by vascular dilation and extensive vascular leakage among other effects. These early responses can transition into a late-phase reaction hours later that is associated with an influx of circulating cell types, which can promote further inflammation.

Although classically anaphylaxis is considered mediated by IgE/antigen through cross-linking of FcεRI, anaphylaxis can also occur through nonclassical mechanisms involving immunoglobulin receptors other than FcεRI in mast cells, at least in animal models, as reviewed by Finkelman et al.³³ Anaphylatoxin receptors are also present on human mast cells, and complement activation can play a role in certain cases of human anaphylaxis, particularly in synergizing with antibody-induced anaphylaxis.³³ In this section we will summarize evidence for newly recognized receptors that might be implicated in non-IgE-mediated mast cell activation and immediate local or systemic reactions and receptors whose importance in the pathology of allergic diseases lies partly in the modulation of mast cell responses.

MRGPRX2, the receptor for cationic mast cell activators

Among the antibody-independent substances that can uniquely activate mast cells are a number of cationic amphiphilic substances. In addition to the prototypic compound 48/80, other cationic mast cell activators include a variety of pharmacologic agents (tubocurarine, atracurium, icatibant, ciprofloxacin, and other fluoroquinolone antibiotics), components of insect venom (eg, mastoparan and *Polistes* kinin), antimicrobial peptides (eg, α- and β-defensins and cathelicidins), secreted eosinophil products (eosinophil peroxidase and major basic protein), and neuropeptides (eg, substance P, vasoactive intestinal peptide, neuropeptide Y, somatostatin, and cortistatin). Although

many of these compounds were originally described as “histamine liberators” or mast cell secretagogues because of their ability to cause mast cell degranulation, they also stimulate production of prostaglandin D₂ and chemokines and cytokines, including IL-2, IL-3, IL-4, IL-6, IL-31, TNF-α, and GM-CSF.^{34–36} They act independently of FcεRI in a G_{iα} subunit-dependent manner, resulting in activation of phospholipase Cβ, phosphatidylinositol-4,5-bisphosphate 3-kinase, and calcium mobilization.³⁷ Recent studies have revealed that these cationic mast cell activators act on human mast cells through MRGPRX2, although the first report that human cord blood CD34⁺-derived mast cells are activated through MRGPRX2 was in 2006.³⁸ In these experiments mast cells were differentiated into 2 major human mast cell subtypes through manipulation of culture conditions. The human mast cell subtype MC_{TC} expressed almost 4000 higher copy numbers of MRGPRX2 RNA than MC_{Ts}, which did not respond to these stimulants. Indeed, it appears that these compounds activate only specific subsets of mast cells, such as CTMCs but not MMCs in rodents and MC_{TCs} but not MC_{Ts} in human subjects.⁵ The mouse orthologue of MRGPRX2 was identified as *Mrgprb2*.³⁹ Both human (MRGPRX2) and mouse (*Mrgprb2*) receptors are activated by the same stimulants, although the human MRGPRX2 receptor exhibits a 10- to 100-fold greater sensitivity to these activators.^{39,40}

By using a dynamic imaging system, it has been reported that in human and mouse mast cells, cytoplasmic granule secretion induced by stimulation of MRGPRs and other GPCRs follows dynamic and physical features that differ from those induced by FcεRI. MRGPR stimulation caused rapid and transient intracellular calcium peaks, followed by immediate secretion of what appeared to be individual secretory granules. Instead, engagement of immunoglobulin receptors led to sustained calcium flux and slightly delayed degranulation caused by granule-to-granule fusion before exteriorization of the combined granule content (compound exocytosis). These distinct patterns of degranulation were also observed in a mouse model of immediate skin hypersensitivity, where the reactions induced by substance P (a MRGPR agonist) were more rapid than those induced by IgE/antigen and the granules secreted by stimulation of *Mrgprb2* were less likely to be transported or persist in draining lymph nodes, suggesting potential physiologic consequences or differences in mast cell function, depending on the nature of the stimulus.⁴¹

The presence of MRGPRX2 in mast cells might contribute to the well-established roles of mast cells in innate and adaptive immunity; chronic allergic diseases, such as chronic idiopathic urticaria and asthma; and potentially neurogenic inflammation, pain, and itch, as recently reviewed.⁵ The pathologic implications have yet to be explored in detail. A significant increase in number and percentage (45% vs 22%) of MRGPRX2⁺ mast cells has been described in the skin of patients with chronic urticaria compared with that of nonatopic control subjects.⁴² Also, an increase in mast cell numbers expressing MRGPRX2 are present in the gingiva of patients with periodontitis.⁴³ In addition to better understanding the involvement of MRGPRX2 in disease, further research is needed to identify mechanisms that regulate the expression of this receptor on human mast cells. Because mast cell MRGPRX2 receptors are increasingly shown to have biologic/pathologic relevance, selective antagonists will be of additional therapeutic and investigational interest and help in understanding how mast cells contribute to homeostasis.

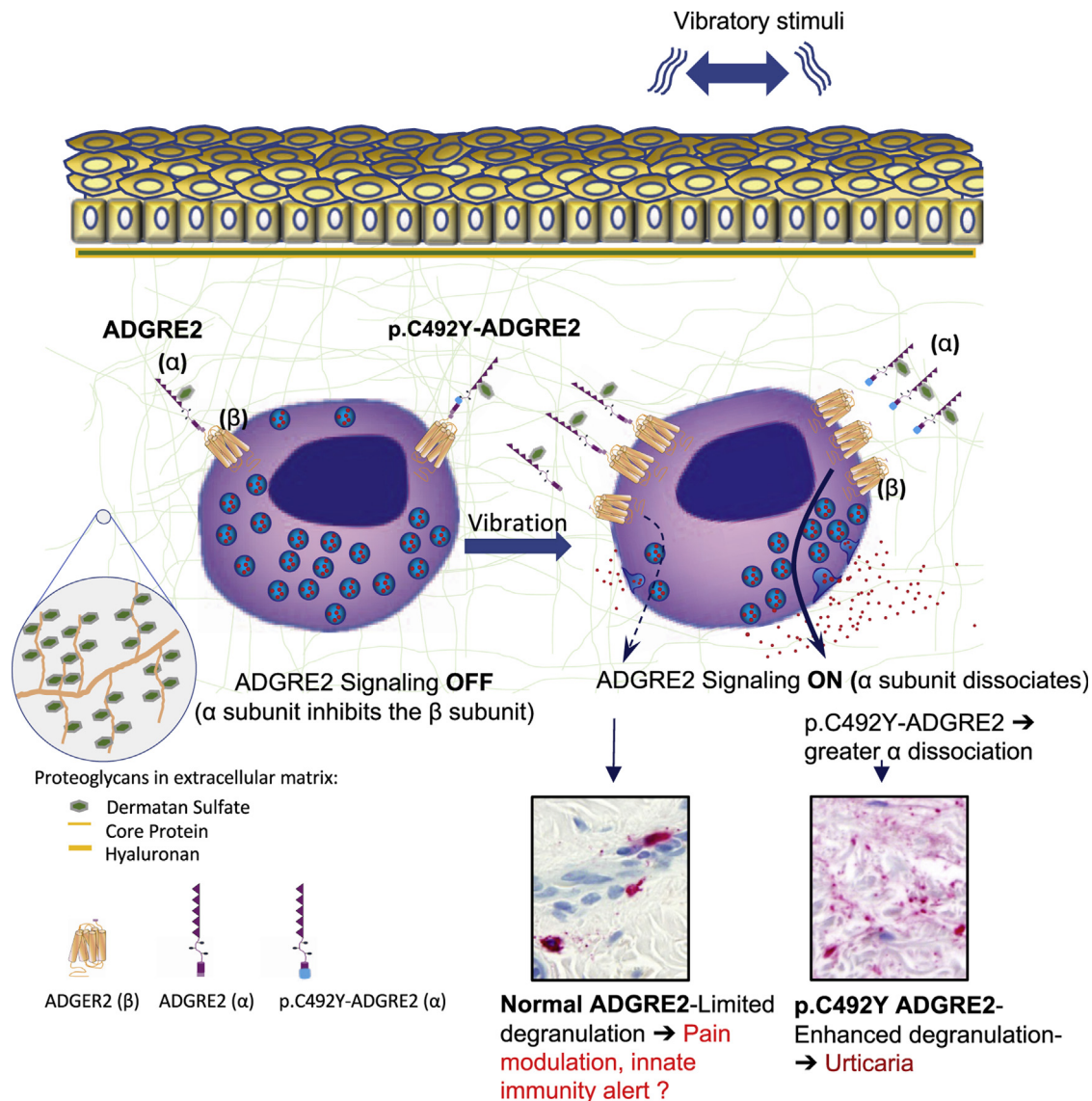


FIG 1. Proposed mechanism for mechanical stimulation of mast cells through ADGRE2 in healthy subjects and patients with VU. ADGRE2, through its α -subunit, binds dermatan sulfate, the most abundant proteoglycan in the skin. The α -subunit is noncovalently bound to the β -subunit (a 7TM domain) of ADGRE2. A mutation in the α -subunit (represented as a blue dot in the α -subunit) found in patients with familial VU facilitates dissociation between the 2 subunits after mechanical stimulation, allowing the β -subunit to become active and inducing calcium fluxes and degranulation. In healthy subjects dissociation of the subunits is not as efficient, and degranulation is not extensive. This response might be beneficial as an innate host defense mechanism, although the significance is still unknown. Dissociation of subunits on vibration is represented on the right side. Normal ADGRE2 receptors are on the left side of the mast cell and mutated ADGRE2 are represented on the right side of the mast cell. Immunohistochemistry tryptase staining of skin from an unaffected subject or an affected patient after vibration show the extent of degranulation in these samples. Dermatan sulfate is illustrated as a green hexagon, and a representation of the structure of proteoglycans containing dermatan sulfate in the skin is shown magnified on the left.

Adhesion G protein-coupled receptor E2, a sensor of mechanical stimulation in human mast cells

Recent findings have associated a missense substitution from cysteine to tyrosine (pC492Y) in the adhesion G protein-coupled receptor E2 (ADGRE2) as the only nonsynonymous variant cosegregating with autosomal dominant vibratory urticaria (VU). VU is a type of physical urticaria clinically and pathophysiologically distinct from dermatographism and characterized by localized hives and systemic manifestations in response to a local stimulus of frictional nature.⁴⁴ In patients

with VU, activation of mast cells after a vibratory stimulus is evidenced by the extensive diffuse tryptase staining in histologic samples of the affected sites in contrast to the intense tryptase staining within mast cells in previbrated skin (Fig 1) and by the rapid increase in histamine levels in venous blood from the affected areas.⁶ Although it has been known that mast cells are involved in the pathology of VU, the mechanisms of such a response, which are dependent on mechanical vibration but IgE independent, have remained a mystery. Studies by Boyden et al⁶ provided first evidence for the function of ADGRE2 in

mechanosensation by demonstrating that patient-derived mast cells or human mast cell lines expressing pC492Y-ADGRE2 degranulate extensively on mechanical vibration, recapitulating the VU phenotype. In contrast, human mast cells expressing nonmutated ADGRE2 show mild responses similar to the somewhat limited responses in healthy subjects after a vibratory challenge.

ADGRE2, also known as EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2) or CD132, belongs to a large family of adhesion GPCRs. Adhesion GPCRs generally contain a 7-transmembrane (7TM) domain (β -subunit), whose sequence provides the basis for classification of adhesion GPCRs into subfamilies and a large extracellular domain (α -subunit), which facilitates interactions with proteins from the extracellular matrix or expressed on the surfaces of other cells. Ligands for most of these receptors are not known, and even if they are identified, only a few are actual agonists that can evoke an intracellular response mediated by the 7TM domain.^{45,46}

ADGRE2 binds dermatan sulfate, the predominant glycosaminoglycan in the skin. However, binding of ADGRE2 to dermatan sulfate does not elicit, by itself, a detectable mast cell activation response. As is true for other adhesion GPCRs involved in mechanosensation, a mechanical force is needed in addition to dermatan sulfate binding to trigger mast cell degranulation.

By way of background, the α - and β -subunits of ADGRE2 are translated into a single polypeptide precursor, but early during trafficking of the receptor to the plasma membrane, this protein undergoes autocatalytic cleavage within its G-protein proteolytic site motif,⁴⁷ rendering 2 subunits that, for the most part, remain noncovalently bound.⁴⁸ This feature appears important for the mechanism of activation of these receptors.⁴⁶ Indeed, mutation of the cleavage site negates the hyperreactive phenotype of the pC492Y mutation, indicating the importance of the 2 separate subunits. During mechanical vibration of mast cells attached to dermatan sulfate, the α -subunit dissociates from the β -subunit, allowing it to signal. Thus it appears that mechanical forces activate this receptor by separating the α - and β -subunits. In patients with VU, the p.C492Y mutation destabilizes the inhibitory interaction between the α - and β -subunits, thereby increasing the susceptibility of these mast cells to vibration-induced degranulation (Fig 1).⁶

Although the physiologic relevance of the limited mast cell responses to friction in healthy subjects is not completely understood, possibilities are that ADGRE2 might subtly alert both resident and immune cells to combat potential injury and wound healing, play a role in pain modulation, and perhaps help sense a parasite migrating through dermal tissues (Fig 1).

IL-33 receptor in mast cells and its regulation of allergic inflammation

Ample experimental and clinical evidence has implicated IL-33, one of the IL-1/IL-18 family of cytokines, as a central player in T_H2 immune responses and the pathogenesis of allergic diseases. In support, genes encoding for IL-33 and its receptors have been identified as susceptibility loci in asthma in large-scale genome-wide association studies.⁴⁹⁻⁵¹ IL-33 is retained within the nucleus associated with chromatin in IL-33-producing cells, and it acts predominantly as an “alarmin” when released from these cells after cell damage induced by either injury or environmental agents.^{52,53} Although IL-33 is

produced in epithelial and other stromal cells, its receptor, ST2 (also referred to as the ST2L to differentiate it from the soluble form of ST2 [sST2]), is expressed in a variety of immune cells predominantly of the innate immune system, including mast cells and basophils. IL-33 binding to ST2L induces differentiation, survival, and chemotaxis of these cells and, most prominently, cytokine production.⁵³⁻⁵⁵ Mouse and human mast cell progenitor cells also express ST2L during development, even before expression of the IgE receptor.^{56,57} It appears that the progenitors of mast cells, basophils, and eosinophils produce T_H2 and proinflammatory cytokines in response to IL-33 more abundantly than the corresponding mature cells, suggesting these myeloid progenitors could also initiate inflammatory IL-33-mediated responses (Fig 2).⁵⁸

It is becoming apparent that IL-33 is a critical player orchestrating a variety of allergic inflammatory conditions through innate immune cells. Recent studies indicate that IL-33 is constitutively produced in developing lungs shortly after birth and at an early age, when the lung is remodeling. However, in the presence of inhaled allergens, IL-33 production is enhanced, causing accumulation of T_H2 innate immune cells, mast cells, eosinophils, and basophils. The authors of this work proposed that this axis of IL-33 production and T_H2 skewing by the activity of the recruited immune cells might be important for the development of asthma at an early age.⁵⁹ Similarly, IL-33 levels are found to be increased in the airways after intratracheal exposure to *Staphylococcus aureus*-derived serine protease-like protein D⁶⁰ and in the gastrointestinal tracts of mice with food allergy and promotes inflammation through its effects on innate immune cells.⁶¹

ST2L in mast cells as a receptor promoting allergic inflammation. Mast cells are emerging as critical amplifiers of IL-33-mediated inflammation (Fig 2) in the conditions described above, as well as in others, such as chronic asthma.⁶² For instance, mast cells were found to exacerbate IL-33-mediated airway constriction in mice through enhanced serotonin secretion⁶³ and IL-13 production.⁶⁴ Along these lines, studies on aspirin-exacerbated respiratory disease have suggested that overgeneration of cysteinyl leukotrienes induced by aspirin in these patients causes IL-33 production, which in turn activates mast cells and promotes bronchoconstriction.⁶⁵ Furthermore, mast cells are critical for IL-33-mediated recruitment of leukocytes in a model of skin inflammation⁶⁶ and for IL-33-mediated IL-9 production and acquisition of IgE-mediated food allergy susceptibility.⁶⁷ In the guts of mice with food allergy, increased IL-33 levels also activate innate lymphoid cells to produce IL-4, which further augments mast cell responses and reduces allergen-specific regulatory T (Treg) cell suppressive functions, thus promoting food allergy. In contrast to these roles for IL-33 and mast cells enhancing unwanted allergic inflammation, during intestinal helminth infections, mast cells have been reported to act as amplifiers of IL-33 production, group 2 innate lymphoid cell activation, and worm expulsion.⁶⁸

IL-33 released in the epithelia can also exacerbate responses to food or airway allergens through mast cells. For example, in a mouse model of food allergy, transcutaneous sensitization to a food allergen on injured skin barrier (by using tape stripping) caused increases in levels of IL-33, which potentiated anaphylaxis on oral antigen challenge. The mechanism involved IL-33-mediated activation of ST2L in mast cells and

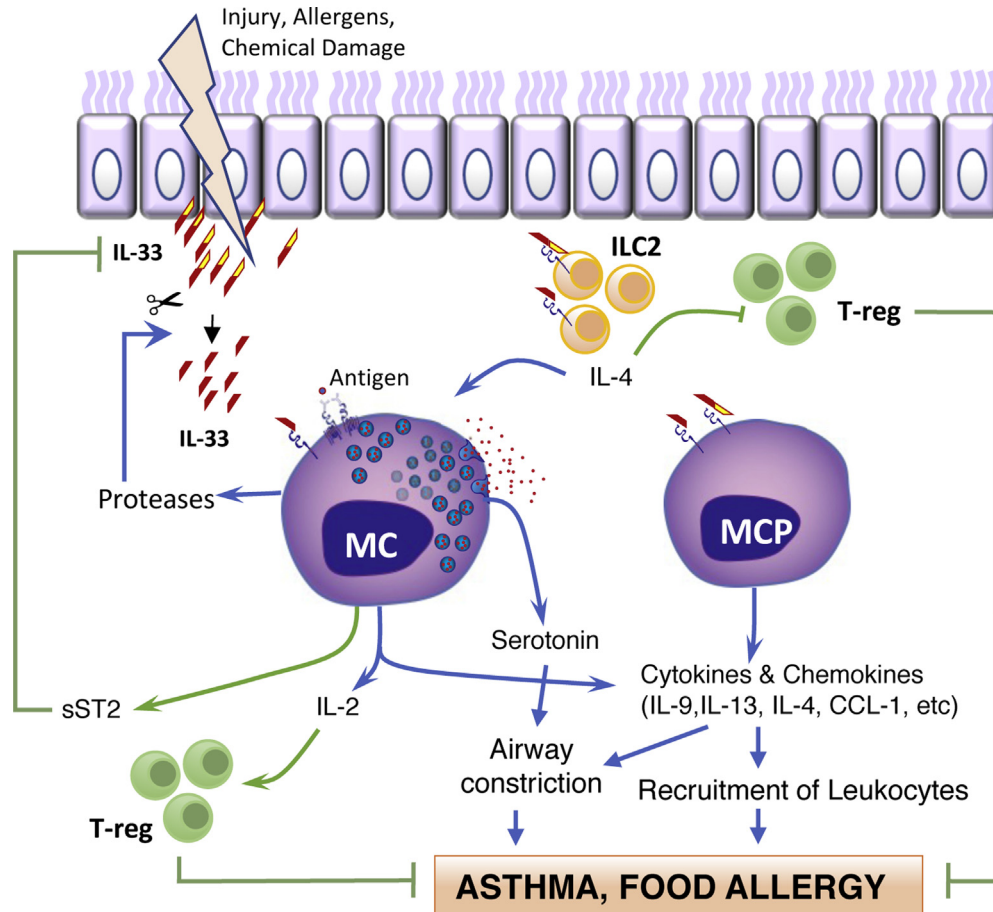


FIG 2. Mast cells modulate IL-33-induced allergic inflammation through ST2L. IL-33 is released from epithelial and other cells (eg, smooth muscle cells and fibroblasts) on tissue damage or stimulation. Mast cells can amplify (blue lines) or reduce (green lines) IL-33 responses. Mast cells and their progenitors (MCP), as well as group 2 innate lymphoid cells (ILC2) and other innate immune cells not represented here, respond to IL-33 through its receptor, ST2L, to produce a variety of T_H2 cytokines that promote allergic inflammation. In addition, IL-33 enhances antigen-induced release of vasoactive mediators by mast cells, which exacerbates asthma or food allergy symptoms. Proteases released by mast cells, in addition to other functions, cleave IL-33 into more active fragments. IL-4 produced by ILC2s in response to IL-33 can also enhance mast cell responses to antigen at the same time that inhibit Treg cell function, exacerbating allergic inflammation. On the other hand, IL-2 and sST2 produced by mast cells ameliorates inflammation by stimulating Treg cells and quenching IL-33 function, respectively. These positive and negative signals can be favored or disfavored in the *in vivo* environment, depending on other environmental cues, shaping the balance of these responses.

augmentation of allergen-specific, IgE-mediated mast cell degranulation.⁶⁹ This mechanism might be relevant in patients with a disrupted skin barrier, including those with atopic dermatitis, and might link cutaneous sensitization to food allergens and food-induced anaphylaxis.⁶⁹ Confocal studies in cultured human mast cells showed that potentiation of FcεRI-mediated responses by means of IL-33 pretreatment is the result of enhancement in the magnitude of individual responses, as well as in the frequency of cells that respond to FcεRI cross-linking.⁷⁰

Potentiation of antigen-mediated mast cell responses by IL-33 production have also been observed in other allergy models. In a mouse model that might resemble the atopic march in human subjects, subcutaneous sensitization to a protease allergen and IgE/IgG₁ production (via a mast cell-dependent process) followed by re-exposure to the protease allergen by

means of inhalation (which induces IL-33 production) caused greater inflammatory airway responses and antibody production than seen in mice exposed only to repeated airway exposures.⁷¹

Other studies have implicated not only IL-33 but also thymic stromal lymphopoietin and IL-25 in the induction and maintenance of IgE-mediated responses in a mouse model in which food anaphylaxis is induced by means of oral gavage. Although neutralization of either of these cytokines suppressed food allergy development in this model, neutralization of all 3 cytokines was needed to suppress established food anaphylaxis.⁷²

Mast cell tryptase and chymase released during allergic reactions (Fig 2) can also contribute to promoting the IL-33 inflammatory potential by cleaving IL-33 to generate more active fragments than the full-length IL-33.⁷³

ST2L in mast cells mediating anti-inflammatory functions. Although mast cells are key contributors to the proinflammatory functions of IL-33, evidence also suggests that mast cells can ameliorate IL-33-mediated inflammatory effects under certain circumstances. Stimulation of mast cells with IL-33 in the absence of IgE cross-linking can induce Treg cell expansion by producing IL-2, and this has been shown to limit inflammation in a papain-induced innate-type airway inflammation model.⁷⁴

In addition to IL-2-mediated effects on Treg cells, both human and mouse mast cells produce substantial amounts of sST2 when activated through ST2L, FcεRI, or KIT.⁷⁵ sST2 is a decoy receptor that neutralizes the actions of IL-33. Thus mast cells can act to limit the later phases of the allergic inflammatory response by releasing this receptor.

These dual actions of mast cells as potent enhancers of the inflammatory action of IL-33 but also in counteracting excessive inflammation are consistent with the immunomodulatory role of mast cells in constraining an inflammatory process and re-establishing tissue integrity. One can speculate that the balance between the proinflammatory and anti-inflammatory roles of mast cells might be regulated by additional microenvironmental cues, depending on the pathophysiologic situation. For example, production of lactic acid,⁷⁶ a byproduct of anaerobic glycolysis present in pathologic environments that promote IL-33 production (inflammation, wound healing, and tumors) and TGF-β1,⁷⁷ levels of which are increased in patients with chronic inflammatory conditions and certain allergic diseases, markedly reduced IL-33-mediated mast cell inflammatory responses.

Even circadian rhythm can affect the production of cytokines by mast cells in response to IL-33 by transcriptionally modulating the expression of ST2.⁷⁸ The authors suggested that augmented IL-33-mediated mast cell responses observed in the resting phases of the day (the night in human subjects) could be a contributory factor to explain the development of nocturnal symptoms in patients with asthma and virus-induced exacerbations.

MAST CELL PROTEASES: AN UPDATE

Proteases are stored in mast cell granules and represent a high fraction of all protein content. Whole-transcriptome analysis suggests that expression of transcripts for serine proteases constitutes the most significant category of gene products that differentiate tissue-resident mast cells from other immune cells.²⁷ These proteases, together with other granule contents, are released into the interstitial space on mast cell activation, where they exert their biologic functions. Indeed, nonbiased proteomic analysis of mast cell releasate identified proteases as the predominant type of proteins secreted.⁷⁹ In addition to releasing the intracellular contents of its granules as soluble mediators, recent data indicate that mouse mast cells, particularly on activation of FcεRI, release small extracellular vesicles that contain proteases, which in the case of carboxypeptidase 3 (Cpa3) constituted about 10% of the total Cpa3 released. This mode of release of encapsulated material can be potentially important for immune regulation and transport of combined cargo to distant cells.⁸⁰

Yet the notion that mast cell proteases exclusively function once released into the extracellular media has been challenged by findings of tryptase in nuclei in association with mast cell chromatin, suggesting an intracellular function.⁸¹ These authors

found that chromatin-associated tryptase truncates histones 3 and histone 2B and modulates acetylation of histone 2B. This tryptase-mediated epigenetic modification appears to be important for maintenance of the mast cell lineage signature, morphology, and proliferative control of mast cells, adding another layer to the complexity of mast cell protease biological functions.⁸¹

Mast cell proteases cleave a number of functionally diverse protein substrates through recognition of specific peptide sequences. Proteolytic cleavage of these substrates can result in either activation or inhibition, and thus their specific roles in specific physiopathologic conditions are complex and depend on the specific environment.⁸² For instance, proteases released from mast cells have been linked to angiogenesis in tissue repair, cancer and bone homeostasis, and inflammation in patients with allergic diseases and other inflammatory conditions, such as inflammatory bowel disease and arthritis.^{9,82-86} Mast cells also play protective roles in host defense against parasites and bacteria, as reviewed by Caughey,⁸² and in defense of vertebrates to animal venoms, including honey bees, scorpions, and reptile venoms, as reviewed by Tsai et al.⁸⁷ Venom-induced innate activation of mast cells results in release of proteases, such as Cpa3 and mast cell protease 4 (homolog of human chymase), that neutralize certain venoms and reduce morbidity and mortality to these venoms in mice.⁸⁷ Furthermore, venom-specific IgE antibodies and IgE-mediated mast cell responses after re-exposure to venoms contribute to protection against lethal doses of these toxic venoms.^{88,89} An interpretation of these observations in mouse models is that anaphylaxis, when appropriately regulated, is beneficial rather than detrimental in the pathology associated with envenomation.⁸⁷

The *TPSAB1* and *TPSB2* mast cell tryptase loci in human subjects encode α- and β-tryptases. Although both loci can express β-tryptases, α-tryptase is believed to be restricted to *TPSAB1*, resulting in α-tryptase/β-tryptase-encoding sequence ratios of 0:4, 1:3, or 2:2 in different subjects. Despite the homology between the 2 tryptases, mature β-tryptase is proteolytically active as a homotetramer, but mature α-tryptase appears to be enzymatically inactive.⁸⁶ Although about a fourth of the general population is deficient in α-tryptase without any noticeable manifestations, recent studies suggest that germline duplications and triplications of *TPSAB1* encoding α-tryptase are linked to subjects with dominantly inherited increased basal serum tryptase levels and with multisystem disorders in cases in which clonal mast cell disease or mast cell activation syndrome is not evident.⁹⁰ The symptom complexes in these patients include irritable bowel syndrome, cutaneous flushing, connective tissue abnormalities, and dysautonomia. In the cohort subjects with duplications on both alleles or a triplication on a single allele showed higher tryptase levels in serum and were more symptomatic than subjects with duplications on only 1 allele, suggesting a gene-dose effect.⁹⁰ The authors further studied whether a second coinherited genetic variant might help explain the syndromic presentation of α-tryptasemia and found that 3 functional variants of the α 1H subunit of the T-type calcium channel Ca_v3.2, *CACNA1H*, were frequently coinherited with *TPSAB1* duplications. Despite the association of this channel with irritable bowel syndrome and heightened anxiety in other studies, the variants did not show a clear enhancement of clinical phenotypes associated with *TPSAB1* duplications at least in a relatively small cohort.⁹¹ Further studies are needed to understand the function of

α -tryptase and how duplications of the α -tryptase gene might cause the associated symptomatology in these patients.

HUMANIZED MOUSE MODELS TO STUDY HUMAN MAST CELL FUNCTION *IN VIVO*

Mast cell-deficient mouse models, including *Kit* mutant mice that lack the receptor function necessary for mast cell growth and survival, mice with deletions in mast cell proteases, and mice with a conditional deletion of the Fc ϵ RI β -chain,^{92–94} have been critical to identify and characterize mouse mast cell functions *in vivo* in conjunction with adoptive transfer of cultured mast cells into these mice. In the last few years, a new direction has been the development of humanized mouse models in which human hematopoietic progenitors are engrafted into immunodeficient mice and develop into adaptive and innate human cells, providing an opportunity to study human mast cell responses in a humanized physiologic context and to investigate the use of human-specific therapies.^{95–98}

Two similar strains of nonobese diabetic severe combined immunodeficient mice lacking the cytokine receptor common γ chain (NOG [NOD.*cg-Prkdc^{scid}Il2rg^{tm1Sug}*] and NSG [NOD.*Cg-Prkdc^{scid}Il2rg^{tm1Wjl}*] mice) have been successfully used for engraftment of human mast cells. Even though engraftment of human mast cells appears to be somewhat limited,^{95,97} these mice have been used for the study of mast cell-related diseases, such as systemic mastocytosis, in which cells of a human mast cell line with a mutation in D816V (ROSAKITD816V-Gluc) were injected into NSG mice, reproducing characteristics of this disorder⁹⁸; in a model of allergen-driven gut inflammation in which PBMCs from allergic subjects were injected into these mice and challenged orally or rectally with the allergen to measure allergic colitis⁹⁹; and in a model of peanut allergy in which NSG mice were inoculated with mononuclear cells from patients with peanut allergy and then sensitized and challenged with peanut extract to reproduce a human anaphylactic phenotype that was prevented with a single administration of an adeno-associated virus expressing persistent levels of anti-human IgE.¹⁰⁰

Transgenic expression of human cytokines into these immunodeficient mice, particularly human IL-3 and GM-CSF,⁹⁷ SCF,^{96,101} or a combination of IL-3, GM-CSF, and SCF,⁹⁵ seems to significantly improve the engraftment of human mast cells. Transplantation of human stem cells into NOG mice expressing human IL-3 and GM-CSF showed passive cutaneous anaphylaxis reactions in response to human IgE and intradermal antigen challenge with mast cells engrafted in various tissues, including the skin.⁹⁷ Mice with human membrane-bound SCF also successfully engrafted human mast cells, outnumbering mouse mast cells in the small intestine, stomach, and skin, which resulted in engraftment of the T- and B-cell compartments. This allowed active oral sensitization to peanut, resulting in increased numbers of mast cells in the spleen and jejunum and production of peanut-specific human IgE. On challenge, the humanized mice presented anaphylactic reactions accompanied by increases in human tryptase levels in plasma, overall recapitulating features of human food-induced anaphylaxis.⁹⁶ Additionally, NSG mice expressing a combination of the cytokines IL-3, GM-CSF, and SCF have also been engrafted with CD34⁺ human stem cells after implantation of these mice with autologous thymic and liver human fragments (BLT model), resulting in robust populations

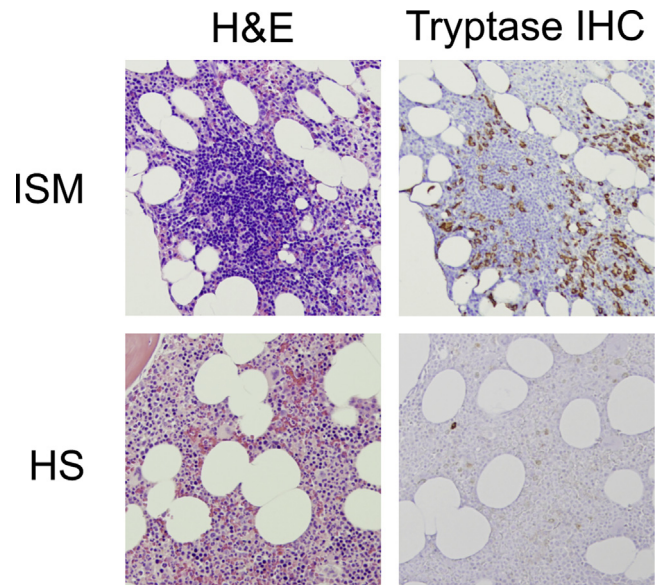


FIG 3. Increase in mast cell counts in the marrow of a patient with a mast cell proliferative disorder. Bone marrow biopsy specimen from an adult patient with indolent systemic mastocytosis (ISM; upper panels) compared with a bone marrow biopsy specimen from an adult with recurrent unexplained anaphylaxis (lower panels; $\times 200$ magnification) stained with hematoxylin and eosin (H&E; left panels) or for mast cell tryptase (right panels). A prominent mast cell infiltrate is apparent in the tryptase-stained biopsy specimen from the patient with ISM. Courtesy of Dr Melody Carter. HS, Healthy subject; IHC, immunohistochemistry.

of myeloid cells and human immune cells in mucosal and peripheral tissues. In this model passive systemic and cutaneous anaphylactic reactions to human IgE were observed. Furthermore, a large population of human mast cells was obtained from the peritoneal cavities of these mice, which could be expanded in culture and used for *ex vivo* studies.⁹⁵

A challenge using these models is the large number of progenitors needed to adoptively transfer a substantial group of mice and the time required for proper engraftment (>10 weeks) and active sensitization protocols (when applicable), which in combination might also be costly. In addition, models like the BLT model⁹⁵ require implantation of autologous liver and thymus fragments before injection of blood progenitors, and these samples and procedures might not be easily accessible. Nevertheless, despite these and other limitations intrinsic to the model, studies have unlocked an exciting new approach to study human mast cell biology *in vivo* and address more predictable outcomes in preclinical studies in allergic reactions and other disorders in which mast cells are key.

MAST CELL PROLIFERATIVE DISORDERS

Mastocytosis is a clonal disorder of mast cells and often associated with gain-of-function mutations in *KIT*. It is classified into variants from indolent to aggressive disease based on the extent and characteristics of pathologic findings (Fig 3). As classification evolved, it became clear that adult and pediatric patients exhibited diverse types of cutaneous lesions. To standardize terminology, an international task force within the European Competence network on Mastocytosis in collaboration with the American Academy of Allergy, Asthma & Immunology

and the European Academy of Allergology and Clinical Immunology proposed new and revised consensus definitions and criteria for cutaneous manifestations of mastocytosis in a consensus document published recently in the *Journal of Allergy and Clinical Immunology*.¹⁰² Specifically, the group recommended that maculopapular cutaneous lesions, previously referred to as urticaria pigmentosa, be subdivided into variants. The first is a monomorphic variant with small maculopapular cutaneous lesions. This variant is typically seen in adult patients and, when it develops in children, is more likely to persist into adulthood. The second is a polymorphic variant with larger lesions of variable size and shape that is more likely to resolve around puberty. The recommendations also included removing telangiectasia macularis eruptive perstans from the current classification of cutaneous mastocytosis.

Several recent clinical studies focusing on the mast cell compartment in patients with mastocytosis have reported novel findings that might relate to future therapeutic intervention. In one study expression of CD123, the α -subunit of the IL-3 receptor, was determined in bone marrow–derived mast cells from 58 patients with systemic mastocytosis.¹⁰³ CD123 expression on these mast cells was found to be 100% in patients with aggressive systemic mastocytosis, 61% in patients with indolent systemic mastocytosis, and 57% in patients with systemic mastocytosis with an associated hematologic malignancy. Focal proliferation of plasmacytoid dendritic cells was observed around mast cell aggregates and was greater in patients with CD123⁺ mast cells. The authors suggested that targeting CD123 in systemic mastocytosis could have antitumor actions through direct effects on mast cells and indirect effects through plasmacytoid dendritic cells.

Programmed cell death protein 1 (PD-1) is a checkpoint protein on activated T cells, B cells, and cutaneous mast cells. PD-1 acts as a type of off switch to prevent these cells from attacking other cells by binding to programmed cell death ligand 1 (PD-L1) expressed on activated T cells, dendritic cells, and monocytes or PD-L2 on dendritic cells and monocytes. Some malignant cells express substantial amounts of PD-1, which helps them escape immune attack. Monoclonal antibodies that target this interaction might enhance the immune response against cancer cells. Two examples are pembrolizumab (Keytruda) and nivolumab (Opdivo).

Examining this PD-1–PD-L axis in the sera of patients with mastocytosis, one group found that serum levels of PD-L1 were significantly increased in adult patients with mastocytosis, and levels were greater in those with SM-AHD, aggressive systemic mastocytosis, and mast cell leukemia.¹⁰⁴ They also found that mast cells from the bone marrow and skin of patients with mastocytosis were PD-L1 positive. They concluded that PD-L1 is a diagnostic marker of disease progression in adult patients with mastocytosis and that the efficacy of antibodies to PD-1 and PD-L1 should be explored in the treatment of advanced mastocytosis.

Like PD-L1, serum levels of IL-6 are a biomarker of disease in patients with mastocytosis and correlate with serum total tryptase levels. The possible contribution of IL-6 to mast cell proliferation has been clarified by a report that IL-6 enhances mast cell proliferation, maturation, and reactivity by reducing suppressor of cytokine signaling 3.¹⁰⁵ These findings related to the methylation of the suppressor of cytokine signaling 3 promotor and increased expression and activation of signal transducer and

activator or transcription 3 (STAT3). Data supported the possibility that IL-6 blockade can ameliorate mast cell–dependent pathology in mastocytosis. Interestingly, mast cell mediator–induced anaphylaxis is blunted in STAT3 mutant mice and in wild-type mice subjected to small-molecule STAT3 inhibition.¹⁰⁶ These observations, along with the IL-6 data, suggest targeting STAT3 might have a beneficial role in lessening the effects of systemic mediator release in patients with mastocytosis.

NEW INTERVENTIONS THAT ALTER THE MAST CELL COMPARTMENT

Many of the observations made in studying the pathogenesis and treatment of mast cell proliferative disorders also relate to developing approaches to treating allergic disease. Long-term treatment of patients with chronic myeloid leukemia with imatinib resulted in a reduction of bone marrow mast cell numbers to 5% of pretreatment values.¹⁰⁷ This was accompanied by a significant decrease in serum tryptase levels. Other myeloid lineages were not affected. The authors found no evidence of a clinical syndrome attributable to drug-induced mast cell deficiency. Specifically, there was no increase in the frequency of thromboembolic events, cancer, or severe bacterial or fungal infections.

This study showing the effects of imatinib on the mast cell compartment is echoed in a randomized double-blind clinical trial that examined the effect of imatinib in patients with poorly controlled severe asthma.¹⁰⁸ In this 24-week trial, imatinib was shown to decrease airway hyperresponsiveness and reduce levels of serum tryptase, although airway mast cell counts decreased in both groups. Use of imatinib was associated with a higher likelihood of muscle cramps and hypophosphatemia. One patient in the imatinib group discontinued participation because of neutropenia thought by investigators to be related to the study agent. Nevertheless, use of imatinib might be considered in other diseases in which mast cells have recently been implicated in pathogenesis, such as celiac disease.¹⁰⁹

Thus pharmacologic approaches directed at decreasing tissue mast cell numbers or perhaps ablating the mast cell compartment might be of benefit in instances in which mast cells are major contributors to disease development. However, the evolutionary advantage and circumstances in which mast cells might be critical in human biology are only partly understood. From mouse models, it is clear that mast cells play important roles in host responses against helminths and venoms and other aspects of host defense and perhaps tissue repair. Thus targeting mast cells might be inconsequential if the host is not exposed to those situations or other arms of the immune system can compensate for the lack of mast cells. This dictates the essential need to carefully monitor subjects for the consequences of therapeutic approaches targeted at ablation of the mast cell compartment.⁹³

In addition to existing drugs repurposed for treatment of diseases involving mast cells by decreasing mast cell number and activation, 2 recent reports of novel therapeutic approaches have also been reported. In the first of these articles, a recently identified technique referred to as exon skipping has been specifically applied to target and downregulate IgE receptor expression on mast cells.¹¹⁰ This approach affects specifically the mast cell/basophil compartment, unlike use of tyrosine kinase

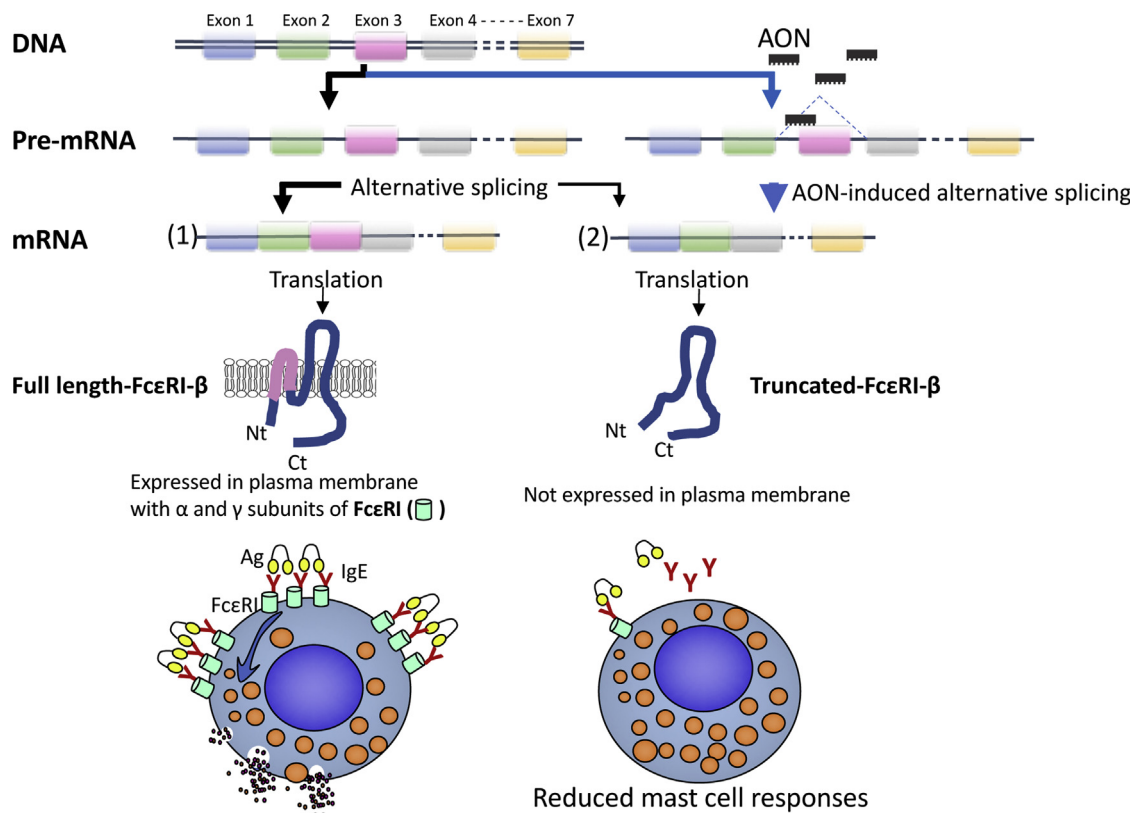


FIG 4. Antisense oligonucleotide (AON)-mediated exon skipping of the β -subunit of Fc ϵ RI to specifically target and downregulate IgE receptor expression in mast cells. The membrane spanning 4A2 gene (*MS4A2*) codes for the β -subunit of Fc ϵ RI (Fc ϵ RI β). Fc ϵ RI β contributes to IgE-dependent mast cell signaling by trafficking the Fc ϵ RI receptor complex to the cell surface and amplifying its signaling. The *MS4A2* gene has 7 exons (not all exons are depicted in this illustration). In mast cells an alternative splice variant of Fc ϵ RI β missing exon 3 (in pink) has been described. Such a variant is translated into a truncated Fc ϵ RI β that lacks the first transmembrane domain of Fc ϵ RI β . This transmembrane domain is required for trafficking the IgE receptor complex to the plasma membrane. Although this variant is not abundantly expressed, *MS4A2* splicing can be altered to favor the expression of truncated Fc ϵ RI β by using AON-mediated exon skipping (blue arrows). Specific AONs promote splicing of exon 3 and thus the disproportionate expression of truncated Fc ϵ RI β , which renders mast cells unable to express cell-surface receptor or respond to an antigen challenge.

inhibitors that target receptors or pathways present in multiple cell types. This approach used antisense oligonucleotide-mediated exon skipping of the β -subunit of Fc ϵ RI to eliminate surface expression of Fc ϵ RI β , which in turn eliminates surface expression of Fc ϵ RI. Mast cells are then rendered unresponsive to IgE-mediated activation (Fig 4). This approach worked *in vivo*, as shown in mouse models of IgE-dependent allergic dermatitis.¹¹⁰

Another novel approach depended on engineering a mechanism-based SCF partial agonist.¹¹¹ Normally, SCF is a growth factor that acts through KIT to promote hematopoietic progenitor cell expansion. However, when SCF is administered *in vivo*, it can lead to mast cell degranulation through dimerization of KIT on the mast cell surface. However, the SCF partial agonist retains the ability to activate hematopoietic progenitors while exhibiting virtually no anaphylactic off-target effects. It remains to be determined whether administration of this SCF partial agonist would block SCF effects in promoting mast cell releasability and thus find application to management of mast cell diseases in general.

CONCLUDING REMARKS

Early after the description of mast cells by Paul Ehrlich, it was understood that mast cell function might rely on the unique content of their granules and releasability. The morphologic uniqueness of mast cells is mirrored by a signature of transcripts that sets them apart from other cells and is to some degree defined by granule content, enzymes involved in their synthesis, and certain cell-surface receptors. The “histamine liberators,” such as compound 48/80, which were found to induce mast cell degranulation in the 1950s provided the first clues on the involvement of mast cells in anaphylaxis. However, it was not until recently that the enigmatic mode of action of these histamine liberators has been resolved with the discovery of Mas-related receptors. Findings related to these receptors,⁵ together with their relative promiscuity and the fact that the ancestral mast cell prototype in urochordates and in zebrafish also respond to compound 48/80, suggest beneficial and evolutionarily conserved functions for mast cells in innate immunity. Other receptors, such as ADGRE2, present in human mast cells cause pseudoallergic reactions in response to vibration in certain subjects, and this

sensing of friction-like perturbations by mast cell ADGRE2 might also be related to a role in innate immunity.

In addition to the view of mast cells as culprit cells in allergic reactions through the IgE receptor, an emerging recent perception incorporates the interpretation that these ancient cells in evolution have a protective role for the host, even during acquired immunity in which mast cell–released products might serve to protect against animal venoms or poisonous substances. The downside is that widespread activation of mast cells might be lethal, perhaps aberrantly so, because of alterations in mast cells, their mediators, or local tissue signals. Newer research emphasizes that the responsiveness of mast cells is not solely dictated by a single receptor that triggers mast cell activation, but by a compilation of signals in the pathologic environment that are modifiers of such responses and that might tilt the balance toward a lethal or simply unwanted outcome. This is illustrated by a recent demonstration in a mouse model that food-induced reactions require, in addition of antigen-specific IgE, production of IL-9 by an immature population of mucosal mast cells (MMC9) that drive their own differentiation and whose program require certain tissue signals.⁶⁷ Another aspect of active investigation is the identification and understanding of function of inhibitory receptors in mast cells. Overall, the identification of receptors that promote mast cells to be activated or inactivated or that modify their responses to antigen, as well as the understanding of allergic and pseudoallergic responses, will help in understanding how to address these unwanted mast cell reactions.

SUMMARY

- Transcriptomic and functional analysis define phenotypic differences within constitutive mast cells from different tissues, reflecting the plasticity of mast cells.
- Local tissue cytokines, such as IL-33, which are present in a pathologic environment, reprogram mast cells or mold their responsiveness to antigen and might explain observed dissociations between levels of allergen-specific IgE and manifestation of allergic reactions.
- Further identification of these factors in mast cell–related disorders and how inflammatory environments affect mast cell programs will aid in our understanding of aberrant mast cell biology.
- MRGPRX2 and ADGRE2 are examples of non-IgE-mediated activation of mast cells that can drive pseudoallergic reactions seen in the clinic and a further understanding of their roles and mechanisms might aid in providing better management of these diseases.
- Humanized models of mast cell–related diseases are now being used and will prove useful as more accurate preclinical models for mast cell–related diseases.

We dedicate this review to the memory of Michael A. Beaven, who passed away unexpectedly last April 2017 while working on this review. He was an expert in mast cell biology and a beloved mentor and colleague. Dr Beaven worked at the National Heart, Lung, and Blood Institute (National Institutes of Health [NIH]) for 47 years before retiring from his position as the Deputy Chief of the Laboratory of Molecular Immunology to become an NIH Scientist Emeritus in 2010. As such, and to our benefit, he continued to work full time in collaboration with the Laboratory of Allergic Diseases (LAD),

National Institute of Allergy and Infectious Diseases (NIH). We are greatly indebted to Dr Beaven for his dedication to the projects and for his sound advice and expertise on mast cell biology. His extensive contributions to the mast cell field and to the LAD mission are reflected by his long list of publications, including the publications we were honored to coauthor.

REFERENCES

1. Mukai K, Tsai M, Starkl P, Marichal T, Galli SJ. IgE and mast cells in host defense against parasites and venoms. *Semin Immunopathol* 2016;38:581-603.
2. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth allergens, parasite-specific IgE, and its protective role in human immunity. *Front Immunol* 2014;5:61.
3. St John AL, Abraham SN. Innate immunity and its regulation by mast cells. *J Immunol* 2013;190:4458-63.
4. Haidl ID, Marshall JS. Human mast cell activation with viruses and pathogen products. *Methods Mol Biol* 2015;1220:179-201.
5. Subramanian H, Gupta K, Ali H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J Allergy Clin Immunol* 2016;138:700-10.
6. Boyden SE, Desai A, Cruse G, Young ML, Bolan HC, Scott LM, et al. Vibratory urticaria associated with a missense variant in ADGRE2. *N Engl J Med* 2016;374:656-63.
7. da Silva EZ, Jamur MC, Oliver C. Mast cell function: a new vision of an old cell. *J Histochem Cytochem* 2014;62:698-738.
8. Krystal-Whittemore M, Dileepan KN, Wood JG. Mast cell: a multi-functional master cell. *Front Immunol* 2015;6:620.
9. Ribatti D, Ranieri G. Tryptase, a novel angiogenic factor stored in mast cell granules. *Exp Cell Res* 2015;332:157-62.
10. Galli SJ. The mast cell-IgE paradox: from homeostasis to anaphylaxis. *Am J Pathol* 2016;186:212-24.
11. Johnzon CF, Ronnberg E, Pejler G. The role of mast cells in bacterial infection. *Am J Pathol* 2016;186:4-14.
12. Graham AC, Temple RM, Obar JJ. Mast cells and influenza A virus: association with allergic responses and beyond. *Front Immunol* 2015;6:238.
13. Saluja R, Metz M, Maurer M. Role and relevance of mast cells in fungal infections. *Front Immunol* 2012;3:146.
14. Oldford SA, Marshall JS. Mast cells as targets for immunotherapy of solid tumors. *Mol Immunol* 2015;63:113-24.
15. Oskeritzian CA. Mast cell plasticity and sphingosine-1-phosphate in immunity, inflammation and cancer. *Mol Immunol* 2015;63:104-12.
16. Russi AE, Walker-Caulfield ME, Brown MA. Mast cell inflammasome activity in the meninges regulates EAE disease severity. *Clin Immunol* 2016 [Epub ahead of print].
17. Xu Y, Chen G. Mast cell and autoimmune diseases. *Mediators Inflamm* 2015; 2015:246126.
18. Crivellato E, Travan L, Ribatti D. The phylogenetic profile of mast cells. *Methods Mol Biol* 2015;1220:11-27.
19. Cavalcante MC, de Andrade LR, Du Bocage Santos-Pinto C, Straus AH, Takahashi HK, Allodi S, et al. Colocalization of heparin and histamine in the intracellular granules of test cells from the invertebrate *Styela plicata* (Chordata-Tunicata). *J Struct Biol* 2002;137:313-21.
20. Wong GW, Zhuo L, Kimata K, Lam BK, Satoh N, Stevens RL. Ancient origin of mast cells. *Biochem Biophys Res Commun* 2014;451:314-8.
21. Akula S, Mohammadamin S, Hellman L. Fc receptors for immunoglobulins and their appearance during vertebrate evolution. *PLoS One* 2014;9:e96903.
22. Da'as S, Teh EM, Dobson JT, Nasrallah GK, McBride ER, Wang H, et al. Zebrafish mast cells possess an FcεRI-like receptor and participate in innate and adaptive immune responses. *Dev Comp Immunol* 2011;35:125-34.
23. Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. *Curr Opin Immunol* 2007;19:31-8.
24. Dahlin JS, Malinowski A, Ohrvik H, Sandelin M, Janson C, Alving K, et al. Lin-CD34hi CD117int/hi FcεpsilonRI+ cells in human blood constitute a rare population of mast cell progenitors. *Blood* 2016;127:383-91.
25. Dahlin JS, Heyman B, Hallgren J. Committed mast cell progenitors in mouse blood differ in maturity between Th1 and Th2 strains. *Allergy* 2013;68:1333-7.
26. Bankova LG, Dwyer DF, Liu AY, Austen KF, Gurish MF. Maturation of mast cell progenitors to mucosal mast cells during allergic pulmonary inflammation in mice. *Mucosal Immunol* 2015;8:596-606.
27. Dwyer DF, Barrett NA, Austen KF. Immunological Genome Project Consortium. Expression profiling of constitutive mast cells reveals a unique identity within the immune system. *Nat Immunol* 2016;17:878-87.
28. Furedi W, Agis H, Willheim M, Bankl HC, Maier U, Kishi K, et al. Differential expression of complement receptors on human basophils and mast cells. Evidence

- for mast cell heterogeneity and CD88/CD5aR expression on skin mast cells. *J Immunol* 1995;155:3152-60.
29. Motakis E, Guhl S, Ishizu Y, Itoh M, Kawaji H, de Hoon M, et al. Redefinition of the human mast cell transcriptome by deep-CAGE sequencing. *Blood* 2014;123:e58-67.
 30. Wang Z, Mascarenhas N, Eckmann L, Miyamoto Y, Sun X, Kawakami T, et al. Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes. *J Allergy Clin Immunol* 2017;139:1205-16.e6.
 31. Gilfillan AM, Beaven MA. Regulation of mast cell responses in health and disease. *Crit Rev Immunol* 2011;31:475-529.
 32. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012;18:693-704.
 33. Finkelman FD, Khodoun MV, Strait R. Human IgE-independent systemic anaphylaxis. *J Allergy Clin Immunol* 2016;137:1674-80.
 34. Kulka M, Sheen CH, Tancowny BP, Grammer LC, Schleimer RP. Neuropeptides activate human mast cell degranulation and chemokine production. *Immunology* 2008;123:398-410.
 35. Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K, et al. Antimicrobial peptides human α -defensins and cathelicidin LL-37 induce the secretion of a proinflammatory cytokine IL-31 by human mast cells. *J Immunol* 2010;184:3526-34.
 36. Yu Y, Blokhuis BR, Garssen J, Redegeld FA. Non-IgE mediated mast cell activation. *Eur J Pharmacol* 2015;778:33-43.
 37. Ferry X, Brehin S, Kamel R, Landry Y. G protein-dependent activation of mast cell by peptides and basic secretagogues. *Peptides* 2002;23:1507-15.
 38. Tatemoto K, Nozaki Y, Tsuda R, Konno S, Tomura K, Furuno M, et al. Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors. *Biochem Biophys Res Commun* 2006;349:1322-8.
 39. McNeil BD, Pundir P, Meeker S, Han L, Undem BJ, Kulka M, et al. Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions. *Nature* 2015;519:237-41.
 40. Zhang T, Che D, Liu R, Han S, Wang N, Zhan Y, et al. Typical antimicrobials induce mast cell degranulation and anaphylactoid reactions via MRGPRX2 and its murine homologue MRGPRB2. *Eur J Immunol* 2017;47:1949-58.
 41. Gaudenzio N, Sibillano R, Marichal T, Starkl P, Reber LL, Cenac N, et al. Different activation signals induce distinct mast cell degranulation strategies. *J Clin Invest* 2016;126:3981-98.
 42. Fujisawa D, Kashiwakura J, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014;134:622-33.e9.
 43. Gupta K, Idahosa C, Roy S, Lee D, Subramanian H, Dhingra A, et al. Differential regulation of Mas-related G protein coupled receptor X2-mediated mast cell degranulation by antimicrobial host defense peptides and *Porphyromonas gingivalis* LPS. *Infect Immun* 2017;85; <https://doi.org/10.1128/IAI.00246-17>.
 44. Abajian M, Schoepke N, Altrichter S, Zuberbier T, Maurer M. Physical urticarias and cholinergic urticaria. *Immunol Allergy Clin North Am* 2014;34:73-88.
 45. Kishore A, Hall RA. Versatile signaling activity of adhesion GPCRs. *Handb Exp Pharmacol* 2016;234:127-46.
 46. Scholz N, Monk KR, Kittel RJ, Langenhan T. Adhesion GPCRs as a putative class of metabotropic mechanosensors. *Handb Exp Pharmacol* 2016;234:221-47.
 47. Lin HH, Chang GW, Davies JQ, Stacey M, Harris J, Gordon S. Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. *J Biol Chem* 2004;279:31823-32.
 48. Huang YS, Chiang NY, Hu CH, Hsiao CC, Cheng KF, Tsai WP, et al. Activation of myeloid cell-specific adhesion class G protein-coupled receptor EMR2 via ligation-induced translocation and interaction of receptor subunits in lipid raft microdomains. *Mol Cell Biol* 2012;32:1408-20.
 49. Makrinioti H, Toussaint M, Jackson DJ, Walton RP, Johnston SL. Role of interleukin 33 in respiratory allergy and asthma. *Lancet Respir Med* 2014;2:226-37.
 50. Savenije OE, Mahachie John JM, Granell R, Kerkhof M, Dijk FN, de Jongste JC, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol* 2014;134:170-7.
 51. Gordon ED, Palandra J, Wesolowska-Andersen A, Ringel L, Rios CL, Lachowicz-Scroggins ME, et al. IL1RL1 asthma risk variants regulate airway type 2 inflammation. *JCI Insight* 2016;1:e87871.
 52. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol* 2016;16:676-89.
 53. Saluja R, Zoltowska A, Ketelaar ME, Nilsson G. IL-33 and thymic stromal lymphopoietin in mast cell functions. *Eur J Pharmacol* 2016;778:68-76.
 54. Nagarkar DR, Ramirez-Carrozzi V, Choy DF, Lee K, Soriano R, Jia G, et al. IL-13 mediates IL-33-dependent mast cell and type 2 innate lymphoid cell effects on bronchial epithelial cells. *J Allergy Clin Immunol* 2015;136:202-5.
 55. Chhiba KD, Hsu CL, Berdnikovs S, Bryce PJ. Transcriptional heterogeneity of mast cells and basophils upon activation. *J Immunol* 2017;198:4868-78.
 56. Moritz DR, Rodewald HR, Gheyselink J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol* 1998;161:4866-74.
 57. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: the ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *J Immunol* 2007;179:2051-4.
 58. Tsuzuki H, Arinobu Y, Miyawaki K, Takaki A, Ota SI, Ota Y, et al. Functional interleukin-33 receptors are expressed in early progenitor stages of allergy-related granulocytes. *Immunology* 2017;150:64-73.
 59. de Kleer IM, Kool M, de Bruijn MJ, Willart M, van Moorlegheem J, Schuijs MJ, et al. Perinatal activation of the interleukin-33 pathway promotes type 2 immunity in the developing lung. *Immunity* 2016;45:1285-98.
 60. Teufelberger AR, Nordengrun M, Braun H, Maes T, De Grove K, Holtappels G, et al. The IL-33/ST2 axis is crucial in type 2 airway responses induced by *Staphylococcus aureus*-derived serine protease-like protein D. *J Allergy Clin Immunol* 2018;141:549-59.e7.
 61. Noval Rivas M, Burton OT, Oettgen HC, Chatila T. IL-4 production by group 2 innate lymphoid cells promotes food allergy by blocking regulatory T-cell function. *J Allergy Clin Immunol* 2016;138:801-11.e9.
 62. Gordon ED, Simpson LJ, Rios CL, Ringel L, Lachowicz-Scroggins ME, Peters MC, et al. Alternative splicing of interleukin-33 and type 2 inflammation in asthma. *Proc Natl Acad Sci U S A* 2016;113:8765-70.
 63. Sjöberg LC, Gregory JA, Dahlen SE, Nilsson GP, Adner M. Interleukin-33 exacerbates allergic bronchoconstriction in the mice via activation of mast cells. *Allergy* 2015;70:514-21.
 64. Kaur D, Gomez E, Doe C, Berair R, Woodman L, Saunders R, et al. IL-33 drives airway hyper-responsiveness through IL-13-mediated mast cell: airway smooth muscle crosstalk. *Allergy* 2015;70:556-67.
 65. Liu T, Kanaoka Y, Barrett NA, Feng C, Garofalo D, Lai J, et al. Aspirin-exacerbated respiratory disease involves a cysteinyl leukotriene-driven IL-33-mediated mast cell activation pathway. *J Immunol* 2015;195:3537-45.
 66. Drube S, Kraft F, Dudeck J, Muller AL, Weber F, Gopfert C, et al. MK2/3 are pivotal for IL-33-induced and mast cell-dependent leukocyte recruitment and the resulting skin inflammation. *J Immunol* 2016;197:3662-8.
 67. Chen CY, Lee JB, Liu B, Ohta S, Wang PY, Kartashov AV, et al. Induction of interleukin-9-producing mucosal mast cells promotes susceptibility to IgE-mediated experimental food allergy. *Immunity* 2015;43:788-802.
 68. Shimokawa C, Kanaya T, Hachisuka M, Ishiwata K, Hiseada H, Kurashima Y, et al. Mast cells are crucial for induction of group 2 innate lymphoid cells and clearance of helminth infections. *Immunity* 2017;46:863-74.e4.
 69. Galand C, Leyva-Castillo JM, Yoon J, Han A, Lee MS, McKenzie AN, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J Allergy Clin Immunol* 2016;138:1356-66.
 70. Joulia R, L'Faqihi FE, Valitutti S, Espinosa E. IL-33 fine tunes mast cell degranulation and chemokine production at the single-cell level. *J Allergy Clin Immunol* 2017;140:497-509.e10.
 71. Kamijo S, Suzuki M, Hara M, Shimura S, Ochi H, Maruyama N, et al. Subcutaneous allergic sensitization to protease allergen is dependent on mast cells but not IL-33: distinct mechanisms between subcutaneous and intranasal routes. *J Immunol* 2016;196:3559-69.
 72. Khodoun MV, Tomar S, Tocker JE, Wang YH, Finkelman FD. Prevention of food allergy development and suppression of established food allergy by neutralization of TSLP, IL-25 and IL-33. *J Allergy Clin Immunol* 2018;141:171-9.e1.
 73. Lefrancais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc Natl Acad Sci U S A* 2014;111:15502-7.
 74. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, et al. An interleukin-33-mast cell-interleukin-2 axis suppresses papain-induced allergic inflammation by promoting regulatory T cell numbers. *Immunity* 2015;43:175-86.
 75. Bandara G, Beaven MA, Olivera A, Gilfillan AM, Metcalfe DD. Activated mast cells synthesize and release soluble ST2-a decoy receptor for IL-33. *Eur J Immunol* 2015;45:3034-44.
 76. Abebayehu D, Spence AJ, Qayum AA, Taruselli MT, McLeod JJ, Caslin HL, et al. Lactic acid suppresses IL-33-mediated mast cell inflammatory responses via hypoxia-inducible factor-1 α -dependent miR-155 suppression. *J Immunol* 2016;197:2909-17.

77. Ndaï VS, Ababayehu D, Spence AJ, Paez PA, Kolawole EM, Taruselli MT, et al. TGF- β 1 suppresses IL-33-induced mast cell function. *J Immunol* 2017;199:866-73.
78. Kawauchi T, Ishimaru K, Nakamura Y, Nakano N, Hara M, Ogawa H, et al. Clock-dependent temporal regulation of IL-33/ST2-mediated mast cell response. *Allergol Int* 2017;66:472-8.
79. Shubin NJ, Glukhova VA, Clauson M, Truong P, Abrink M, Pejler G, et al. Proteome analysis of mast cell releasates reveals a role for chymase in the regulation of coagulation factor XIIIa levels via proteolytic degradation. *J Allergy Clin Immunol* 2017;139:323-34.
80. Groot Kormelink T, Arkesteijn GJ, van de Lest CH, Geerts WJ, Goerdal SS, Altelaar MA, et al. Mast cell degranulation is accompanied by the release of a selective subset of extracellular vesicles that contain mast cell-specific proteases. *J Immunol* 2016;197:3382-92.
81. Melo FR, Wallerman O, Paivandy A, Calounova G, Gustafson AM, Sabari BR, et al. Tryptase-catalyzed core histone truncation: a novel epigenetic regulatory mechanism in mast cells. *J Allergy Clin Immunol* 2017;140:474-85.
82. Caughey GH. Mast cell proteases as pharmacological targets. *Eur J Pharmacol* 2016;778:44-55.
83. Grujic M, Paivandy A, Gustafson AM, Thomsen AR, Ohrvik H, Pejler G. The combined action of mast cell chymase, tryptase and carboxypeptidase A3 protects against melanoma colonization of the lung. *Oncotarget* 2017;8:25066-79.
84. Lind T, Gustafson AM, Calounova G, Hu L, Rasmussen A, Jonsson KB, et al. Increased bone mass in female mice lacking mast cell chymase. *PLoS One* 2016;11:e0167964.
85. Stevens RL, McNeil HP, Wensing LA, Shin K, Wong GW, Hansbro PM, et al. Experimental arthritis is dependent on mouse mast cell protease-5. *J Biol Chem* 2017;292:5392-404.
86. Galli SJ, Tsai M, Marichal T, Tchougounova E, Reber LL, Pejler G. Approaches for analyzing the roles of mast cells and their proteases in vivo. *Adv Immunol* 2015;126:45-127.
87. Tsai M, Starkl P, Marichal T, Galli SJ. Testing the 'toxin hypothesis of allergy': mast cells, IgE, and innate and acquired immune responses to venoms. *Curr Opin Immunol* 2015;36:80-7.
88. Marichal T, Starkl P, Reber LL, Kalesnikoff J, Oettgen HC, Tsai M, et al. A beneficial role for immunoglobulin E in host defense against honeybee venom. *Immunity* 2013;39:963-75.
89. Starkl P, Marichal T, Gaudenzio N, Reber LL, Sibilano R, Tsai M, et al. IgE antibodies, Fc ϵ RI α , and IgE-mediated local anaphylaxis can limit snake venom toxicity. *J Allergy Clin Immunol* 2016;137:246-57.e11.
90. Lyons JJ, Yu X, Hughes JD, Le QT, Jamil A, Bai Y, et al. Elevated basal serum tryptase identifies a multisystem disorder associated with increased TPSAB1 copy number. *Nat Genet* 2016;48:1564-9.
91. Lyons JJ, Stotz SC, Chovanec J, Liu Y, Lewis KL, Nelson C, et al. A common haplotype containing functional CACNA1H variants is frequently co-inherited with increased TPSAB1 copy number. *Genet Med* 2017 [Epub ahead of print].
92. Dahdah A, Gautier G, Attout T, Fiore F, Lebourdais E, Msallam R, et al. Mast cells aggravate sepsis by inhibiting peritoneal macrophage phagocytosis. *J Clin Invest* 2014;124:4577-89.
93. Reber LL, Marichal T, Galli SJ. New models for analyzing mast cell functions in vivo. *Trends Immunol* 2012;33:613-25.
94. Rodewald HR, Feyerabend TB. Widespread immunological functions of mast cells: fact or fiction? *Immunity* 2012;37:13-24.
95. Bryce PJ, Falahati R, Kenney LL, Leung J, Bebbington C, Tomasevic N, et al. Humanized mouse model of mast cell-mediated passive cutaneous anaphylaxis and passive systemic anaphylaxis. *J Allergy Clin Immunol* 2016;138:769-79.
96. Burton OT, Stranks AJ, Tamayo JM, Koleoglou KJ, Schwartz LB, Oettgen HC. A humanized mouse model of anaphylactic peanut allergy. *J Allergy Clin Immunol* 2017;139:314-22.e9.
97. Ito R, Takahashi T, Katano I, Kawai K, Kamisako T, Ogura T, et al. Establishment of a human allergy model using human IL-3/GM-CSF-transgenic NOG mice. *J Immunol* 2013;191:2890-9.
98. Bibi S, Zhang Y, Hugonin C, Mangean MD, He L, Wedeh G, et al. A new humanized in vivo model of KIT D816V+ advanced systemic mastocytosis monitored using a secreted luciferase. *Oncotarget* 2016;7:82985-3000.
99. Weigmann B, Schughart N, Wiebe C, Sudowe S, Lehr HA, Jonuleit H, et al. Allergen-induced IgE-dependent gut inflammation in a human PBMC-engrafted murine model of allergy. *J Allergy Clin Immunol* 2012;129:1126-35.
100. Pagovich OE, Wang B, Chiuchiolo MJ, Kaminsky SM, Sondhi D, Jose CL, et al. Anti-IgE gene therapy of peanut-induced anaphylaxis in a humanized murine model of peanut allergy. *J Allergy Clin Immunol* 2016;138:1652-62.e7.
101. Takagi S, Saito Y, Hijikata A, Tanaka S, Watanabe T, Hasegawa T, et al. Membrane-bound human SCF/KL promotes in vivo human hematopoietic engraftment and myeloid differentiation. *Blood* 2012;119:2768-77.
102. Hartmann K, Escobedo L, Grattan C, Brockow K, Carter MC, Alvarez-Twose I, et al. Cutaneous manifestations in patients with mastocytosis: consensus report of the European competence network on mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. *J Allergy Clin Immunol* 2016;137:35-45.
103. Pardanani A, Reichard KK, Zblewski D, Abdelrahman RA, Wassie EA, Morice Ii WG, et al. CD123 immunostaining patterns in systemic mastocytosis: differential expression in disease subgroups and potential prognostic value. *Leukemia* 2016;30:914-8.
104. Rabenhorst A, Leja S, Schwaab J, Gehring M, Forster A, Arock M, et al. Expression of programmed cell death ligand-1 in mastocytosis correlates with disease severity. *J Allergy Clin Immunol* 2016;137:314-8.e5.
105. Desai A, Jung MY, Olivera A, Gilfillan AM, Prussin C, Kirshenbaum AS, et al. IL-6 promotes an increase in human mast cell numbers and reactivity through suppression of suppressor of cytokine signaling 3. *J Allergy Clin Immunol* 2016;137:1863-71.e6.
106. Hox V, O'Connell MP, Lyons JJ, Sackstein P, Dimaggio T, Jones N, et al. Diminution of signal transducer and activator of transcription 3 signaling inhibits vascular permeability and anaphylaxis. *J Allergy Clin Immunol* 2016;138:187-99.
107. Cerny-Reiterer S, Rabenhorst A, Stefanzi G, Herndlhofer S, Hoermann G, Mullauer L, et al. Long-term treatment with imatinib results in profound mast cell deficiency in Ph+ chronic myeloid leukemia. *Oncotarget* 2015;6:3071-84.
108. Cahill KN, Katz HR, Cui J, Lai J, Kazani S, Crosby-Thompson A, et al. KIT inhibition by imatinib in patients with severe refractory asthma. *N Engl J Med* 2017;376:1911-20.
109. Frossi B, Tripodo C, Guarnotta C, Carroccio A, De Carli M, De Carli S, et al. Mast cells are associated with the onset and progression of celiac disease. *J Allergy Clin Immunol* 2017;139:1266-74.e1.
110. Cruse G, Yin Y, Fukuyama T, Desai A, Arthur GK, Baumer W, et al. Exon skipping of Fc ϵ RI β eliminates expression of the high-affinity IgE receptor in mast cells with therapeutic potential for allergy. *Proc Natl Acad Sci U S A* 2016;113:14115-20.
111. Ho CCM, Chhabra A, Starkl P, Schnorr PJ, Wilmes S, Moraga I, et al. Decoupling the functional pleiotropy of stem cell factor by tuning c-Kit signaling. *Cell* 2017;168:1041-52.e18.