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Perspective

Beyond classical immunity: Mast cells as signal converters between tissues and neurons

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SUMMARY

Mast cells are regarded as effectors in immune defense against parasites and venoms and play an essential role in the pathology of allergic diseases. More recently, mast cells have been shown to receive stimuli derived from type 2 immunity, tissue damage, stress, and inflammation. Mast cells then rapidly convert these diverse signals into appropriate, organ-specific protective reflexes that can limit inflammation or reduce tissue damage. In this review, we consider functions of mast cells in sensations—such as pain, itch, and nausea—arising from tissue insults and inflammation and the ensuing protective responses. In light of emerging data highlighting the involvement of mast cells in neuroimmune communication, we also propose that mast cells are "signal converters" linking immunological and tissue states with nervous system responses.

INTRODUCTION

Mast cells belong to the hematopoietic system.[1] They develop from progenitors in yolk sac[2] and fetal blood[3] and from adult bone marrow.[2] Mature mast cells can be viewed as tissue-resident cells, located mainly at the body's interface to the environment. Mast cells are absent from bone marrow and blood. Mast cells often reside in close proximity to blood vessels[4] and neurons[5,6] and they have long lifespans (between 3 and 10 months in rodents).[7,8] Mast cells are present in many species, including amphibians, reptiles, and mammals. "Mast-cell-like cells" have been described in the invertebrate *Styela plicata* (Chordata-Tunicata),[9] dating back approximately 680 million years, making it evolution's oldest known organism with such cells. Therefore, mast cells predate the emergence of adaptive immunity by more than 100 million years.[10] In brief, mast cells are tissue-resident, long-lived, evolutionarily ancient cells that have been maintained in mice and humans, which are the focus of this review.

Mast cells were discovered by their "metachromasia" (turning purple upon staining with blue dyes) and their distinctive, granule-filled morphology.[11] The granules within mast cells store large amounts of biologically active compounds, including proteoglycans such as heparin and chondroitin sulfate (which are responsible for the metachromasia); specific proteases, like chymases (e.g., mast cell protease [Mcpt] 4 or 5), tryptase (e.g., Mcpt6), and carboxypeptidase A3 (Cpa3); and the monoamines histamine and serotonin (5-hydroxytryptamine or 5-HT).[12,13] In mice and humans, mast cells are generally classified into mucosal (e.g., in the mucosal layers of the gastrointestinal tract and the airways) and connective-tissue mast cells (e.g., in skin).[12] Apart from their location, both subsets differ in their produced mediators, responsiveness to stimulating ligands, and signals required for their growth and maturation. For instance, mucosal mast cell numbers are generally low and depend on microbial and inflammatory signals, whereas connective-tissue mast cells are constitutively present at steady state and their numbers also increase in response to inflammation.[12,14] With the advent of sensitive single-cell RNA sequencing technology, it is now being recognized that mast cells express tissue-specific transcriptomes[15] that may define further subsets beyond the classical mucosal versus connective-tissue phenotypes.

The best-studied mast cell trigger is activation of the high-affinity immunoglobulin E (IgE) receptor (FcεRI) by antigen.[16] FcεRI is a tetrameric complex ($\alpha\beta\gamma_2$) composed of α, β, and γ chains.[16] The α-subunit contains the binding site for IgE, whereas β and γ chains are important for signal transduction.[16] The γ chain is not specific for FcεRI but also serves as a signaling subunit for FcγRI and FcγRIII, which can also be expressed by mast cells.[17] The γ chain, functionally redundant with the T cell antigen receptor CD3 ζ chain,[18] possesses immunoreceptor tyrosine-based activation motifs (ITAMs)[19] within its intracellular domain. Following receptor activation, tyrosine residues within the ITAM are targeted by *Src* family kinases, initiating intracellular signaling events resulting in calcium mobilization as well as activation of PLCγ_1 and PI-3 kinase.[16,17] Following these events, mast cells rapidly release preformed mediators stored in secretory granules and newly synthesized molecules such as lipid mediators derived from arachidonic acid, cytokines, and chemokines.[12,13,20] Mast-cell-expressed FcεRIα binds with high affinity to the Fc-portion of IgE (dissociation constant 10^{-10} M),[21] leading to stably bound IgE on the mast cell surface for up to several weeks.[22] *In vivo*, mast cells are probably never devoid of bound IgE. FcγRI and FcγRIII on mast cells can be triggered by immunoglobulin G (IgG)-antigen immune complexes.[23] However, the dissociation constant of IgG for FcγRI and FcγRIII is considerably higher than for IgE bound to FcεRIα, which means that, unlike IgE, IgG is not retained on the mast cell surface.[17] Co-aggregation of FcεRI, FcγRI, or FcγRIII with FcγRIIb inhibits mast cell activation, as the intracellular domain of FcγRIIb contains

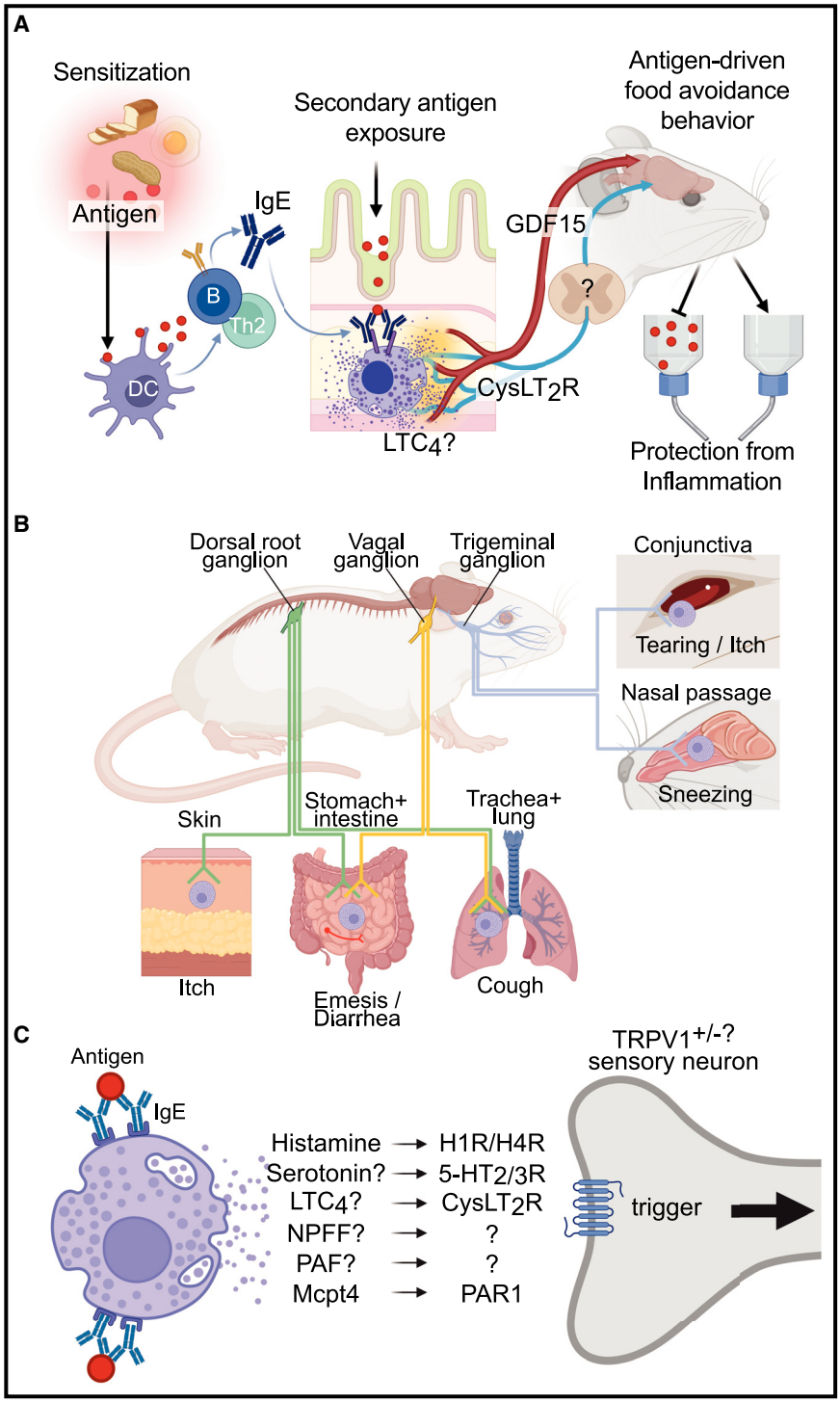

Figure 1. Mast cell - neuronal pathways in the framework of the toxin hypothesis

(A) Barrier breach allows for antigen (red dots) uptake by dendritic cells (DC), leading to type 2 immune responses. T helper 2 cells (Th2) are activated, and B cells (B) produce antigen-specific IgE, sensitizing mast cells, which, on re-encounter with antigens, promote avoidance via leukotriene C4 (LTC_4), Cysteinyl leukotriene receptor 2 ($CysLT_2R$), and GDF15. Heeding avoidance protects mice from antigen-driven intestinal inflammation.

(B) Innervation of mast cell-bearing tissues with tissue-specific allergic responses that may confer protective reflexes.

(C) Ligand-receptor pairs connecting IgE-activated mast cells and neurons. NPFF = Neuropeptide FF; PAF = Platelet activating factor; PAR1 = Protease activated receptor 1; H1R/H4R = Histamine receptors 1/4; $5\text{-}HT_{2/3}R$ = Serotonin receptor 2/3; TRPV1 = transient receptor potential cation channel subfamily V member 1; $CysLT_2R$ = Cysteinyl leukotriene receptor 2; Mcpt4 = Mast cell protease 4. Question marks indicate lack of data on definitive involvement of receptor-ligand signaling pairs.

immunoreceptor tyrosine-based inhibitory motifs, which recruit phosphatases that blunt signaling.[17] Although FcγRI/III aggregation on mast cells may contribute to anaphylaxis when FcεRI is absent,[24] clinical observations from allergy patients treated with the IgE-specific neutralizing antibody Omalizumab[25] indicate that, in humans, antigen-dependent mast cell responses are largely mediated through the IgE-FcεRI pathway.

Antigen-specific IgE antibodies can be elicited during a type 2 immune response,[20] which can occur when antigen crosses tissue barriers. Antigen-presenting cells process the antigen, migrate to draining lymph nodes, and activate helper T cells,[20] which differentiate into T helper 2 cells (Th2). Type 2 immunity is also promoted by innate lymphocytes 2 (ILC2). Th2 cells and ILC2s release cytokines (interleukin [IL]-4 and IL-13) that stimulate B cells to undergo isotype-class switching and differentiation into plasma cells producing IgE antibodies[20] (Figure 1A). IL-4 is crucial for class switching to IgE,[22] whereas IL-13, produced by follicular helper T cells in the germinal center, can facilitate the production of high-affinity IgE antibodies.[26] IgE affinity maturation shows a notable convergence of antibody V region sequences and hypermutations, as seen in the high-avidity peanut-specific IgE responses among unrelated individuals.[27] Following primary antigen exposure, few IgE^+ memory B cells are generated and IgE-producing plasma cells are short-lived.[28] However, during repeated antigen exposure, IgG memory B cells can switch to IgE and differentiate into long-lived plasma cells producing high-affinity IgE.[28]

Although mast cells clearly promote pathology associated with type 2 immunity and allergy, there is evidence that mast cells can mediate protection against noxious substances, i.e., toxins, and allergens via neuroimmune signaling. Despite the well-known close association between mast cells and nerve

fibers—which may enable bidirectional communication[29]—the scope and implications of interactions between mast cells and the nervous system leading to host protection through behavioral responses has received less attention. In this review, we discuss the communication between mast cells and neurons in host-protective avoidance behavior and expulsion reflexes and present a concept of mast cells as converters of signals from IgE and other stimuli into organ-specific or systemic reflexes. We also address crucial open questions and highlight avenues for future investigations in this rapidly evolving field.

MAST CELLS—THE HEMATOPOIETIC UNICORN

Mast cells are hematopoietic cells strategically located in barrier tissues, which has been taken to suggest that they sense invading pathogens and promote innate immunity. However, unlike other classical immunity cell types—such as macrophages, neutrophils, and eosinophils—mast cells express markedly distinct transcriptomes[15,30–32] and proteomes,[33] and they lack broad pattern recognition receptor expression.[30,33] Although there is evidence for a contributing role of IgE-mediated mast cell activation in adaptive immunity[34] and defense against venom toxicity,[35,36] there are no immunodeficiencies in the absence of IgE and mast cells.[37] Thus, questions remain as to whether classical immunological protection—akin, for example, to the functions of neutrophils and IgG antibodies in destruction and neutralization of pathogens—represents a fundamental role of mast cells and IgE. On the other hand, two independent studies[38,39] highlight that mast cells and IgE have specific and crucial roles in host-protective behavior. This can be viewed as non-classical immunological protection. The data support the "toxin hypothesis,"[40] which proposes that allergic reactions act as a defense mechanism against allergens—antigens that induce immunological sensitization and the production of specific IgE. Sensitization to allergens may occur when barrier integrity is compromised[41] due to prolonged or heightened antigen exposure, physical[42] or chemical insults (e.g., detergents or solvents[43]), or infections.[44] Other factors, such as altered microbiome diversity,[45] may also contribute to globally rising sensitization rates. Allergens can be innocuous (non-toxic substances)[46] or may be toxic via proteolytic properties (e.g., papain from papaya[47] or Der p 1 from house dust mites[48]). Repeated exposure to innocuous allergens, often found in occupational environments, can lead to persistent inflammatory responses. Expulsion and avoidance behaviors may hence protect the host not only from futile inflammation and immunopathology induced by harmless allergens but also in mitigating damage when toxic allergens are encountered.

Understanding these and other neuroimmune functions of mast cells presents a major challenge due to the complexities associated with the specificity and limitations of commonly used experimental tools. The use of *Kit*-deficient, mast-cell-deficient mice in immunological or behavioral research introduced significant complications. The *Kit* gene is expressed in multiple cell types beyond mast cells and plays essential roles in the normal function of many lineages,[49,50] including several neuronal populations, particularly transient receptor potential cation channel subfamily V member 1^+ (TRPV1; the capsaicin receptor) nociceptive neurons.[51] Neuronal defects in *Kit*-mutant mice may explain the anxiety-like behaviors observed in these animals,[52] which are not present in *Kit*-mutation-independent $Cpa3^{Cre/+}$ mast-cell-deficient mice.[38] Thus, prior claims regarding functions of mast cells based on experiments in *Kit*-deficient mice must be re-evaluated in models with unimpaired *Kit* function.[37] A large body of the literature on mast cells in neuro-immunology is based on work done in *Kit* mutants. Despite the fact that these data are not definitive, we refer to these reports here as a blueprint for verification. Beyond genetic models, pharmacological experiments using the mast cell "stabilizer" cromoglicic acid (cromolyn) are not specific for mouse mast cells[53] but instead inhibit neurons,[54] whereas ketotifen, another drug commonly used for mast cell inhibition,[55] lacks specificity[56] and affects other cell types as well. As a result, conclusions about mast cell functions drawn from studies using these inhibitors require closer scrutiny.

ANTIGEN AVOIDANCE

According to the toxin hypothesis, antigen avoidance can prevent repeated antigen exposure.[40] This theory has been extended and broadened in subsequent considerations.[46,57] Experimentally, avoidance of dietary antigens has been linked to the immune status, as ovalbumin (OVA)-immunized mice develop aversion to solutions containing OVA, even when sweetened.[58] Such food aversion can be reversed by inducing OVA-specific unresponsiveness through oral tolerance[58] or transferred to naive mice via passive transfer of serum or spleen cells.[59] Studies have elucidated how type 2 immunity drives this avoidance behavior to food allergens.[38,39] Immunization-induced OVA avoidance does not occur in mast-cell-deficient $Cpa3^{Cre/+}$ or mast-cell-depleted $Ms4a2^{DTR}$ (diphtheria toxin receptor [*DTR*]-expression driven by the FcεRIβ [*Ms4a2*]-promoter) mice, demonstrating that mast cells link immune sensing to antigen avoidance behavior.[38,39] Hence, this is a non-redundant mast cell function. Upstream components of type 2 immunity, IL-4Rα, IgE, and FcεRI, are required for avoidance. Breaking avoidance drives local and systemic inflammation,[38] supporting the notion that antigen avoidance protects against continuous and unnecessary immune responses. In summary, type 2 immunity leads to the generation of antigen-specific IgE, which sensitizes mast cells. Upon reencounter of antigen, mast cells function as important sensor cells, detecting OVA via IgE bound to FcεRI and converting the antigen signal to host-protective avoidance behavior (Figure 1A). The loss of avoidance in the absence of mast cells resembles other key deficiencies in an immune system lacking specific cell types, including antibody deficiency in the absence of B cells.

Mice sense dietary antigen by mucosal mast cells in the stomach and small intestine. Potential roles for connective-tissue-type mast cells in antigen avoidance need to be addressed in future studies. OVA avoidance can develop rapidly (within minutes),[38,39] a time frame compatible with neuronal transmission of avoidance signals from the gut to the brain. Possible neural pathways in the gut involve neurons of the enteric nervous system, along with mucosal endings of vagal ganglion (VG) and dorsal root ganglion (DRG) sensory neurons[60] (Figure 1B). However, luminal antigen stimulation in gut tissues of OVA-immunized mice does not trigger activation in enteric neurons[38] and avoidance behavior remains unaffected by surgical vagotomy,[38,39]

suggesting that avoidance signals are transmitted independent of enteric and vagal neurons. Antigen sensing in the gut can be transmitted to DRG neurons, as luminal antigen exposure in immunized rats induces action potentials in mesenteric nerve bundles.[61] Oral antigen challenge of food-sensitized mice results in mast-cell-, IgE-, and histamine 1 receptor (H1R)-dependent nociception upon intestinal distension and augmented activation of splanchnic nerves following mechanical stimulation of the mucosa.[44] Both mesenteric nerve bundles and splanchnic nerves contain gut-innervating DRG neurons, which include both $TRPV1^+$ and $TRPV1^-$ subsets. Given that avoidance is not affected by the systemic depletion of $TRPV1^+$ neurons,[38,39] the possibility remains that DRG neurons lacking TRPV1 signal avoidance to the central nervous system (CNS). Alternatively, the entire enteral innervation may not be involved.

Genetic or pharmacological blockade of histamine, serotonin, substance P, and mast cell proteases (Cpa3, Mcpt5, and Mcpt6) does not impair avoidance. Inhibition of leukotriene synthesis (inhibitors Zileuton, MK-886, and leukotriene C4 synthase *Ltc4s*$^{-/-}$) or blockade of the leukotriene C4-receptor, cysteinyl leukotriene receptor 2 ($CysLT_2R$), interferes with antigen avoidance.[38,39] Hence, leukotriene C4 and its receptor appear to be involved in the avoidance signal. Leukotriene signaling through $CysLT_2R$ initiates the itch reflex in the skin,[62,63] and mast cells may initiate avoidance through a similar pathway in the gut. Moreover, fast-acting circulating humoral factors released following antigen exposure could transmit avoidance signals to the brain. Growth/differentiation factor 15 (GDF15) is rapidly induced by antigen challenge downstream of mast cells and leukotrienes.[39] Injection of a GDF15-blocking antibody in OVA-immunized mice does not interfere with avoidance behavior immediately (day 1) but on day 2 of antigen exposure.[39] The necessity for leukotriene signaling via $CysLT_2R$ during the initial phase of avoidance raises the possibility of first, GDF15-independent, avoidance signal(s). Subsequently, mast-cell-induced GDF15 could sustain avoidance and may also cause conditioning. The roles of both leukotrienes and GDF15 in antigen avoidance require further study. In addition to avoidance, GDF15 is a humoral molecule known to contribute to conditioning in taste aversion experiments.[64,65] In this context, it was observed that immunized rats exposed to conditioning, where antigen stimulation is paired with external (audio-visual) cues, release mucosal mast cell protease into the bloodstream in response to the conditioned stimulus.[66] This suggests that remembering antigen exposure may be sufficient to trigger mast cell activation and avoidance behavior, even in the absence of the actual antigen.[66] The insular cortex region of the brain is known to store memories, and it is intriguing to speculate that these include "allergic memories" and other memories of specific immune responses.[67] Further evidence supporting a connection between avoidance and conditioning comes from studies of allergic asthma. OVA-inhalation challenge of OVA-sensitized mice leads to an IgE-dependent avoidance of the chamber where the challenge took place, even in the absence of OVA,[68,69] and pairing of antigen inhalation with a neutral cue in asthma patients causes allergic reactions in response to the cue.[70] However, there is no evidence that mast-cell-mediated avoidance is a result of conditioning. In fact, when given the free choice, mice that are actively avoiding do not completely abstain; instead, they continuously sample small quantities of antigen-containing water.[38] This small amount of antigen signal, which is below a pathogenic threshold, may constantly renew the avoidance behavior. Hence, any link between avoidance and conditioning is likely complex. Future studies should investigate whether avoidance mechanisms in the airways are similar to mast-cell-dependent avoidance of ingested antigens and clarify the link between avoidance behaviors and conditioning.

IgE-ACTIVATED MAST CELLS IN ITCH, TEARING, SNEEZING, COUGH, DIARRHEA, AND EMESIS

Itch (pruritus) is a sensation that elicits the urge to scratch, a reflex thought to protect the body by removing harmful allergens and parasites from the skin surface.[40,71] Active and passive immunization of mice against OVA, followed by intradermal injection of OVA, leads to scratching behavior.[62] This response is reduced in *Kit*-mutant mice, hence mast cells may detect injected OVA through specific IgE and transmit this antigen signal to neurons, resulting in itch. IgE may also play a role in heat nociception.[72,73] The skin is innervated by sensory neurons with cell bodies situated in the DRG and, in the case of the skin covering the head, within the trigeminal ganglia (TG)[71] (Figure 1B). Histamine is a key pruritogen released from IgE-activated mast cells that, when injected alone, can induce itch in both mice and humans.[71] IgE-mediated activation of dermal mast cells induces activation of DRG neurons that could be blocked by H1R and H4R antagonists.[74] These same neurons also responded to direct intradermal injections of histamine and capsaicin, indicating that mast cell products trigger neuron subsets characterized by expression of TRPV1 and H1R/H4R.[74] All three receptors are expressed on the NP2/NP3 subtypes of DRG neurons, which, based on their responsiveness to stimulation, as well as protein and RNA expression profiles, are itch neurons.[71,75] Collectively, the data support a model in which histamine released by IgE-activated mast cells stimulates H1R/H4R receptors on NP2/NP3 DRG neurons, signaling itch to the CNS, which may underlie allergic itch. The potential roles of neuron-expressed FcεRI[76] and mast-cell-independent histamine sources, such as basophils[10] and neutrophils,[77] in antigen-induced itch require further investigation.

Tearing, which can be triggered by allergic reactions such as allergic conjunctivitis,[78] may serve a protective function by aiding removal of antigens from the eye.[40] Experimental evidence in both mice and humans supports a role of mast cells in antigen-induced tearing. When the eyes of allergic conjunctivitis patients are exposed to antigen, increased tearing, conjunctival redness, and itching occur within 5 min.[78] Over half of the patients show increases in histamine levels in their tear fluid after antigen challenge, indicating histamine release, potentially from mast cells.[78] In mice, active immunization against ragweed, followed by application of ragweed on the eye's surface, leads to multiple inflammatory reactions, including tearing.[79] This response is reduced in *Kit*-mutant mice.[79] Tearing can be induced by TG sensory neurons that innervate the eye[80] (Figure 1B). TG neurons exhibit molecular characteristics similar to DRG neurons, notably the expression of H1R.[81] Existing data support a model in which histamine released from IgE-activated mast cells triggers TG neuron activation, rapidly leading to tearing.

Sneezing, a common allergic symptom in the upper airways—for example, in hay fever or allergic rhinitis—may serve a protective function by expelling antigens from the nasal cavity.[40] Two recent studies provide evidence for the involvement of mast cells in the sneezing reflex.[82,83] Intranasal OVA challenge of antigen-sensitized mice induces pronounced sneezing, a response abrogated in *Kit*-mutant mice.[82] Similar to the conjunctiva, the nasal epithelium is innervated by TG sensory neurons (Figure 1B). Genetic ablation of a subset of TG neurons, marked by MrgprC11 expression, abolishes sneezing responses to aerosolized histamine, 5-HT, or neuropeptide FF (NPFF).[83] NPFF can be released from mast cells upon activation.[84] In a mouse model of allergic rhinitis, depletion of MrgprC11^{+}[83] or TRPV1^{+} neurons[82] abrogated allergen-triggered sneezing responses. MrgprC11 and TRPV1 are co-expressed by a specific population of nose-innervating TG neurons.[83] Hence, mast cells can detect antigen in the nasal mucosa and convert antigen signals into secreted mediators acting in concert to stimulate MrgprC11^{+} TRPV1^{+} TG neurons, which induces sneezing.

Coughing, which can be associated with allergic reactions, may protect the lower airways by expelling antigens.[40] Guinea pigs have been extensively used to study allergic cough, primarily due to their respiratory physiology, which bears similarities to humans, and their heightened sensitivity to tussive (cough-inducing) stimuli.[85] Guinea pigs immunized with OVA and alum, and subsequently challenged with OVA inhalation, cough, which depends on signaling through H1R[86] and TRPV1.[87] Whereas the respiratory tract is innervated by VG and DRG sensory neurons (Figure 1B), mast cell mediators may stimulate only a subset (TRPV1^{+} SST^{+}) of VG neurons, akin to chemical-induced cough.[83] From these neurons, signals are transmitted to the brainstem, initiating the cough event.[88] Coughing commences with the concerted relaxation and contraction of airway muscles,[88] and mast cells are also involved in this phase of coughing. Isolated trachea from OVA-immunized wild-type mice exhibit rapid and short-lived muscle contraction upon OVA exposure, which could be replicated by stimulation with 5-HT[89] that can be secreted from airway mast cells.[90] Antigen-induced contractions are inhibited by ketanserin, a 5-HT$_2$ receptor antagonist.[89,90] Trachea isolated from immunized *Kit*-mutant mice do not contract following antigen stimulation,[89] a phenotype also reported in mast-cell-deficient *Cpa3*$^{Cre/+}$ mice.[90] Airway muscle contractions are controlled by cholinergic neurons of the parasympathetic nervous system.[91] In support of this mechanism, antigen-induced tracheal contractions are inhibited by atropine, an inhibitor of muscarinic acetylcholine receptors.[89] Moreover, intracellular recordings of parasympathetic neuron membrane potential in mouse tracheal ganglia demonstrate a ketanserin-sensitive depolarization triggered by 5-HT, along with a similar response elicited by OVA challenge.[89] Collectively, these studies suggest that mast cells and their mediators are involved in both induction and execution of the cough reflex, with the strongest evidence of a role for mast-cell-derived 5-HT signaling to cholinergic parasympathetic neurons during the airway contraction phase. In contrast, evidence for mast cell dependency in the initiation of cough through triggering of sensory neurons following antigen inhalation appears less conclusive.

Diarrhea and vomiting can be triggered by allergic reactions in the gastrointestinal tract, commonly known as food allergies, and may serve a protective function by expelling ingested antigens and gastrointestinal parasites.[40] In mice, mast cells have been linked to antigen-induced diarrhea. Immunization with OVA and alum, followed by repeated OVA gavage, results in diarrhea, a response that can be prevented by IgE-neutralization,[92] anti-Kit-antibody-mediated mast cell depletion,[92] and genetic mast cell deficiency (*Cpa3*$^{Cre/+}$ mice).[38] Antigen-induced diarrhea is also suppressed by antagonizing a combination of serotonin and platelet-activating factor (PAF) signaling,[92] suggesting involvement of these mast-cell-expressed mediators. The precise mechanisms through which mast cells contribute to diarrhea remain uncertain. Diarrhea occurs when chloride (Cl^-) ions and water are lost through the epithelial barrier into the gut lumen.[92–94] *In vitro* antigen stimulation of intestinal tissue segments excised from immunized mice increases short-circuit currents (indicative of Cl^- secretion), which are diminished in *Kit*-mutant mice.[95] Hence, mast-cell-activation-induced epithelial Cl^- secretion may be part of the mechanism. In mouse models of food allergy, multiple oral antigen challenges are required to induce diarrhea,[38,92] conditions that also produce gut inflammation.[38,39,92] Therefore, inflammation-driven changes in the gut may be involved in mast-cell-induced diarrhea; however, the precise cellular and molecular pathways downstream of mast cells contributing to inflammation and diarrhea in food allergy remain incompletely understood.

Vomiting (emesis), typically accompanied or preceded by nausea, is the forceful expulsion of stomach and intestinal contents through the mouth.[96] There is evidence for the contribution of IgE and mast cells to vomiting; however, mechanistic experiments are complicated by rodents' inability to vomit.[96] Emetic species such as house musk shrews (*Suncus murinus*) and common marmosets (*Callithrix jacchus*) exhibit profound vomiting upon oral administration of the bacterial superantigenic toxin staphylococcal enterotoxin type A (SEA).[97,98] SEA binds to submucosal mast cells in the gut and triggers their degranulation (i.e., loss of metachromatic staining) and histamine release from the intestine.[97,98] However, SEA can also activate immune cells other than mast cells. Vagotomy or pharmacological blockade of H1R and 5-HT$_3$ receptors prevents emesis.[97,98] This suggests that mast cells could be activated by SEA, which may trigger vomiting via 5-HT$_3$ receptors on gut-innervating vagal sensory neurons. A secondary line of experimentation linking mast cells to vomiting involves studies in mice that, even though non-emetic, display pica behavior; i.e., they ingest nonfood substances, like kaolin, following emetic stimulation.[99] Systemic administration of GDF15, which is a stress-response cytokine that activates glial-derived neurotrophic factor receptor alpha-like (GFRAL) on brainstem neurons,[96] induces emesis in musk shrews and triggers pica behavior in mice.[64] In a mouse model of food allergy, OVA-sensitization and intragastric OVA challenge induce a rapid (within 1 h) increase in systemic GDF15 levels, which is further elevated upon successive OVA challenges.[39] Mice deficient in IgE or mast cells do not show this systemic GDF15 increase, even following multiple gavages.[39] Taken together, mast cells and IgE may promote emesis and pica independent of the vagus nerve, which may involve the GDF15-brainstem-GFRAL pathway.

In summary, itching, tearing, sneezing, coughing, diarrhea, and vomiting are host-protective reflexes. These responses

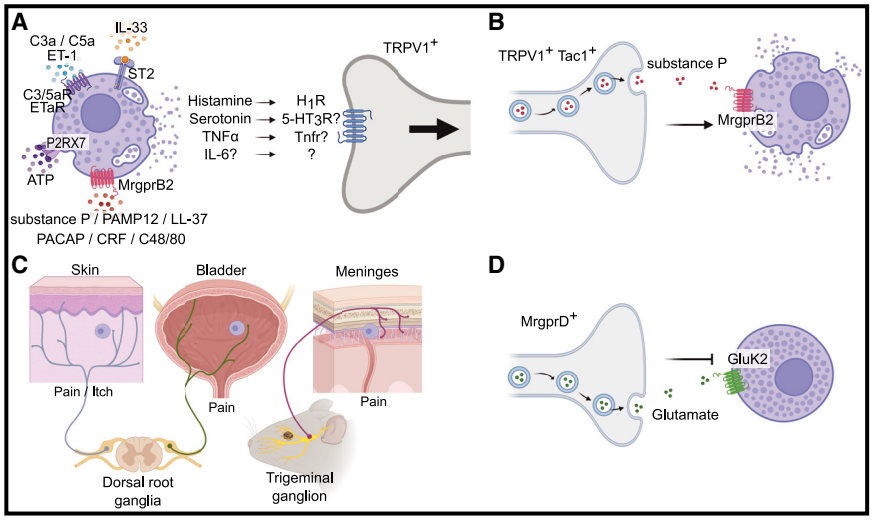

Figure 2. Bi-directional intercellular signaling between mast cells and neurons

(A) Mast cells can be activated by ligation of the following receptors in an IgE-independent manner: complement receptors 3/5 (C3/5aR) by complement components C3a/C5a; ST2 (IL-33R) by interleukin-33 (IL-33); P2X-purinoceptor 7 (P2RX7) by adenosine triphosphate (ATP); or Mas related g-protein coupled receptor B2 (MrgprB2) via substance P, Proadrenomedullin N-terminal 20 peptide (PAMP-12), the Cathelicidin antimicrobial peptide LL-37, pituitary adenylate cyclase activating peptide (PACAP) and corticotropin-releasing factor (CRF), or C48/80. Displayed between mast cells and neurons are ligand-receptor pairs likely that could be involved in mast cell-dependent, but IgE-independent, reflexes, such as in nociception and pain. H1R = histamine receptor 1; 5-HT_3R = serotonin receptor 3; Tnfr = tumor necrosis factor receptor; TRPV1 = transient receptor potential cation channel subfamily V member 1; TNFα = tumor necrosis factor alpha, and IL-6 = interleukin-6.

(B) $TRPV1^+Tac1^+$ neurons produce substance P which can activate mast cell-expressed MrgprB2 to induce IgE-independent activation.

(C) Innervation of mast cell-bearing tissues with tissue-specific IgE-independent responses that may confer protective reflexes.

(D) $MrgprD^+$ neurons produce glutamate which can activate the metabotropic glutamate receptor GluK2 to inhibit mast cells.

can be rapidly initiated by tissue mast cell sensing of antigen through IgE, and subsequent signaling to neural cells, transmitting the information to the CNS. Hence, in this scenario, mast cells act as converters of antigen signals into host-protective reflexes. Mast cell products and their receptors on sensory neurons are depicted in Figure 1C. For example, antigen sensing by mast cells can trigger the release of serotonin and histamine, which have specific receptors on neurons (Figure 1C). Different tissues receive distinct neural innervation (Figure 1B), which, together with distinct mast cell phenotypes, likely imparts tissue-specificity of allergic reflexes. Clearly, the repertoire of mast-cell-derived neuroactive mediators requires further study.

ANAPHYLAXIS AS HOST PROTECTION?

The discovery of anaphylaxis[100] was a founding event in immunology, yet, to this date, the physiological "purpose" of this drastic form of hyperreactivity remains obscure. In nature, snake or arthropod envenomation can cause venom to spread throughout the body, which can trigger systemic mast cell activation, potentially leading to anaphylaxis. Anaphylaxis accompanies the release of mast cell proteases, which can degrade venom toxins.[101,102] Although carrying inherent risks, it has been proposed that non-fatal anaphylaxis may confer protection by mitigating the effects of envenomation.[103] Systemic injection of antigen into IgE-sensitized mice causes IgE-mediated mast cell degranulation and rapid hypothermia, a response that is completely abrogated in mast-cell-deficient *Cpa3*$^{Cre/+}$ mice.[14] High-affinity IgE-antigen interactions lead to mast cell activation and robust mast cell mediator release *in vitro*.[104] This high-affinity binding serves as a key signal for both local[104] and systemic[26] anaphylaxis *in vivo*. In addition, factors such as antigen concentration and avidity[105] of IgE-antigen interactions may also play a role. Future studies should explore the physiological roles of high- versus low-affinity IgE in regulating mast cell function and physiological outcomes.

Anaphylaxis is accompanied by hypothermia and hypotension, which are clinical hallmarks. Activation of neurons downstream of mast cells is thought to drive this reduction of body temperature.[106] Anaphylaxis-induced hypothermia is reduced in TRPV1-deficient mice, and selective activation of $TRPV1^+$ sensory neurons is sufficient to induce hypothermia.[106] This suggests that mast cells induce $TRPV1^+$ neurons to promote hypothermia. Deficiency of the chymase Mcpt4, or pharmacological blockade of protease-activated receptor 1 (PAR1; a target of Mcpt4) reduces hypothermia.[106] Hence, the results are in agreement with a model in which IgE-activated mast cells promote hypothermia via Mcpt4-PAR1 signaling to $TRPV1^+$ sensory neurons. In the context of host protection, one could speculate that anaphylaxis-triggered hypothermia and hypotension reduce the metabolic rate as well as the systemic spread of toxins.

MrgprB2: A LIGAND-VERSATILE RECEPTOR SENSING TISSUE INSULTS EXPRESSED BY MAST CELLS

Mast cells express cell surface receptors beyond those for IgE, which are capable of sensing signals released during tissue insults and stress, e.g., in the course of infection, inflammation, or injury (Figure 2A). The most ligand-versatile receptor capable of recognizing many different, mostly positively charged, endogenous and exogenous compounds[107,108] is the Mas-related G-protein-coupled receptor B2[109] (MrgprB2; in humans MRGPRX2[110]). MrgprB2 is expressed in connective-tissue-type mast cells but not in mucosal mast cells.[15] Endogenous ligands include host-defense peptides (e.g., cathelicidins such as LL-37), neuropeptides (including substance P and somatostatin), and peptide hormones such as proadrenomedullin N-terminal 12 peptide (PAMP12), pituitary adenylate cyclase activating peptide (PACAP), and corticotropin-releasing factor (CRF),[107,108] all of which can be induced during cell stress, infection, or inflammation. Exogenous MrgprB2 ligands include the widely used artificial MrgprB2 ligand compound 48/80

(C48/80),[109] arthropod venom peptides (e.g., mastoparan), and microbial products (such as quorum-sensing molecules and competence-stimulating peptides). C48/80 can activate mast-cell-like "test cells" in *Styela plicata*,[9] suggesting that MrgprB2-mediated mast cell sensing represents a primordial function.

Binding of ligands to the extracellular domains of MrgprB2 induces conformational changes in the receptor, resulting in canonical activation of G-protein-signaling pathways.[111] Certain ligands with a distinct binding mode may activate both G proteins and the β-arrestin signaling pathway, potentially leading to divergent responses.[111] A further level of complexity arises from the fact that the Mrg receptor family consists of 22 members with considerable sequence and structural similarities.[108] For example, MrgprB2 ligand's substance P, C48/80, and potentially others, can also activate MrgprA1,[112] which is expressed by dendritic cells,[113] neutrophils,[114] and various subsets of DRG neurons.[75] Hence, rather than mast-cell-expressed MrgprB2, MrgprA1 activation on immune cells might account for the adjuvant effect attributed to C48/80,[115] whereas neuronal expression of MrgprA1 may explain the ability of C48/80 to directly stimulate DRG neurons in culture.[54,116] Given the complexities arising from many different ligands, divergent ligand binding modes, and the expression of structurally similar Mrg receptors across different cell types, it will be important to disentangle the mast-cell-dependent and mast-cell-independent roles of Mrgpr activation in tissues.

An example of mast cell sensing of tissue damage through MrgprB2 is seen after cutaneous exposure of mice to a combination of house dust mite allergen extract and staphylococcal enterotoxin B, which induces atopic dermatitis (AD)-like skin inflammation.[117] Mice deficient in mast cells (*Cpa3-Cre Mcl1*$^{fl/fl}$), or lacking *MrgprB2* or substance P (*Tac1*$^{-/-}$), are protected from AD-like inflammation. Substance P expression is restricted to skin innervating TRPV1^{+} neurons, and ablation of these neurons protects mice from inflammation.[117] A further example of how the MrgprB2-substance P axis may contribute to inflammation is diabetes, which is associated with increased substance P expression in TRPV1^{+} DRG neurons in the skin.[118] Again, neuron-released substance P activates mast cells via MrgprB2, causing impaired blood flow in the skin microvasculature.[118] Collectively, these studies support a model in which TRPV1^{+} sensory neurons secrete substance P, which subsequently activates mast cells through MrgprB2, ultimately leading to skin inflammation and vascular leakage (Figure 2B).

MAST CELL SENSING OF TISSUE INSULTS PROMOTES NOCICEPTION, PAIN, AND ITCH

A symptom of inflammation is nociception and pain,[119] a reflex with possible mast cell involvement. For the occurrence of pain, which is defined as "an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions,"[120] nociceptive neurons innervating the barrier tissue must be stimulated to convey the sensation to the CNS (i.e., nociception). Analogous to innervation patterns governing allergic reflexes, skin and bladder tissues are innervated by DRG neurons whereas the meninges are innervated by TG neurons (Figure 2C). Pain in animal models is assessed by measuring protective motor and avoidance responses, e.g., by observing withdrawal of rodent paws following thermal or mechanical stimulation.

MrgprB2-dependent mast cell activation in the skin, initiated by stress- or damage-associated molecules released during injury or inflammation, could play a role in nociception. Injection of mice with complete Freund's adjuvant (CFA; consisting of a blend of mineral oil and heat-killed *Mycobacterium tuberculosis*) triggers mechanical and thermal hyperalgesia (increased sensitivity to noxious stimuli) along with robust inflammation and the release of damage-associated molecules and neuropeptides, including substance P.[121] However, mast cell involvement in CFA nociception is controversial. CFA pain is reduced in *Kit*-mutant mice,[122] and in mice where *MrgprB2*-expressing cells are ablated via diphtheria toxin injection (*MrgprB2-Cre ROSA26*^{iDTR} [inducible DTR] mice),[121] but remains unaffected in mast-cell-depleted *Mcpt5-Cre ROSA26*^{iDTR} mice.[116] Nociceptive defects in *Kit*-mutant mice[51] and the expression of MrgprB2 outside of the mast cell lineage[123] may underly the observed CFA pain inconsistencies. Nevertheless, a substance-P-MrgprB2-mast-cell axis in nociception may function in a mouse model of post-operative (i.e., incision-induced) pain. Skin incision rapidly induces substance P release as well as mechanical and thermal hypersensitivity in wild-type mice, which is abrogated in *MrgprB2*-deficient mice,[121] in mast-cell-deficient *Mcpt5-Cre ROSA26*^{DTA} (diphtheria toxin alpha) mice,[124] and in mice with 5-HT-deficient mast cells.[124]

CFA injection and skin incision can enhance the sensitivity of nociceptive neurons to mast cell mediators, which may lower the threshold for host-protective reflexes. However, even under non-inflammatory conditions, activation of skin mast cells by injection of MrgprB2 ligands may induce pain and itch. For instance, C48/80 injected into the skin causes thermal and mechanical hyperalgesia in naive wild-type mice, a response that is abrogated in *Kit*-mutant mice.[125] Furthermore, injection of C48/80 and PAMP12 induces itch in wild-type, but not in *MrgprB2*$^{-/-}$, mice.[74] However, the dependency of MrgprB2-induced itch on mast cells remains unclear, as studies with *Kit*-mutant mice show inconsistent results, with some reporting reduced itch[74] and others normal levels of itch.[126,127] Clarifying the specific role of MrgprB2 and mast cells will require further investigation using mast-cell-specific tools. Nevertheless, in a gain-of-function experiment, IgE-independent activation by clozapine-n-oxide (ligand for hM3Dq) of skin mast cells by the genetically introduced artificial G-protein-coupled designer receptor hM3Dq (*Mcpt5-Cre CAG-LSL-hM3Dq*) causes itch,[128] suggesting that mast cell activation via G-protein-coupled receptors is sufficient to induce itch. Thus, skin mast cells may trigger host-protective pain and itch, even in the absence of overt inflammation.

Beyond skin, MrgprB2-activation of meningeal mast cells may promote pain. Both repeated restraint stress and alcohol withdrawal in mice induces systemic increases in PACAP[129] and CRF[130] levels, respectively, and facial mechanical hypersensitivity similar to migraine headaches. PACAP- and CRF-induced pain is diminished in *MrgprB2*$^{-/-}$ animals,[129–131] suggesting that both PACAP and CRF mediate their effects via this receptor. Alcohol withdrawal induces tumor necrosis factor alpha (TNF-α),

a mediator produced by mast cells,[132] in the dura mater of wild-type, but not *MrgprB2*$^{-/-}$, animals.[130] Pharmacological inhibition of TNF receptor or TRPV1 following restraint stress or alcohol withdrawal reduces mechanical hyperalgesia.[129,130] Dural injection of PACAP or CRF increases spontaneous as well as mechanical and capsaicin-driven TG neuron activity.[129,130] Hence, the stress-response mediators PACAP and CRF may induce MrgprB2-mediated activation of meningeal mast cells, subsequently eliciting mechanical hyperalgesia through TRPV1$^+$ TG neurons. However, formal proof for a role of mast cells is lacking.

MrgprB2 activation of bladder mast cells may contribute to pain. Injection of LL-37 into the bladder, which activates MrgprB2 on mast cells, induces mechanical hyperalgesia—a response reduced in *Kit*-mutant mice.[133] Furthermore, repeated bacterial urinary tract infections in *Kit*-independent mast-cell-deficient mice (*Mcpt5-Cre ROSA*^{iDTR} mice) lead to reduced mechanical hyperalgesia in the pelvic area surrounding the bladder.[134] In this infection model, substance P$^+$ neurons accumulate in the bladder and *Trpv1*-deficiency or pharmacological inhibition of histamine or bradykinin prevents hyperalgesia. Instilling histamine or bradykinin into the bladder is sufficient to induce pain in naive wild-type mice.[134] In support of mast-cell-independent bladder pain, *MrgprB2* transcripts are expressed in the bladders of *Kit*-mutant mice, and bladder smooth muscle contractility is similar in both wild-type and *Kit*-mutant mice following C48/80 instillation.[123] This suggests that non-mast-cell-expressed MrgprB2 may also play a significant role in bladder function and points to a broader involvement of MrgprB2 beyond mast cells. Together, these findings suggest that mast-cell- and non-mast-cell-expressed MrgprB2 contributes to bladder pain and contractility.

Overactivity of the mast-cell-MrgprB2 axis in the inflammation and pain pathway could lead to autoimmune-like pathology, highlighting a need for regulatory mechanisms to control it. One such mechanism involves nonpeptidergic MrgprD$^+$ neurons in the skin that reduce mast cell responsiveness to MrgprB2 ligands through the release of glutamate.[135] Genetic depletion of MrgprD$^+$ neurons increases C48/80-mediated mast cell degranulation, and administration of the MrgprD agonist, β-alanine, which enhances the excitability of MrgprD$^+$ neurons and their production of glutamate, suppresses C48/80-induced mast cell responses.[135] Genetic reduction of glutamate release from MrgprD$^+$ neurons, or pharmacological blockade of the glutamate receptor GluK2 (GluR6), also increases C48/80-mediated mast cell degranulation.[135] Hence, glutamate released from MrgprD$^+$ neurons may serve as a mechanism to continuously suppress skin mast cell activation and maintain tissue homeostasis (Figure 2D).

Trauma, tissue damage, and inflammation induce complement components C3a/C5a,[136] endothelin-1 (ET-1),[137] ATP,[138] or IL-33[139] (Figure 2A), all of which are also implicated in pain hypersensitivity. Binding of C3a/C5a and ET-1 to their specific G-protein-coupled receptors C3aR/C5aR[140] and ET_A receptor[141] on mast cells results in degranulation. Although direct evidence linking mast cell activation via C3a/C5a or ET-1 to pain and nociception is lacking, several experiments suggest a connection. In mice, hind paw incision nociception correlates with increased skin C5a levels, and pharmacological inhibition of C5aR1 alleviates hyperalgesia.[136] Moreover, injection of purified C3a or C5a is sufficient to induce mechanical and thermal hyperalgesia,[142] suggesting a contribution of mast cells to nociception via the C5a-C5aR1 axis. Similarly, paw injection of ET-1 results in ET_A-receptor-dependent paw licking (indicative of overt pain) as well as thermal hyperalgesia.[143] Pharmacological blockade of ET_A receptors alleviates both mechanical and thermal hyperalgesia in the CFA model.[144] These findings suggest a potential contribution of mast cells to nociception through the ET-1-ET_A receptor axis. Degranulation via ET-1 induces the secretion of carboxypeptidase A3 from mast cells, which then proteolytically inactivates ET-1,[102] representing a specific, most likely local, ET-1-degrading mechanism of yet unknown physiological significance.

Mast cells can detect alarmins like ATP and IL-33 through the P2RX7[138] ion channel and ST2 (IL-33R),[145] respectively. In healthy tissues, extracellular nucleotide concentrations—particularly ATP—are typically low, but they can increase in inflamed tissues.[138] ATP-mediated mast cell degranulation is associated with pain in two studies. Injection of high concentrations of ATP into mouse skin induces mechanical hyperalgesia, a response that is reduced in *Kit*-mutant mice and prevented by blocking P2RX7 receptors.[146] Using an *ex vivo* model to record TG neuron activity, ATP application to the meninges activates TG neurons. This activation is diminished in tissues from *Kit*-mutant mice or when a serotonin receptor 3 (5-HT_3R) antagonist is applied.[147] Together, these findings may indicate that ATP-activated mast cells contribute to pain.

The IL-33 receptor, ST2, is not only expressed by mast cells but also on various other cells—including Th2 cells, type 2 innate lymphoid cells, eosinophils, and basophils. Hence, the specific role of mast cells in IL-33 signaling remains complex. In mast cells, IL-33 does not induce degranulation but instead promotes the production of cytokines such as IL-13[148] and IL-6.[149] Mast-cell-derived IL-13 may lower the activation threshold of sensory neurons, thereby enhancing itch[148] and pain[150] responses. Similarly, IL-6 not only lowers neuronal activation thresholds but can also directly induce pain.[151] Following injection of IL-33, mast-cell-derived IL-6 may contribute to mechanical hyperalgesia of the skin.[152] Furthermore, injection of antigen into immunized mice induces mechanical hyperalgesia, which can be blocked by pre-treatment with soluble ST2, which acts as a decoy receptor for IL-33.[152] However, mast cells can also produce soluble ST2[153] and induce IL-33 release from neighboring epithelial cells.[154] Further research is needed to clarify the role of mast cells in IL-33 pain regulation.

In response to tissue insults, mast cell activation—through receptors such as MrgprB2, C5aR, ET_AR, P2RX7, and ST2—is likely involved in nociception, pain, and itch via signaling through sensory neurons. However, the specific roles and mechanisms by which mast cells and their mediators contribute to these processes are complex and, in part, controversial, highlighting the need for further investigation.

CONCLUSIONS AND OUTLOOK

Mast cells are strategically located within barrier tissues and can detect a vast array of foreign antigens through IgE as well as signals released during tissue insults and stress via the ligand-versatile receptor MrgprB2, the IL-33-receptor ST2, and others.

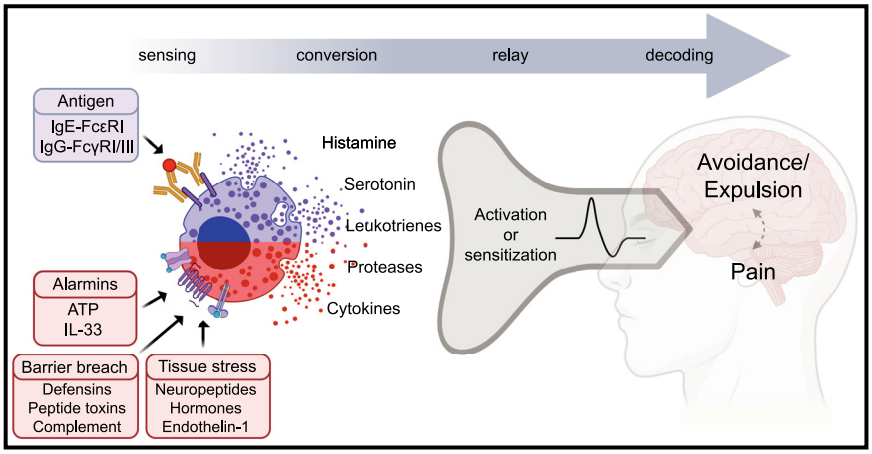

Figure 3. Mast cells as converters of antigen- and tissue- signals into host protective behaviors

Mast cells express FcεRI, FcγRI and FcγRIII, and through IgE and IgG can sense a broad diversity of antigens, representing current immunity, and memory of past B cell immune responses. In addition, mast cells express receptors for sensing signals released during tissue insults and stress, e.g. in the course of infection, inflammation, or injury, including ATP, IL-33, defensins, peptide toxins, complement proteins, neuropeptides, hormones, and endothelin-1. Mast cells can convert these inputs into output signals, akin to neurotransmitters, that are readily detected by sensory neurons. Among the mediators produced by mast cells are histamine, serotonin, leukotrienes, proteases, and cytokines, which can induce action potentials and/or lower the activation threshold of sensory neurons. In this model, electrical signals from neurons are transmitted to the central nervous system that decodes the information and mounts appropriate, organ-specific, host protective behavior.

There is experimental evidence for mast cells receiving immunological and tissue signals and relaying them to cells from the nervous system. Remarkably, mast cells link immune sensing to antigen avoidance behavior. More generally, in host-protective reflexes, mast cell sensing is converted into messenger molecules, akin to neurotransmitters (Figure 3). Sensory neurons respond to mast cell signals and generate electrical output (action potentials), relaying the information from mast cells to the CNS. There, the signals are decoded to elicit host-protective behavioral responses. Organ specificity of these responses could be achieved by distinct tissue innervation patterns as well as tissue-specific mast cell phenotypes. In this model, mast cells act as converters of antigen and tissue-insult signals to neuronal output (Figure 3). In contrast to afferent signaling, mast cells can also receive stimuli originating from the nervous system and relay them into tissues, for example, during inflammation. In both axes, mast cells would be crucial sensor cells. Further experiments will be needed to dissect such mast cell functions and to explore whether they are non-redundant.

Regarding the mechanism underlying mast-cell-mediated avoidance of antigen-containing foods, future research shall elucidate the gut-brain signaling pathways. Furthermore, putative avoidance pathways in the airways are of great interest. Although initial studies have suggested neuroimmune mechanisms of IgE-dependent behavioral reflexes involving mast cells, including itch, tearing, sneezing, cough, diarrhea, and emesis, many questions remain. Key areas include the identification of the mediators produced by mast cells that drive reflexes—and the neural cells responding to these mediators. Mast cell activation leads to the simultaneous release of various classes of mediators, each potentially possessing distinct stimulatory activities toward neurons.[155] This is complicated by the fact that mediators that activate mast cells in response to tissue injury can also directly stimulate sensory neurons. Therefore, future research should aim to disentangle the effects of individual mediators and their cooperative interactions, both locally within tissues and at the systemic level. Data on IgE-independent mast cell activation in nociception is also rapidly emerging. It has been long known that IgE-dependent and -independent mast cell activation differs in kinetics and release of mediators.[74,156,157] However, the biological meaning of these divergent responses remains unclear. Neurons contain distinct types of intracellular vesicles, such as synaptic vesicles and dense-core granules, which store specific neurotransmitters and are released in a stimulus-dependent manner.[158] A similar layer of secretion regulation may operate in mast cells. To fully understand the impact of different mast cell granule species on responses (reflexes) *in vivo*, it will be critical to more comprehensively understand the conditions dictating potential selective release. Finally, the development of new tools to track mast-cell-neuron interactions *in vivo* may accelerate our understanding of mast cell functions in host-protective behavior. The future appears bright for the mast cell field as we enter an exciting era of research at the crossroads of immunology and neuroscience.

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AUTHOR CONTRIBUTIONS

T.P. and H.-R.R. wrote the manuscript with input from T.B.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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