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REVIEW

The role of G protein-coupled receptors in mast cell activation by antimicrobial peptides: is there a connection?

Priyanka Pundir^{1,2} and Marianna Kulka^{1,2}

Antimicrobial peptides (AMPs) are ancient and essential elements of the host defense system, which are found in a wide variety of species. They show antimicrobial activity against a wide range of pathogenic microorganisms. In addition, AMPs are expressed by different immune cells and have an important function in host innate immune response against pathogens by mechanisms that are different from those involved in direct microbial cytotoxicity. One host innate immune response that is directly activated by AMPs involves induction of localized inflammation through interaction with mast cells. Activation of mast cells releases pre-formed mediators, cytokines, chemokines and eicosanoids, which influence recruitment, survival, phenotype and functions of many immune cells. Mast cells can respond to AMPs independent of antigen and Fc epsilon receptor 1 stimulation. One of these pathways involves G protein-coupled receptor signaling, which can lead to mast cell degranulation. Whether AMPs activate G proteins in mast cells through a receptor-dependent or a receptor-independent mechanism remains poorly understood and there are a great many questions that have yet to be answered. In this review, we will discuss the possible involvement and role of GPCRs in mast cells activation by AMPs and the gaps in our current understanding of this important interaction.

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Antimicrobial peptides (AMPs) act as important mediators of innate immune responses because they have microbicidal activity against invading pathogens. However, AMPs, in addition to exhibiting a direct antimicrobial activity, possess a wide range of immunomodulatory properties. AMPs interact with cells of innate immune system such as neutrophils, monocytes, dendritic cells, T cells, B cells and mast cells to indirectly eradicate pathogens by activating or inhibiting cellular functions such as production of cytokines and chemokines, alteration of gene expression, chemotaxis, modulation of cell-surface receptors and initiation of downstream signaling cascade (Figure 1).¹ Mast cells are tissue resident cells that have a crucial role in innate and adaptive immunity through the release of stored or *de novo*-synthesized inflammatory mediators. Two major pathways recognized for mast cell activation are 'immunoglobulin E (IgE) dependent'^{2–3} and 'IgE independent'.⁴ Having been overshadowed by the classical IgE-dependent mechanism, the importance of IgE-independent mechanisms of mast cell activation is receiving more attention, especially in the context of bacterial and viral infection.

The immediate microenvironment surrounding mast cells contains AMPs that, under specific conditions, may activate mast cells through IgE-independent receptors such as G protein-coupled receptors

(GPCRs). GPCRs are 7-*trans*-membrane receptors, which are coupled to heterotrimeric GTP-binding proteins (G proteins). G proteins exist as a complex of G α and G $\beta\gamma$ subunits (Figure 2).⁵ After ligand binding, G α exchanges GTP for GDP and detaches from the G $\beta\gamma$ dimer and each activated component interacts with different effectors to initiate various cellular responses (Figure 2).^{5–6}

Many GPCRs have been theorized to bind and be activated by AMPs (Figure 2). However, the nature of these protein–receptor interactions and signaling pathways, in context of mast cell activation, is largely unknown. This review will highlight recent advances in GPCR signaling, focusing on the importance of AMP–mast cell interaction through GPCRs.

ANTIMICROBIAL PEPTIDES

AMPs are a unique and diverse group of gene-encoded molecules, which are highly conserved in their structure, function and mechanism of action. AMPs are synthesized as precursor molecules, which consist of a signal sequence followed by a propeptide region. Precursor peptides undergo proteolytic cleavage to release mature, biologically active peptide.⁷ AMPs are grouped into four main classes according to similarities in charge, sequence homology, structure and functional

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Figure 1 Mast cell-derived mediators. Activation of mast cells releases mediators, which fall under three major categories: (i) preformed granule-associated mediators; (ii) cytokines and chemokines and; (iii) newly generated lipid mediators. These mediators contribute in pro-inflammatory responses and host defense.

Figure 2 GPCR signaling cascade. The active G α -GTP subunit stimulates PLC β , which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃).¹²⁰ Ins(1,4,5)P₃ binds IP₃ receptor present on the surface of internal calcium stores, mainly the smooth endoplasmic reticulum and opens calcium channels. Release of intracellular calcium leads mast cell degranulation.⁴⁸ G $\beta\gamma$ dimer activates RAS-RAF pathway, which phosphorylates mitogen-activated protein kinases, extracellular-signal-regulated kinases and c-Jun N-terminal kinases. These, together with PLC β and PI3K γ lead to cytokine and chemokine generation. PKC stimulate generation of eicosanoids through cytosolic phospholipases A₂ (cPLA₂).¹²¹

Table 1 Classification of antimicrobial peptides

Class of antimicrobial peptides	Example
Linear α -helical short anti-microbial peptide	Cercopins Maganins, berrinin-1 BMAP, SMAP, PMAP Cathelicidins/LL-37
Specific amino acids rich cationic peptides	Bactenecins, PR-39 rich in arginine and proline Indolicidins rich in tryptophan Small histidine -rich histatins
β -pleated sheets, high cysteine residues containing anionic and cationic peptides	Protegrins Human α - and β -defensins
Anionic peptides	Maximins Dermicidins

similarity. These four classes are (1) linear cationic α -helical peptides, (2) cationic peptides enriched with specific amino acids, (3) anionic and cationic peptides that contain cysteine and form disulphide bonds, and (4) anionic peptides (Table 1).⁸ Most AMPs are cationic in nature and their structure is organized such that the hydrophobic region lies opposite to the cationic (charged) surfaces. The association between cationic AMPs and anionic microbial cell surface facilitates AMPs to penetrate the microbial membrane, leading to membrane disruption and cytolysis. Various models have been proposed to explain microbial membrane disruption, but in all of these models, AMPs insert perpendicularly into the lipid bilayer of microbial membrane, forming transmembrane pores leading to membrane disintegration.⁹ Beside these transmembrane pore-forming models, different modes of intracellular killing of microbes by AMPs have also been documented, such as the inhibition of cell wall synthesis by mersacidin,¹⁰ inhibition of nucleic acids and protein synthesis by human neutrophil defensins¹¹ and inhibition of cellular enzymatic activity.¹²

AMP expression

The expression of AMPs by different tissues is tightly regulated and corresponds to their gene structure. AMPs are predominantly expressed by epithelial cells and immune cells. Their expression can be constitutive, inducible or both. Human α -defensins, also known as human neutrophil defensins HNP1-4, are expressed constitutively by mature neutrophils. Human defensins-5 and -6 are expressed in intestinal paneth cells.¹³⁻¹⁴ The first human β -defensin, hBD-1, was isolated from hemofiltrate¹⁵ and is constitutively expressed in epithelial cells, urogenital tissues and respiratory tract.¹⁸⁻¹⁹ Expression of hBD-1 does not change with inflammatory signals¹⁶ suggesting that its function is independent of host inflammatory response to infection.

hBD-2 was originally isolated from psoriatic skin¹⁷ and is primarily expressed by epithelial cells such as those lining the respiratory tract.¹⁶ Currently, four types of human β -defensins have been characterized and are referred to as hBD1-4, which are produced pre-dominantly by epithelial tissue. Human β -defensins are also expressed by neutrophils, monocytes and dendritic cells.¹⁸ In contrast to constitutive expression of hBD-1, hBD-2-4 can be induced by infectious and inflammatory agents such as Gram-positive and Gram-negative bacteria, lipopolysaccharides, peptidoglycans, tumor necrosis factor (TNF), interleukin (IL)-1 β and interferon- γ .¹⁹⁻²⁰ Expression of hBD-3 can be induced by microorganisms, TNF, IL-1 β and interferon- γ ,²¹ whereas the expres-

sion of hBD-4 is induced by phorbol-12-myristate-13-acetate (PMA) and bacterial infection.²²

Besides defensins, another important family of AMP is the cathelicidins. A variety of cathelicidins have been identified in animals. However, humans express only one cathelicidin, hCAP-18/LL-37,²³ produced mainly by immune cells such as neutrophils, monocytes, NK cells, B cells,²⁴⁻²⁵ squamous epithelia, keratinocytes and airway epithelia.²⁶ The expression of LL-37 is significantly increased in inflamed skin.²⁷ Upregulation of defensin and cathelicidin gene expression depends on the activation of nuclear factor (NF)- κ B, activation protein (AP)-1, janus kinase (JAK)-2 and signal transducer and activator of transcription (STAT)-3 signaling pathways.²⁸

Immune activities of AMPs

High expression level of AMPs by epithelial cells is advantageous because pathogens first try to breach these barriers to overcome the host defense system. AMPs regulate inflammation and promote wound healing, tissue repair, angiogenesis, vascularization and epithelialization. AMPs are chemoattractants for leukocytes and other immune cells and they influence cell proliferation and direct T helper responses to initiate the adaptive immune system.²⁹ Decreased expression of AMPs increases susceptibility to infectious diseases. For example, decreased expression of LL-37 and hBD-1 is associated with gastrointestinal infections³⁰ and respiratory disorders, such as cystic fibrosis.³¹ Patients with atopic dermatitis show decreased expression of LL-37, hBD-2 and -3.³²

Receptors for AMPs have been proposed although specific receptor/ligand interactions are poorly characterized. Putative receptors include innate immune pattern recognition receptors such as toll-like receptors (TLRs). TLRs are pattern recognition receptors, which bind pathogen-associated molecular patterns such as bacterial lipopolysaccharides, flagellin, lipoteichoic acid, viral peptidoglycans and nucleic acids.³³ The epithelium lining mucus membranes in the lung and GI tract express very specific TLRs on their apical or basolateral surfaces such that they can respond to specific infections. hBD mediates its antimicrobial activity through TLR-2 present on keratinocytes.³⁴ hBD-3 induces expression of TLR-1 and -2 on monocytes and myeloid dendritic cells.³⁵ LL-37 increases TLR-4 expression in human mast cells.³⁶ The airway epithelium also expresses several other innate immune receptors such as CD14, TNF receptors, IL-1 receptors and inter-cellular adhesion molecule capable of initiating responses to microorganisms and inducing secretion of AMPs.³⁷ The majority of AMPs are believed to act through GPCRs as the cellular functions induced by AMPs could be inhibited by pretreatment of the target cells with pertussis toxin, a toxin capable of inhibiting GPCR signaling by G α_i subunit.³⁸ AMPs may use chemotactic GPCRs such as formyl peptide receptor-like-1 (FPRL-1) to migrate immune cells.³⁹ GPCR pathways seem to have a central role in AMP-mediated signaling in host defense.

AMPs and mast cells

AMPs establish an antimicrobial barrier at epithelial interfaces and initiate pro-inflammatory responses. Numerous studies have reported increased levels of AMPs in a number of inflammatory states. For example, inflamed intestinal epithelium exhibits high levels of hBD-2 compared with normal colon⁴⁰ and patients with irritable bowel syndrome show elevated levels of hBD-2.⁴¹ Interestingly, initiation of inflammation at these epithelial barriers seems to be dependent on activation of mast cell histamine release.⁴²

Mast cells are hematopoietic stem cells derived from bone marrow progenitor stem cells.⁴³ Typically, considered as 'allergy cells,' mast cells

Table 2 Mast cell-derived pro-inflammatory mediators

<i>Class of Product</i>	<i>Examples</i>	<i>Biological effects</i>
Preformed mediators/ Toxic products	Histamine, heparin	Increase vascular permeability Cause smooth muscle contraction
Enzymes	Tryptase, chymase, cathepsin G, carboxypeptidase	Remodel tissue matrix
Cytokines	IL-4, IL-13	Stimulate and amplify Th2-cell response
	IL-3, IL-5, GM-CSF	Promote eosinophil production and activation
	TNF	Promotes inflammation, stimulates cytokine production by immune cells
Chemokines	CCL2, CCL3, CCL4, CXCL1, CXCL2, CXCL3, CXCL10	Attracts monocytes, macrophages and neutrophils
Lipid mediators	Prostaglandin D ₂ , E ₂ Leukotriene B ₄ , C ₄	Cause smooth muscle contraction, increase vascular permeability, stimulate mucus secretion
	Platelet-activating factor	Attracts leukocytes, amplifies production of lipid mediators, activates neutrophils, eosinophils and platelets

Abbreviations: CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; TNF, tumor necrosis factor.

are also critically involved in the defense against invading pathogens. Mast cells are distributed in the tissues throughout the body, particularly in close proximity to blood vessels, nerves, smooth muscle cells and more notably on the surfaces exposed to constant environmental challenge such as the skin, gut and airways.^{44–45} After pathogen invasion, epithelial surfaces release AMPs, which can activate nearby mast cells through cell-surface receptors. Mast cells rapidly respond to these pathogenic stimuli by releasing pro-inflammatory mediators. Mast cell-derived pro-inflammatory mediators protect the host by limiting microbial invasion (Table 2). Mast cells also clear pathogens by phagocytosis and/or through the secretion of AMPs such as LL-37.⁴⁶ Mast cells can form extracellular traps (MCEs) consisting of a chromatin-DNA backbone with attached AMPs, ultimately forming a net in which microbes are entrapped and killed.⁴⁷ These characteristics of mast cells make them indispensable for a strong defense mechanism.

Mast cell activation by GPCRs and mediator release

After ligand–GPCR binding, mast cells get stimulated and release a diverse array of biologically active mediators, which initiate pro-inflammatory responses (Figure 1) (Table 2). $G\alpha$ -GTP activate phospholipase C (PLC) and phosphokinase C (PKC) resulting in massive influx of extracellular calcium.⁴⁸ High calcium concentrations cause mast cell granule translocation and granule docking.⁴⁹ Interaction between the integral membrane proteins called soluble NSF attachment proteins (SNAREs) present on both, granules (v-SNARE/VAMP) and plasma membrane (t-SNARE) allows close apposition and ultimately fusion between granules and cell membrane leading to exocytosis of granular contents.⁴⁹ PKC further activates the generation

of eicosanoids (for example, leukotrienes C₄ and prostaglandin D₂) through phosphorylation of cytosolic phospholipases A₂ pathway (Figure 2).⁵⁰

$G\beta\gamma$ dimer phosphorylates and activates mitogen-activated protein kinases (MAPKs), extracellular-signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNKs). These, together with PLC- β and PI3K γ , regulate activation of transcription factors leading to cytokine and chemokine generation (Figure 2). This pathway is also calcium dependent.⁵¹

Mast cell-derived mediators such as histamine, TNF and chemokines activate immune cells to migrate and differentiate and promote recruitment of immune cells by upregulating the expression of adhesion molecules by vascular endothelial cells. IL-4 and IL-13 promote IgE production by B cells;^{52–53} IL-4 and IL-9 induce T helper 2-cell responses.⁵⁴ Mast cell tryptases and chymases oppose inflammation by inactivating allergens and inflammatory neuropeptides and by reducing toxicity of venoms and endogenous peptides.⁵⁵ Leukotriene B recruits effector T cells and immature mast cells.⁵⁶

AMPs TRIGGER GPCR SIGNALING IN MAST CELLS

Several examples of AMPs have been shown to stimulate and activate mast cell mediator release not only in humans, but also in less evolved invertebrate systems.

Invertebrate AMPs

Stings of social wasps and bees contain several chemical substances, primarily a range of amines, peptides and proteins that together can produce a wide spectrum of biological effects such as severe pain, edema,

Table 3 Antimicrobial peptides from invertebrates activating mast cell degranulation

Peptide	Source	Antimicrobial activity	References
Mastoparan	<i>Vespula lewisii</i>	Broad spectrum	105
Eumenine Mastoparan-AF	<i>Anterhynchium flavomarginatum micado</i>		106
Protopolybia MPI; MPII; MPIII	<i>Protopolybia exigua</i>	Broad spectrum	107
Mastoparan B	<i>Vespa basalis</i>	Broad spectrum	60
Polybia-MPI	<i>Polybia paulista</i>	G+ and G- bacteria	108
Vespa mastoparan MP-VBs & Vespa chemotactic peptide VESP-VBs	<i>Vespa bicolor fabricius</i>	Broad spectrum	109
Decoralin	<i>Oreumenes decoratus</i>	Broad spectrum	110
Eumenitin	<i>Eumenes rubronotatus</i>	G+ and G- bacteria	111
Anoplin	<i>Anoplius samariensis</i>	G+ and G- bacteria	112
Melectin	<i>Melecta albifrons</i>	G+ and G- bacteria	113
Mastoparan PDD- A, PDD-B, MP, PMM	<i>Polistes major major Mischocyttarus phthisicus</i>	G+ and G- bacteria	114
Brevinins-Alb & Temporins-Ala	<i>Amolops loloensis</i>	Broad spectrum	115
Esculentin-1SEa, 1SEb, 2Se, 1Se; Brevinin-1SE & Ranatuerin-2SEa, 2SEb, 2Sec	<i>Rana sevosia</i>	G+ and G- bacteria	116

Abbreviations: G−, Gram negative; G+, Gram positive.

local damage and even death in some cases.^{57–59} The most abundant group of peptides in the venom of social wasps is mast cell degranulator peptides, which include mastoparans and chemotactic peptides.^{59–60} Mast cell degranulator peptides exhibit a broad range antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.⁶¹ Mast cell degranulator peptides is a strong mediator of mast cell degranulation and histamine release.⁶² Mastoparan from vasp venom is an amphiphilic tetradecapeptide and activates mast cell-mediator release by binding G α_i proteins because secretion of histamine by rat peritoneal mast cells is inhibited by pertussis toxin (a selective inhibitor of G α_i protein). In fact, mastoparan is a potent G protein activator and stimulates the GTPase activity of G α_i protein 15–20 above that of constitutive G α_i activity.⁶³ Mastoparans activate PLC to release Ins(1,4,5)P₃, showing that degranulation and cytokine secretion are likely calcium dependent.⁶⁴ Mastoparans also activate PLA₂ to generate eicosanoids.⁶⁵ Several other peptides from insects and frogs have been reported to induce mast cell degranulation (Table 3), showing that association between AMPs and mast cells is an important first line of defense in organisms lacking well-developed adaptive immunity.

AMPs from mammals

The two well-characterized families of mammalian AMPs, defensins and cathelicidins, promote inflammatory innate immune responses by

inducing mast cell-mediator release and mast cell chemotaxis. Human α -defensins induce secretion of histamine from rat peritoneal mast cells⁶⁶ and human mast cells; induce epithelial cell proliferation; promote epithelial wound repair⁶⁷ and serve as chemoattractant for leukemic HMC-1.⁶⁸ hBD-2, -3, -4 and LL-37 induce mast cell degranulation^{36,69} and PGD₂ production,^{69–70} but hBD-3 is more potent than hBD-4 in this regard.⁷⁰ hBD-2, -3, -4 and LL-37 are chemotactic for mast cells.^{70–72} LL-37, hBD-3 and -4 activate mast cells to release histamine, which in turn increases vascular permeability in the skin. This requires phosphorylation of MAPK p38 and ERK 1/2.^{70,73} LL-37 also induces production of the Th1 cytokine IL-2, Th2 cytokines IL-4 and IL-5 and pro-inflammatory cytokines TNF and IL-1 β by laboratory of allergic diseases cells (LAD2) human mast cell line,³⁶ suggesting that LL-37 can mediate both pro-inflammatory and anti-inflammatory effects by activating mast cells to modify host defenses against infection.

To elucidate the signaling mechanisms involved, these studies have largely focused on the use of inhibitors of GPCR signaling pathways like pertussis toxin, U-73122 (PLC inhibitor), wortmannin (PI3-K inhibitor) and R0-31-8220 (PKC inhibitor)^{69–72} suggesting that defensins and cathelicidins stimulate mast cell-mediator release and through the activation of G proteins signaling.⁷⁴ The chemotactic effect of defensins and cathelicidins is suppressed by pertussis toxin, suggesting they use G α_i PCR. However, the information available on

Table 4 Important human antimicrobial peptides expressed during inflammation

Peptide	Effect	Antimicrobial activity	References
Cathelicidins (LL-37)	Induction during inflammation, wound and blisters; antiseptis; reduce cytokine expression; chemotaxis	Broad spectrum	70, 117
Human alpha defensins	Cross talk between immune cells; chemotaxis; promote acquired systemic immunity	Broad spectrum	66, 74
Human beta defensins	Induction during inflammation; chemotaxis; wound healing	Broad spectrum	70, 74
Neuropeptides			
<i>Substance P; Neurokinin A; Vasoactive intestinal peptide; Neuropeptide Y; Calcitonin gene-related peptide</i>	Induction during inflammation; link between mast cells and nervous system in neuroinflammation	Broad spectrum	77, 118-119

specific GPCRs involved in ligand binding is very limited. Initially, it was shown that the chemotactic activity of β -defensins for dendritic cells and memory T cells is mediated by human CCR6, a GPCR.⁷⁵ This finding was later refuted by Soruri *et al.* who reported that in contrast to CCL20 (a ligand for CCR6), human β -defensins are unable to migrate dendritic cells and memory T cells thus arguing against CCR6 as a receptor for human β -defensins.⁷⁶ This is supported by the finding that RBL-2H3 mast cells transfected with human CCR6 did not migrate toward hBD-2 and hBD-3 despite high CCR6 expression levels.⁷⁶ Murine bone marrow-derived mast cells and HMC-1 chemotax in response to hBD-1, -4 and hBD1-4, respectively, and hBD1-4 desensitize each other's chemotactic impact on mast cells in PKC-independent manner indicating that human β -defensins signal through common GPCRs in mast cells.⁷⁶ In similar experiments with human α -defensins HNP-1, HNP-3 and human defensin-5 were found to be strong chemoattractants for HMC-1.⁶⁸ Functional receptor desensitization between HNP-1 and human defensin-5 in PKC-independent manner suggests that human α -defensins also signal through a common receptor in mast cells. α -Defensins desensitized hBD-2-mediated mast cell migration in a PKC-dependent manner, suggesting unique GPCR for both the families.⁶⁸ However, the activating and chemotactic receptors for β -defensins on mast cells are still unknown.

The ability of cathelicidins to induce calcium mobilization in FPRL-1-transfected human embryonic kidney (HEK)-293 cells suggests that FPRL-1, a receptor for formyl-methionyl-leucyl-phenylalanine (fMLP), may be a receptor for LL-37.³⁹ LAD2 cells express mRNA for FPRL-1.³⁶ However, whether LL-37 is a ligand for FPRL-1 remains uncertain as the effect of antagonists of FPRL-1 and knockdown of

FPRL-1 expression on LL-37-mediated activation of mast cells have not been evaluated.

NEUROPEPTIDES AS MAST CELL ACTIVATORS

Neuropeptides can be classified as AMPs because various neuropeptides have been shown to possess microbicidal activity against a range of microorganisms.⁷⁷ Mast cells are located close to neurons and this unique positioning allows mast cells to respond to neuropeptides released from nerve endings.⁷⁸ Thus, neuropeptides participate in innate immune system not only by killing pathogens, but also by modulating inflammatory responses (Table 4).

Substance P is an important neuropeptide found widely distributed in central and peripheral nervous systems and has a key function in pain signal transmission. Its role in host defense has been extensively studied. Substance P is believed to act through tachykinin receptor subfamily of GPCRs known as neurokinin (NK)-1 receptors. NK-2R and NK-3R have also been known to interact with substance P.⁷⁹ In rodent mast cells, there is some evidence for the involvement of a selective substance P receptor in substance P-mediated mast cell activation.⁴ Human skin mast cells degranulate to release pro-inflammatory mediators in response to substance P, express adhesion proteins and produce TNF.⁸⁰ In dispersed human skin mast cells, substance P and vasoactive intestinal polypeptide (VIP) induce dose-dependent release of histamine.⁸¹ Studies with rat peritoneal mast cells showed the histamine-releasing activity of substance P,⁸² neuropeptides Y⁸³ and calcitonin gene-related peptide (CGRP). In these studies, very high concentrations (micromolar range) of substance P was required to activate mast cells,⁸⁴⁻⁸⁵ and NK-1 receptor antagonists were found to act as potent secretagogues.⁸⁵⁻⁸⁶ Pretreatment of mast

cells with pertussis toxin inhibited histamine release.⁸² All of these findings suggested that substance P degranulates rat peritoneal mast cells in a receptor-independent manner, possibly through direct activation of G proteins.⁴ However, a study by Ogawa *et al.*⁸⁷ reported the release of histamine from peritoneal mast cells of a wistar substrain, Std:Wistar rats in response to very low concentrations (nanomolar range) of substance P, which could be significantly blocked by a NK-1 receptor antagonist, CP-96345, suggesting a receptor-dependent (NK-1 receptor-mediated) mechanism of histamine release from rat peritoneal mast cells.

In 2006, it was shown that human mast cells express Mas-related gene (Mrg) GPCR. Substance P stimulated human cord mast cells, expressing MrgX2, to degranulate.⁸⁸ Substance P activated calcium influx in HEK-293 cells transfected with MrgX2, but not in cells lacking MrgX1⁸⁸ indicating that MrgX2 may be a receptor for substance P in HMCs. Whether substance P binds MrgX2 or by passes it to interact with G proteins remains to be determined.

Our previous studies characterized the response of two human mast cell models (primary CD34⁺-derived HuMC and LAD2) to substance P, nerve-growth factor, CGRP and VIP.⁸⁹ Substance P and VIP, but not nerve-growth factor and CGRP, induced degranulation in human mast cells along with the production of cytokines such as TNF, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3, and chemokines such as interferon-inducible protein-10 (IP-10), monocyte chemoattractant protein (MCP)-1, IL-8 and RANTES (regulated on activation, T cell expressed and secreted).⁸⁹ Human mast cell activation was sensitive to pertussis toxin and wortmannin (PI3-K inhibitor). LAD2 cells express receptors for substance P (NK1R, NK2R and NK3R), CGRP (CGRPR) and VIP (VPAC2). HuMCs express only NK-1R, NK-3R and VPAC1 suggesting the presence of functional neuropeptide receptors on HMC.⁸⁹ However, as NK-1 receptor antagonist could only partially block substance P's effect,⁸⁹ these studies suggest that substance P may also activate mast cells in a receptor-independent mechanism, perhaps by direct activation of G proteins.⁹⁰

OTHER ROUTES OF MAST CELL ACTIVATION

Mast cell-derived mediators secreted in response to AMPs can influence mast cells in an autocrine manner. Histamine released by mast cells activates histamine H4 GPCRs expressed on mast cells and enhances migration of murine mast cells.⁹¹ Mast cell-derived cytokines and chemokines can also activate mast cell by autocrine activation of GPCRs. For example, CCR1 that binds MIP-1 α , CCR1, -3 and -4 that bind RANTES to induce chemotaxis in mast cells. Cord-blood-derived human mast cells express cysteinyl leukotriene receptor 1 and 2^{92–93} and EP1-4 for prostaglandin E,⁹⁴ which are coupled to G proteins. These receptors help improve immune responses by increasing mast cell numbers and by enhanced degranulation and mediator release.

FUTURE DIRECTIONS

It is clear that AMPs activate mast cells to initiate pro-inflammatory conditions. However, numerous questions still remain unanswered. Which GPCRs actually bind AMPs directly? Is the interaction physiologically relevant? Do the GPCRs co-localize, and if yes, is there a synergy, competition or inhibition? Are the chemotactic GPCRs equally potent in mediating mast cell-mediator release?

The current literature suggests that GPCR–mast cell interactions are complex. Most of the studies on GPCR signaling in mast cells have used cultured mouse mast cells and information available from human mast cells is limited. As the nature of responses generated by

GPCRs in mouse and human mast cells differs significantly, a careful evaluation of both mouse and human-derived mast cells is important. Besides the interspecies difference, phenotype of mast cells also makes a significant difference in outcomes of mast cell activation. In rodents, two major distinct mast cell populations have been described as mucosal type and connective tissue type.⁹⁵ In humans, the two mast cell subsets are designated as MC_T and MC_{TC}. MC_T subtype is mucosal with granules rich in tryptase whereas the MC_{TC} has a connective tissue phenotype with granules rich in tryptase and chymase.⁹⁶ Connective tissue-type mast cells show higher secretory response to polybasic compounds such as compound 48/80 and polymixin B as compared with mucosal type.⁹⁷ Hence, selection of mast cell type is important. Further studies with improved models, particularly *in vivo* studies, are warranted to determine the proper GPCR-mediated pathways and their contribution to mast cell's role in host innate defense. Use of receptor knockout studies using mice deficient in specific GPCR is also necessary to evaluate the relative contribution of these diverse set of receptors to antimicrobial effects.

There are many other GPCRs that may have a role in both innate immune responses and antimicrobial activation of mast cells. For instance, purinergic P2 receptors have been hypothesized to recruit immune cells to sites of inflammation. Chemotaxis of neutrophils is mediated through P2Y2.⁹⁸ Eosinophil trafficking toward platelets⁹⁹ is mediated possibly through P2X and P2Y receptors.¹⁰⁰ AMPs may also bind purinergic receptors on immune cells to secrete humoral factors. LL-37 stimulates IL-1 β from monocytes by activation of P2X7 receptor thus forming a link between innate and adaptive immunity.¹⁰¹ HNP induced IL-8 production from human lung epithelial cells through the P2Y6 signaling pathway.¹⁰² Mast cells express P2Y1, 12 and 13 mRNA¹⁰³ but whether AMPs can induce mast cell functions through these receptors remains to be elucidated. Similarly, LAD2 and human lung mast cells express P2X1, P2X4 and P2X7.¹⁰⁴ Their role in AMP–mast cell interaction is also unknown.

Future research will need to better clarify the relationship between AMPs and mast cells, identify the specific GPCRs that bind AMPs, investigate the effects of these interactions in *in vivo* models and investigate their role in host defense against infection. Such studies will increase our understanding of the complex relationship between mast cells and AMPs during innate immune responses.

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