Roles of Mas-related G protein–coupled receptor X2 on mast cell–mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases

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Mast cells (MCs), which are granulated tissue-resident cells of hematopoietic lineage, contribute to vascular homeostasis, innate/adaptive immunity, and wound healing. However, MCs are best known for their roles in allergic and inflammatory diseases, such as anaphylaxis, food allergy, rhinitis, itch, urticaria, atopic dermatitis, and asthma. In addition to the high-affinity IgE receptor (FcεRI), MCs express numerous G protein–coupled receptors (GPCRs), which are the largest group of membrane receptor proteins and the most common targets of drug therapy. Antimicrobial host defense peptides, neuropeptides, major basic protein, eosinophil peroxidase, and many US Food and Drug Administration–approved peptidergic drugs activate human MCs through a novel GPCR known as Mas-related G protein–coupled receptor X2 (MRGPRX2; formerly known as MrgX2). Unique features of MRGPRX2 that distinguish it from other GPCRs include their presence both on the plasma membrane and intracellular sites and their selective expression in MCs. In this article we review the possible roles of MRGPRX2 on host defense, drug-induced anaphylactoid reactions, neurogenic inflammation, pain, itch, and chronic inflammatory diseases, such as urticaria and asthma. We propose that host defense peptides that kill microbes directly and activate MCs through MRGPRX2 could serve as novel GPCR targets to modulate host defense against microbial infection. Furthermore, mAbs or small-molecule inhibitors of MRGPRX2 could be developed for the treatment of MC-dependent allergic and inflammatory disorders. (J Allergy Clin Immunol 2016;138:700-10.)

Key words: *G protein–coupled receptor, MRGPRX2, mast cells, host defense peptides, neuropeptides, drug-induced pseudoallergy, chronic urticaria, asthma*

Mast cells (MCs) reside primarily at sites exposed to the external environment, such as the skin, oral/gastrointestinal mucosa, and respiratory tract. Activation of MCs through the cross-linking of high-affinity IgE receptors (FcεRI) results in the release of preformed and newly synthesized mediators, which contribute to signs and symptoms associated with hypersensitive and allergic diseases. MCs are generally classified into 2 types based on the protease content of their secretory granules.[1] In human subjects most MCs that are found in connective tissues, such as the skin, contain tryptase, chymase, carboxypeptidase, and cathepsin, and are known as tryptase and chymase-expressing mast cells (MC_{TC}). In contrast, the majority of MCs found in the lung and gut express only tryptase and are known as tryptase-expressing mast cells (MC_T).[2-4] MCs also differ in their responses to endogenous and exogenous stimuli that promote degranulation. Thus, although both human MC types are activated through aggregation of FcεRI, MC_{TC} respond to complement components C3a, C5a, and compound 48/80, but MC_T do not.[5,6] In mice connective tissue mast cells (CTMCs) resemble MC_{TC}, and mucosal mast cells (MMCs) resemble MC_T.[1,7] Thus murine CTMCs are found in the skin, and MMCs mature in the mucosal tissues, such as the lung and gut. In addition, CTMCs are responsive to compound 48/80, C3a, and C5a for degranulation, but MMCs are not.[8-10] Although MCs have been extensively studied for their role in allergic diseases, recent evidence suggests that they contribute significantly to vascular hemostasis, pain, itch, and host defense, and these responses are most likely mediated through activation of MC_{TC} in human subjects and CTMCs in mice.[11-15]

G protein–coupled receptors (GPCRs) represent the largest family of 7-transmembrane domain receptors that couple through heterotrimeric G proteins to regulate vital cellular functions, including cell proliferation, development, survival, metabolism, and neuronal signal transmission. Homology cloning and bioinformatics analysis of sequence databases have led to the identification of approximately 800 human GPCRs.[16,17] However, endogenous and/or natural exogenous ligands remain unknown for more than 100 receptors, and they are collectively classified as "orphan GPCRs." Dong et al[18] carried out a comparative analysis of the transcriptome of the dorsal root ganglia (DRG) of wild-type and neurogenin-1–deficient mice, which does not develop a subclass of nociceptive neurons. This analysis led to the identification of multiple unknown transcripts, including an entire new subfamily of GPCRs related to the MAS1 oncogene,

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Abbreviations used

C3aR: Anaphylatoxin C3a receptor
CGRP: Calcitonin gene–related peptide
CTMC: Connective tissue mast cell
CU: Chronic urticaria
DRG: Dorsal root ganglia
EC_{50}: Concentration required to produce a 50% response
EPO: Eosinophil peroxidase
GPCR: G protein–coupled receptor
GRK: G protein–coupled receptor kinase
hBD: Human β-defensin
HDP: Host defense peptide
MAPK: Mitogen-activated protein kinase
MBP: Major basic protein
MC: Mast cell
MCDP: Mast cell degranulating peptide
MC_T: Tryptase-expressing mast cells
MC_{TC}: Tryptase and chymase-expressing mast cells
MMC: Mucosal mast cell
Mrgpr: Mas-related G protein–coupled receptor
MRGPRX2 (MrgX2): Mas-related G protein–coupled receptor X2
NK1R: Neurokinin 1 receptor
ORAI: Ca^{2+} release–activated Ca^{2+} channel
PGD_2: Prostaglandin D_2
PMC: Peritoneal mast cell
PTx: Pertussis toxin
SCA: Sickle cell anemia
SP: Substance P
VIP: Vasoactive intestinal peptide

and hence the encoding genes are called Mas-related gene (MRG) and the receptors are called MAS-related GPCRs.[18,19]

The MRG family is composed of approximately 50 members in mouse, rat, human, macaque, and rhesus monkey that can be subdivided into several subfamilies.[18,20-22] Subfamilies A, B, C, D, E, F, and H exist only in rodents, whereas subfamily X is specific to human subjects, macaques, and rhesus monkeys.[23] In human subjects there are 4 MRGX genes, *MRGPRX1* to *MRGPRX4*; the mouse genome contains *MrgprA* (A1-A10), *MrgprB* (B1-B5, B8), *MrgprC* (C11), *MrgprD*, *MrgprE*, *MrgprF*, *MrgprG*, and *MrgprH* genes.[18,20] It now appears that outside the DRG, human MC_{TC} are the only cells that express Mas-related G protein–coupled receptor X2 (MRGPRX2).[24-26] In DRG cortistatin has been identified as a potent ligand for MRGPRX2, and the receptor likely contributes to sleep regulation, locomotion activity, and cortical function.[27] Recent studies have shown that host defense peptides (HDPs) and neuropeptides activate human MC_{TC} through MRGPRX2.[28-31]

In this article we review the possible roles of MRGPRX2 on host defense, drug-induced anaphylactoid reactions, neurogenic inflammation, pain, itch, and chronic inflammatory diseases, such as urticaria and asthma. In addition, we discuss the signal transduction pathway through which peptide ligands activate MRGPRX2 and the mechanism of its regulation.

MRGPRX2 AS A NOVEL GPCR FOR NEUROPEPTIDES AND OTHER CATIONIC PEPTIDES IN HUMAN MC_{TC}

In addition to FcεRI, MCs express a large number of GPCRs for ligands as diverse as lipids, chemokines, adenosine, and the anaphylatoxins C3a and C5a.[8,32-36] Although basic peptides, such as mast cell degranulating peptide (MCDP), neuropeptides, and the synthetic histamine releaser compound 48/80, have long been known to activate MC_{TC} through a G protein–dependent mechanism, the possible involvement of specific GPCRs has been the subject of intense debate. It was previously thought that neurokinin 1 receptor (NK1R) expressed on MCs partly mediates the response to substance P (SP).[37-39] Accordingly, an NK1R inhibitor partially blocks SP-induced responses in human MCs.[38,39] Furthermore, SP translocates rapidly into rodent MCs and is able to initiate secretion when it is introduced directly into the cytosol.[40,41] Based on these findings, it was proposed that the effects of SP on MCs are mediated through 2 pathways, one involving NK1R and the other through insertion of the amphiphilic SP molecule into the cell membrane, thus enabling direct activation of G proteins.[42,43]

Tatemoto et al[25] provided the first demonstration that MRGPRX2 is expressed in MC_{TC} and showed that SP, vasoactive intestinal peptide (VIP), MCDP, and compound 48/80 activate MCs through this receptor. Human cord blood–derived MCs can be cultured under conditions that promote their differentiation into MC_{TC} or MC_T. By using quantitative PCR, it was found that the copy number of *MRGPRX2* in *in vitro*–differentiated MC_{TC} is 17,565 per 5 ng of total RNA, whereas it is only 32 per 5 ng of total RNA in MC_T.[25] This is consistent with PCR and microarray data showing that the transcript for MRGPRX2 is expressed at high levels in human skin MC_{TC} but at low levels in lung MC_T.[24,26] Interestingly, MC_{TC} express MRGPRX1 and MRGPRX2 but not MRGPRX3 and MRGPRX4.[25,44,45] Peptide and chemical libraries were screened by using a reporter gene assay in cells of the PC12 cell line transiently transfected with cDNA encoding MRGPRX2 to identify candidate ligands for MRGPRX2. It was found that MCDP, compound 48/80, SP, and VIP increase reporter gene expression in MRGPRX2-transfected cells.[25] These ligands also induced dose-dependent Ca^{2+} mobilization in human embryonic kidney (HEK293)–expressing MRGPRX2 but not MRGPRX1.[25] The specificity of SP for MRGPRX2 over MRGPRX1 for MC degranulation was later confirmed by using transfected rat basophilic (RBL-2H3) cells.[30] In addition, Fujisawa et al[26] showed that MRGPRX2 is present in both the plasma membrane and at intracellular sites in human skin MC_{TC}. Furthermore, lentiviral small hairpin RNA–mediated knockdown of MRGPRX2 expression results in substantial inhibition of SP-induced MC degranulation and prostaglandin D_2 (PGD_2) generation. In addition, a specific inhibitor of NK1R (CP-96345) does not inhibit SP-induced MC degranulation.[26] These findings suggest that although both NK1R and MRGPRX2 are expressed in MC_{TC}, SP uses MRGPRX2 to promote G protein–dependent MC degranulation.

MrgprB3 AS THE RAT ORTHOLOG OF HUMAN MRGPRX2

Most original studies on the effects of neuropeptides and HDPs on MC activation were performed with rat peritoneal mast cells (PMCs).[46-51] Unlike the human genome, the rat genome possesses one each of the *MrgprA*, *MrgprC*, *MrgprD*, *MrgprE*, *MrgprF*, and *MrgprH* genes and 6 *MrgprB* genes.[19,20] RT-PCR analysis demonstrated that rat PMCs express high levels of MrgprB3 and MrgprB8 and low levels of *MrgprB1*, *MrgprB2*, *MrgprB6*, and *MrgprB9* genes. MCDP and SP caused increased

reporter gene expression in MrgprB3-transfected PC12 cells. Furthermore, these peptides caused a dose-dependent increased Ca^{2+} mobilization in MrgprB3-transfected HEK-293 cells. In contrast, none of the peptides increased reporter gene expression or Ca^{2+} mobilization in cells transfected with the *MrgprA*, *MrgprB2*, *MrgprB6*, *MrgprB8*, *MrgprB9*, or *MrgprC* genes. These findings suggest that although neuropeptides activate human MC_{TC} through MRGPRX2, they activate rat PMCs through MrgprB3.

MrgprB2 IS THE MOUSE ORTHOLOG OF HUMAN MRGPRX2

There are 22 potential MRG GPCR coding genes in mice, which makes it difficult to identify the mouse ortholog of human MRGPRX2. Using stringent RT-PCR in mouse PMCs, McNeil et al[52] showed that these cells express mRNA for MrgprB2 and no other MRG GPCRs. Generation of a transgenic mouse with the td-Tomato reporter under the control of the MrgprB2 promoter demonstrated that expression of this receptor is restricted to CTMCs in the skin, gut, and trachea.[52] The same group also generated mice with a 4-bp deletion in the *MrgprB2* coding region, resulting in a frameshift mutation and early termination of the receptor (shortly after the first transmembrane domain). These MrgprB2 mutant mice have no defect in MC numbers and respond normally to IgE/FcεRI activation. However, PMCs from these mice show a dramatic reduction in Ca^{2+} mobilization and histamine release in response to compound 48/80 and SP *in vitro* and reduced paw edema *in vivo*. These findings demonstrate that MrgprB2 is the mouse ortholog of human MRGPRX2.

Human MRGPRX2 and mouse MrgprB2 share certain unique characteristics because these receptors are expressed in MC_{TC}/CTMCs and are activated by basic ligands, such as neuropeptides and compound 48/80. Surprisingly, MRGPRX2 and MrgprB2 differ substantially with respect to the concentration of the agonists required for their activation. Thus, concentration required to produce a 50% response (EC_{50}) values of most ligands for MrgprB2 are significantly higher than those for MRGPRX2.[25,52] For example, although SP activates MrgprB2 with an EC_{50} of 54 μmol/L, it activates MRGPRX2 with an EC_{50} of 152 nmol/L. This difference is reflected in only approximately 53% overall sequence similarity between these receptors. Furthermore, sequence similarities at the N-terminal 60 amino acids and the C-terminal 80 amino acids are approximately 34% and 47%, respectively.

Although the GPCR superfamily is composed of about 800 human proteins, the crystal structure of only 4 of these proteins have been resolved.[53] Based on these studies, it has been proposed that modules within the GPCR's extracellular and transmembrane extracellular region domains contribute to agonist binding, whereas intracellular domains are involved in G protein coupling.[53] These findings suggest that differences in the amino acid sequences of MRGPRX2 and MrgprB2 contribute to differences in the ability of peptide ligands to activate these receptors.

MRGPRX2 AS A NOVEL GPCR FOR HDPs AND ITS ROLE IN INNATE IMMUNITY

HDPs, such as defensins and cathelicidins, are positively charged amphipathic peptides that are crucial for the clearance of microbial pathogens and thus play an important role in host defense. In humans defensins are divided into α and β families depending on the position of the cysteine residues involved in disulfide linkages.[54] α-Defensins are produced by neutrophils and intestinal Paneth cells, whereas human β-defensins (hBDs) are produced primarily by epithelial cells.[55,56] Of the 4 members of the hBD family (hBD1-4) that have been characterized in detail, hBD1 is expressed constitutively, whereas the others are induced by bacteria, viruses, and cytokines. LL-37 is a cathelicidin produced by neutrophils as an inactive precursor (hCAP18), which is enzymatically cleaved to release the active LL-37. HDPs kill microbes by interacting with the negatively charged phospholipid moieties and by disrupting their membranes.[56] Direct antimicrobial activities were originally considered the primary function of these peptides, hence their name antimicrobial peptides. However, as described below, HDPs also activate MCs and other immune cells, and these features likely contribute to their effectiveness as antimicrobial agents.[57]

It is well documented that MCs play a critical role in host defense.[12,14,58] HDPs (LL-37 and hBDs) act as potent MC chemoattactants[46,59,60] and also increase the expression of Toll-like receptor 4 on their surfaces, which might enhance the ability of MCs to detect invading pathogens.[14,61] MC degranulation plays an important role in host defense by causing increased vascular permeability and initiating the recruitment of neutrophils to the site of infection.[58,62-66] We have recently shown that hBDs, which are derived from epithelial cells, activate human MCs through MRGPRX2.[29] LL-37 is produced from activated MCs and neutrophils, and its antimicrobial activity reflects, at least in part, the activation of MCs through MRGPRX2.[28,31,57,67,68] LL-37 causes release of the T_H2 cytokines IL-4 and IL-5, as well as the proinflammatory cytokine IL-β, and might contribute to the development of both innate and adaptive immunity. Interestingly, LPS generated from gram-negative bacteria inhibits LL-37–induced T_H2 cytokine but not IL-β release.[61] These findings raise the interesting possibility that LL-37 coexisting with bacterial infection switches MC function toward innate immunity.[61]

POSSIBLE ROLES OF MRGPRX2 IN THE ORCHESTRATION OF ADAPTIVE IMMUNITY AND WOUND HEALING

In addition to innate immunity, MCs orchestrate the development of adaptive immunity and play an important role in wound healing.[69-72] Thus, at the sites of microbial infection, mediators released from MCs promote migration of dendritic cells, which are subsequently increased in draining lymph nodes.[73-75] Furthermore, MC-derived histamine directly modulates dendritic cell activation to enhance antigen presentation to T cells.[76] Interestingly, compound 48/80, which activates MCs through MRGPRX2, has been used as a safe and effective vaccine adjuvant in mice.[25,72,77] *Enterococcus faecalis* has emerged as an important cause of life-threatening multidrug-resistant bacterial infections in the hospital setting. Scheb-Wetzel et al[78] recently showed that MCs exert potent antimicrobial effect against this pathogen and that this effect is mediated through their degranulation and release of LL-37. Moreover, LL-37 protects skin from necrotic skin infections and promotes healing.[79] Therefore, it is likely that MRGPRX2 expressed in MCs not only contributes to innate immunity but also provides an important link to adaptive immunity and promotes wound healing (Fig 1).

In addition to MCs, the effects of HDPs on host defense and wound healing might also reflect the activation of leukocytes and

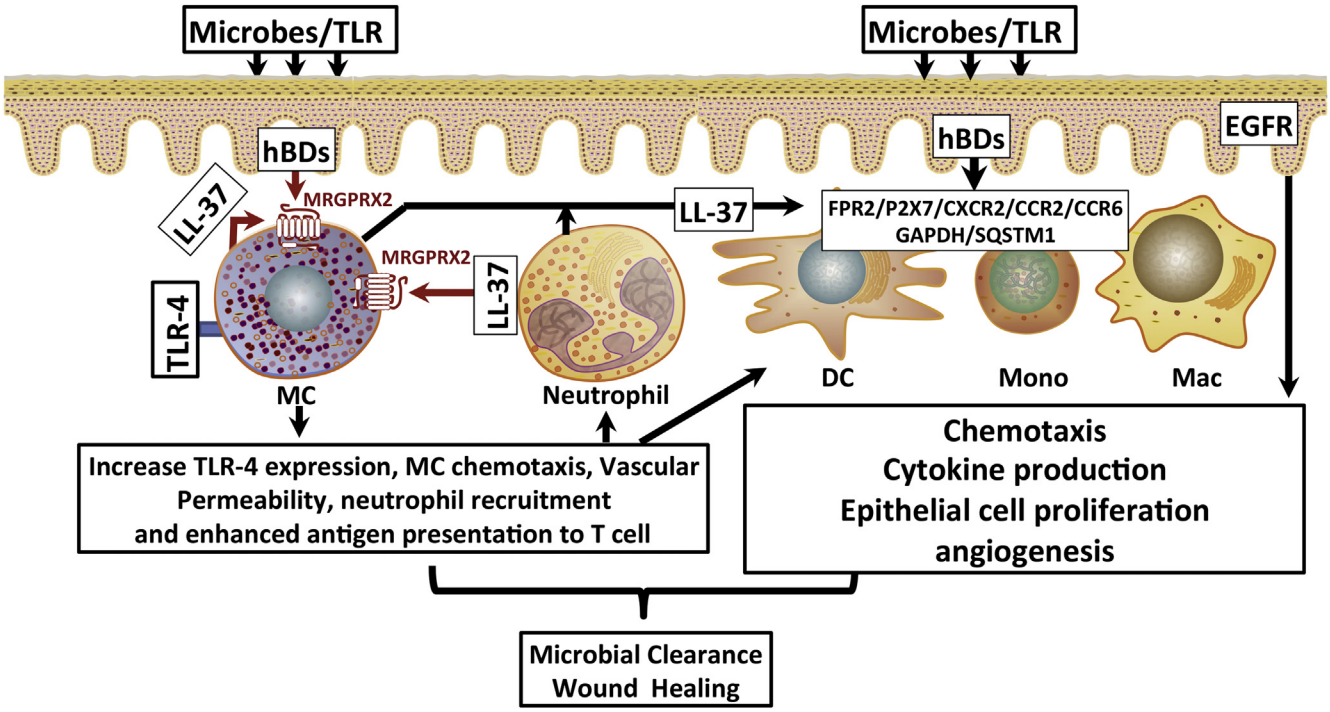

FIG 1. Activation of MRGPRX2 on MCs by HDPs might orchestrate microbial clearance and promote wound healing. Activation of epithelial cells after microbial infection leads to the generation of hBDs, which cause MC chemotaxis and degranulation through MRGPRX2. MC-derived mediators cause increased vascular permeability and promote neutrophil recruitment. LL-37 released from neutrophils also activates MRGPRX2, which enhances Toll-like receptor 4 *(TLR-4)* expression and causes further MC chemotaxis and degranulation. Histamine and TNF-α released from MCs activate dendritic cells *(DC)*, leading to enhanced antigen presentation to T cells. hBDs and LL-37 produced from epithelium and neutrophils, respectively, also promote keratinocyte migration and proinflammatory cytokine production through the transactivation of epidermal growth factor receptor *(EGFR)*. These HDPs also activate other cell-surface GPCRs and intracellular receptors (glyceraldehyde-3-phosphate dehydrogenase *[GAPDH]*/sequestosome 1 *[SQSTM1]*) on monocytes *(Mono)* and macrophages *(Mac)* to induce growth factor and cytokine release to promote microbial clearance and wound healing.

epithelial cells. Thus, LL-37 induces human neutrophil and monocyte chemotaxis through activation of formyl-peptide receptor 2, purinergic receptor P2X7, and chemokine receptor CXCR2.[80-82] Furthermore, hBD3 promotes monocyte chemotaxis through CCR2.[83] hBD3 and LL-37 induce keratinocyte migration, proliferation, and production of proinflammatory cytokines and chemokines through transactivation of epidermal growth factor receptor, which likely promotes the recruitment of additional leukocytes at the site of infection.[84,85] In addition to cell-surface receptors, HDPs activate intracellular receptors, glyceraldehyde-3-phosphate dehydrogenase, and sequestosome 1.[86,87] This leads to stimulation of multiple signaling pathways that are important in innate immunity, including p38, extracellular signal-regulated kinases 1 and 2 (also called mitogen-activated protein kinase [MAPK] 3 and MAPK1, respectively), stress-activated protein kinases/Jun amino-terminal kinases, and nuclear factor κB, thus resulting in augmentation of chemokine production and recruitment of neutrophils and monocytes to the site of infection.[57,88] Thus, it is likely that antimicrobial activities of HDPs largely depend on their ability to activate MCs through MRGPRX2, which orchestrates the development of adaptive immunity, resulting in microbial clearance and enhanced wound healing (Fig 1).

ROLES OF MRGPRX2 AND MrgprB2 ON ANAPHYLACTOID DRUG REACTIONS

Many US Food and Drug Administration–approved peptidergic drugs are known to cause pseudoallergic drug reactions in human subjects. For example, icatibant, a bradykinin B_2 receptor antagonist used for the treatment of hereditary angioedema, causes injection-site erythema and swelling in nearly every patient.[89] Neuromuscular blocking drugs, such as artacurium, mivacurium, tubocurarine, and rocuronium, which are routinely used in surgery to reduce unwanted muscle movement, induce histamine release from skin MCs and cause allergic reactions in surgical settings.[90,91] Furthermore, the fluoroquinolone family of antibiotics, such as ciprofloxacin and levofloxacin, activate MCs to cause histamine release and are associated with allergic reactions.[92-95] These 3 classes of drugs promote Ca^{2+} mobilization in HEK293 cells stably expressing MRGPRX2.[52] Furthermore, they stimulate degranulation and PGD_2 and TNF-α release in the human MC line LAD2, and these responses are attenuated in cells with small interfering RNA (siRNA)–mediated knockdown of MRGPRX2.[52]

Peptidergic drugs also cause Ca^{2+} mobilization and degranulation in PMCs from wild-type mice, which are dramatically reduced in MrgprB2 mutant mice.[52] Additionally, MrgprB2

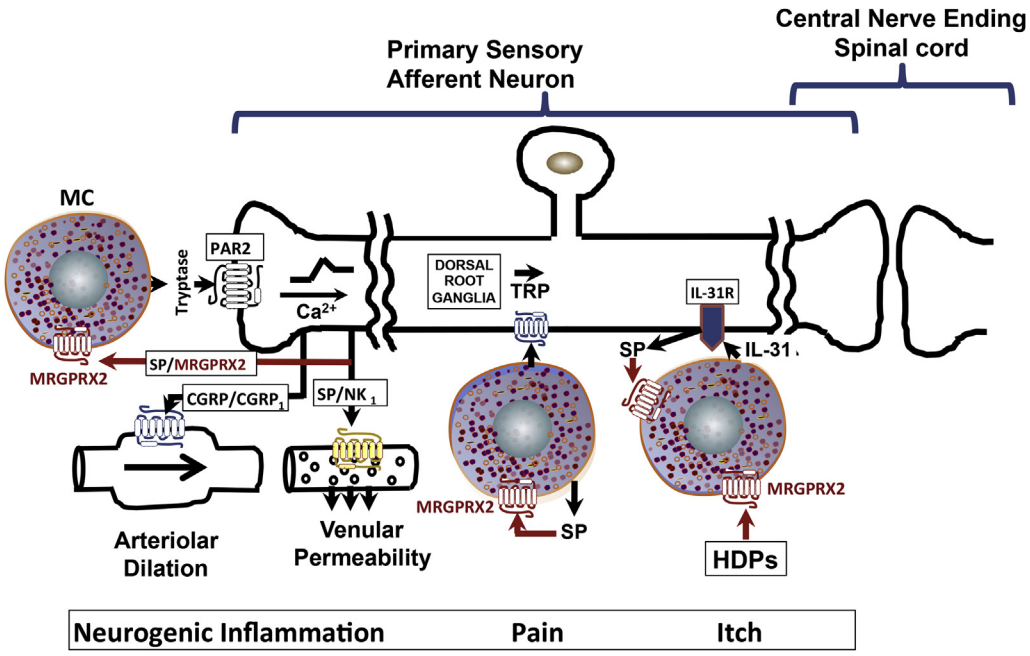

FIG 2. Potential roles for MC-MRGPRX2 on neurogenic inflammation, pain, and itch. For neurogenic inflammation and pain, tryptase released from degranulated MCs activates protease-activated receptor 2 *(PAR2)* on sensory nerve endings, resulting in the release of CGRP and SP, which interact with their receptors, CGRP1 and NK1R, to promote arteriolar dilation and venular permeability, respectively. SP released from sensory nerve ending and activated MCs also acts on MCs themselves, thus promoting a vicious cycle of MC activation through MRGPRX2. Furthermore, the Ca^{2+}-mediated axon potential travels orthodromically along the axon to the central nervous system, ultimately resulting in pain and itch responses. HDPs activate MCs through MRGPRX2, which results in IL-31 release. Activation of DRG neurons through IL-31 receptor leads to SP generation, which further activates MCs through MRGPRX2 to promote the itch response.

mutant mice display reduced paw edema in response to icatibant and atracurium and show significantly blunted systemic anaphylactic response to the fluoroquinolone antibiotics, such as ciprofloxacin, when compared with wild-type mice.[52]

Based on these findings, it has been proposed that human MRGPRX2 is responsible for pseudoallergic drug reactions and that MrgprB2 might serve as a model to develop a potential therapeutic target for drug-induced anaphylactic responses *in vivo*. However, the concentrations of ligands required to activate MrgprB2 are significantly higher than those for MRGPRX2.[25,52] For example, although ciprofloxacin and levofloxacin activate MrgprB2 with EC_{50} values of approximately 126 and 807 μg/mL, respectively, the same drugs activate MRGPRX2 with EC_{50} values of approximately 6.8 and 23 μg/mL, respectively. These findings suggest that there are important species-specific differences between human MRGPRX2 and mouse MrgprB2 and that MrgprB2 mutant mice might not be an appropriate model to screen drugs for human use.

POTENTIAL ROLES OF MRGPRX2 ON NEUROGENIC INFLAMMATION, PAIN, AND ITCH

MCs are found in close proximity to nerve endings that release peptides, such as SP and calcitonin gene–related peptide (CGRP), and appear to serve as a functional homeostatic regulatory unit.[96-99] It is now generally accepted that MC activation by neuropeptides contributes to neurogenic inflammation, pain, and itch.[15,98,100-102] Tryptase released from MCs cleaves and activates proteinase-activated receptor 2 on primary afferent neurons, which promotes Ca^{2+} influx, resulting in release of prestored neuropeptides, such as CGRP and SP.[100] MC-derived histamine and cysteinyl leukotrienes also interact with their specific GPCRs on sensory afferent neurons to cause neuropeptide release.[100,103,104] CGRP interacts with the CGRP1 receptor to induce arteriolar dilation and hyperemia, whereas SP interacts with NK1R on endothelial cells of precapillary venules to cause gap formation and plasma extravasation (Fig 2). SP, VIP, and other neuropeptides released from sensory nerve endings and activated MCs can provide a positive feedback mechanism for further MC activation and neuropeptide release.[100,104-106] The recent demonstration that neuropeptides activate human MC_{TC} through MRGPRX2[25,26,30,52] suggests that this GPCR contributes to the development of neurogenic inflammation. In addition to this local MC–sensory nerve interaction, the sensation of pain is also amplified through the Ca^{2+}-mediated axon potential that travels orthodromically along the axon to the central nervous system (Fig 2).

The effects of MCs on neurogenic inflammation can occur after their activation by both IgE-mediated and non–IgE-mediated pathways. Thus, IgE-mediated activation of cutaneous MCs is markedly decreased in denervated skin when compared with normal skin.[107] This suggests that sensory skin nerves augment MC-driven inflammatory responses by releasing neuropeptides that increase MC degranulation. Sickle cell anemia (SCA) is an inherited disorder associated with inflammation, vascular dysfunction, severe lifelong pain, and significant morbidity.[108,109] Serum SP levels are increased in patients with SCA when compared to healthy control subjects and increase further during

painful crises.[110] Because neuropeptides play an important role in cutaneous neurogenic inflammation through MC-dependent mechanisms, it has been proposed that MC activation and SP release contribute to the proinflammatory milieu and pain in patients with SCA.[106,111] Indeed, this hypothesis has recently been confirmed in a mouse model, and it has been postulated that SP released from nerve endings and MCs acts on MCs themselves, thus promoting a vicious cycle of MC activation.[112] SP activates murine and human MCs through MrgprB2 and MRGPRX2, respectively.[25,52] This raises the interesting possibility that MRGPRX2 could serve as a novel target for the modulation of neurogenic inflammation and pain associated with both allergic and nonallergic conditions, such as SCA (Fig 2).

The MC-dependent mechanisms that promote neurogenic inflammation and pain described above also contribute to itch, a condition defined as an unconscious sensation leading to a desire to scratch. Keratinocytes release a variety of inflammatory and pruritogenic substances and also detect itch-associated signals through expression of proteinase-activated receptor 2, opioid, cannabinoid, and histamine receptors.[113-116] IL-31 is a newly identified cytokine that plays an important role in itch. Thus, mice overexpressing IL-31 have spontaneous atopic dermatitis–like lesions with severe pruritus.[117] Furthermore, in a mouse model of atopic dermatitis, the expression of the IL-31 receptor is increased, and an mAb against IL-31 ameliorates the scratching behavior.[118,119] Moreover, acute allergic contact dermatitis, a skin disease featuring inflammation and pruritus, has been associated with higher IL-31 mRNA levels in DRG neurons than those seen in healthy skin.[120] Furthermore, the IL-31 receptor is expressed on DRG neurons and their activation leads to the release of neuropeptides.[121] Until recently, T cells were thought to be a major source of IL-31.[101,121] However, Niyonsaba et al[122] found that MCs express IL-31, and its expression is increased in psoriatic human skin MCs. In addition, keratinocyte-derived HDPs, such as hBDs, induce the expression and release of IL-31 from human MCs. These HDPs also cause release of other pruritogenic mediators, such as histamine, prostaglandin E_2, and SP, from MCs.[122] The fact that HDPs activate MCs through MRGPRX2 suggests that this receptor could be targeted for the treatment of symptoms associated with itch.

ROLE OF MRGPRX2 IN CHRONIC INFLAMMATORY DISEASES

Chronic urticaria (CU) is characterized by the presence of hives for at least 6 weeks that occurs on a daily basis.[123] About 45% of these patients are known to have autoimmune CU because they produce antibodies against FcεRI and, less commonly, IgE[123,124] and is associated with MC degranulation and generation of lipid-derived mediators and cytokines. The remaining approximately 55% of patients have idiopathic conditions.[123,124] Intradermal administration of SP and VIP causes greater wheal reactions in patients with idiopathic CU when compared with healthy control subjects.[125-127] The number of MCs in the skin and their histamine content do not differ between the 2 groups. Based on this finding, it has been proposed that patients with CU display a defect at the level of MC function rather than their numbers. Given that SP and VIP activate human MCs through MRGPRX2, it raises the interesting possibility that MRGPRX2 expression could be upregulated in patients with CU and that its activation by neuropeptides contributes to the pathogenesis of CU.

Fujisawa et al[26] recently showed that skin MCs in patients with CU express MRGPRX2 at higher levels than healthy subjects. Furthermore, SP causes degranulation and PGD_2 generation in human skin MCs, and these responses are significantly reduced in MRGPRX2-silenced MCs. These findings suggest that increased expression of MRGPRX2 in skin MCs renders them more susceptible to SP and that the resulting MC degranulation and PGD_2 generation contributes to the pathogenesis of idiopathic CU. However, the mechanisms responsible for MRGPRX2 upregulation in skin MCs remain unknown. Addition of histamine, SP, epithelium-derived cytokine IL-33, and thymic stromal lymphopoietin has no effect on the expression of MRGPRX2 on human skin MCs *in vitro*.[26] Thus, further studies will be required to clarify the mechanisms that regulate the expression of MRGPRX2 in MCs.

Eosinophils accumulate in CU lesions, and the presence of major basic protein (MBP) indicates that degranulation of eosinophils contribute to its pathogenesis.[26,128] Fujisawa et al[26] showed that MCs and eosinophils colocalize in urticarial lesions in patients with CU. Furthermore, they demonstrated that eosinophil granule proteins, MBP, and eosinophil peroxidase (EPO) induce histamine release from human skin MCs and that this response is substantially blocked in MRGPRX2-silenced cells. Thus, activation of MCs by SP through MRGPRX2 serves to initiate the wheal response and that recruitment of eosinophils and the subsequent activation of the same receptor by MBP and EPO contribute to the late- or chronic-phase reaction.

MCs are important effector cells that orchestrate the development of airway hyperresponsiveness and inflammation in asthmatic patients through their close interaction with airway smooth muscle cells, T cells, and leukocytes.[129-133] The ability of allergen to cross-link FcεRI on MCs to induce mediator release is well documented.[134-136] MCs found in the normal lung (MC_T) are phenotypically different from those present in the skin (MC_{TC}).[137,138] Interestingly, although normal skin MC_{TC} express MRGPRX2, lung MC_T do not.[24,26] Mild allergic asthma is generally correlated with an increase in MC_T numbers in both the submucosa and smooth muscle, but severe asthma is dominated by the presence of MC_{TC} in the airway and increased PGD_2 levels.[139,140] Levels of SP are increased in the lungs of asthmatic patients when compared with normal lung tissue.[141,142] Furthermore, SP causes degranulation of MCs obtained from bronchoalveolar lavage fluid.[143] These findings suggest that as for CU, MRGPRX2 could participate in the pathogenesis of severe asthma.

Respiratory tract infection by rhinoviruses causes increased asthma severity in both children and adults and is associated with MC degranulation and eosinophil and neutrophil recruitment to the airways.[144-149] Lung epithelial cells are the principal site of rhinovirus infection in both the upper and lower airways. Interestingly, rhinovirus induces hBD production in bronchial epithelial cells,[148,150] which activates MCs through MRGPRX2.[29,31] Thus, it is possible that MRGPRX2 expressed in human MCs contribute to rhinovirus-induced asthma exacerbation by responding to ligands generated from epithelial cells (hBDs),[147,148] eosinophils (MBP and EPO),[26] and neutrophils (LL-37). It is noteworthy that LL-37 not only causes MC degranulation and PGD_2 synthesis but also promotes their chemotaxis and induces the T_H2 cytokines IL-4 and IL-5.[28,61]

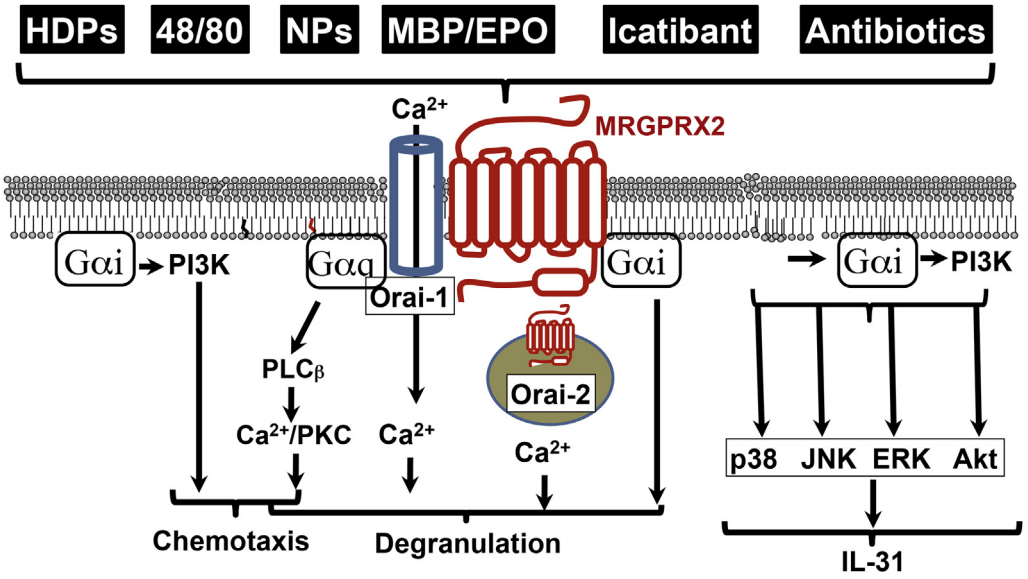

FIG 3. MRGPRX2 signaling in MCs. Ligands for MRGPRX2 activate PTx-dependent Gαi and PTx-independent Gαq pathways for MC responses. MCs express MRGPRX2 on the cell surface, as well as intracellular sites. The extracellular receptor probably uses the ORAI1 Ca^{2+} channel and the PTx-independent Gαq pathway for MC degranulation, whereas the intracellular receptor might use the ORAI2 Ca^{2+} channel to mediate MC responses. Activation of MRGPRX2 also results in Gαi-dependent production of the pruritogenic cytokine IL-31 through the MAPK- and Akt-dependent pathways.

These findings suggest that MRGPRX2 might be involved in asthma exacerbation by rhinovirus infection.

SIGNALING AND REGULATION OF MRGPRX2 IN HUMAN MCs

Most studies on the ability of neuropeptides and HDPs to activate MCs were conducted with rat PMCs,[46-51] human MC line LAD2 cells,[28-30] or *in vitro* $CD34^+$-derived human MCs.[25,29] These studies demonstrated that neuropeptides and HDPs cause MC chemotaxis, degranulation, and cytokine generation through signaling pathways that involve activation of pertussis toxin (PTx)–sensitive G proteins. Although PTx inhibits HDP-induced degranulation in human MCs endogenously expressing MRGPRX2 and transfected RBL-2H3 cells, it has no effect on Ca^{2+} mobilization in response to these ligands.[28,29] La^{3+} (an inhibitor of Ca^{2+} release–activated Ca^{2+} channels [ORAIs]) and 2-aminoethoxydiphenyl borate (a dual inhibitor of inositol 1,4,5-triphosphate receptor and ORAI1/ORAI2) cause substantial inhibition of hBD-induced Ca^{2+} mobilization and degranulation. This suggests that MRGPRX2 couples to both PTx-sensitive and insensitive signaling pathways, most likely involving Gαq and Gαi to induce degranulation. In addition to degranulation, HDPs also induce expression of the potent pruritic cytokine IL-31 through phosphatidylinositol 3-kinase and MAPK p38, Jun amino-terminal kinases, and extracellular signal-regulated kinase in human MCs. Furthermore, treatment of MCs with PTx or inhibitors of MAPKs resulted in the substantial inhibition of hBD/LL-37–induced IL-31.[122]

Until the discovery of MRGPRX2, cationic amphipathic peptides (HDPs, neuropeptides, and MCDPs) were thought to directly interact with PTx-sensitive G proteins (Gαi2 and Gαi3) in a receptor-independent manner to propagate signaling for Ca^{2+} mobilization and degranulation.[151-153] A unique feature of MRGPRX2 that distinguishes it from other GPCRs in MCs is that it is expressed both on the plasma membrane and at intracellular sites.[26] Although the exact intracellular site for MRGPRX2 has not been determined, it appears to colocalize with tryptase, indicating its possible expression in MC granules.[26] However, the mechanism through which amphipathic peptide ligands interact with granule-associated MRGPRX2 is unknown. Increased Ca^{2+} mobilization through ORAI1 and ORAI2 is essential for antigen-induced MC degranulation.[154,155] It is interesting to note that although ORAI1 is found in the plasma membrane, ORAI2 is mainly localized in MC granules.[156] 2-Aminoethoxydiphenyl borate, which attenuates MRGPRX2-mediated Ca^{2+} mobilization and degranulation, inhibits both ORAI1 and ORAI2.[29,157] These findings suggest that MRGPRX2 uses plasma membrane ORAI1 and granule-associated ORAI2 to promote MC mediator release (Fig 3).

Most GPCRs undergo rapid agonist-induced receptor phosphorylation, and this provides an important mechanism for their desensitization and internalization.[158] Although human MCs express a large number of GPCRs, phosphorylation and desensitization of the anaphylatoxin C3a receptor (C3aR) has been studied in most detail. After C3a stimulation, the receptor undergoes rapid phosphorylation, desensitization, and internalization.[159-161] Furthermore, silencing the expression of G protein–coupled receptor kinase (GRK) 2 or 3 causes a more sustained Ca^{2+} mobilization, attenuated C3aR desensitization, and enhanced degranulation. However, unlike C3aR and most other GPCRs, MRGPRX2 is resistant to LL-37–induced receptor phosphorylation, desensitization, and internalization. Also, silencing of either GRK2 or GRK3 has no effect on LL-37–induced MC degranulation.[28] Thus, MRGPRX2 appears to be unique among GPCRs, with regard to its resistance to desensitization. The biological significance of this feature is unknown but could reflect the fact that unlike

other GPCRs expressed in MCs, MRGPRX2 is activated by multiple peptide ligands that are likely to be present at the site of infection or inflammation. Thus, resistance to desensitization may allow the receptor to respond to multiple ligands simultaneously.

CONCLUSION AND FUTURE DIRECTIONS

MRGPRX2 is a noncanonical GPCR that is expressed on human MCs and found both at the plasma membrane and intracellular sites. It likely plays a dual role in promoting MC-mediated host defense and contributing to the pathogenesis of allergic and inflammatory diseases. In addition to the direct kill of microbes by HDPs, their ability to activate MRGPRX2 might serve to orchestrate the development of MC-mediated innate and adaptive immune responses and to promote healing (Fig 1). Thus, harnessing this MC-activating feature of HDPs might provide novel approaches to develop antimicrobial therapy against multidrug resistant bacteria.[162] Most studies evaluating clinical potentials of HDPs for antimicrobial activity involve their topical application at the site of infection.[163] At these sites, it is desirable for a therapeutic agent to display antimicrobial activity and to harness MC host defense and wound-healing properties. Therefore, HDP-mediated activation of MCs through MRGPRX2 and the resulting inflammatory responses in the context of cutaneous microbial infection likely outweighs the risks of adverse reactions.

Based on recent finding that the mouse counterpart of MRGPRX2 (MrgprB2) participates in peptidergic drug–induced pseudoallergic reactions, it has been proposed that mice with functional deletion of MrgprB2 together with their wild-type counterparts could serve as useful tools in the preclinical screening of new peptidergic drugs and candidate small-molecule therapeutics for symptoms of pseudoallergic reactions.[153] However, given the important differences in the potency of neuropeptides and certain peptidergic drugs for MRGPRX2 and MrgprB2, these mice might not be suitable to screen drugs for use in human subjects.[52] Recently, a number of humanized mouse models have been developed through engraftment of human hematopoietic stem cells in immune-deficient mice.[164-166] These mice develop human MCs in the skin, intestine, and lung. Furthermore, human MCs generated in these mice express human FcεRI and are responsive to human anti-IgE/IgE and serum from patients with Japanese cedar pollinosis for strong passive cutaneous anaphylaxis *in vivo*.[166] We have shown that human MCs that develop in humanized mice express MRGPRX2 (unpublished data), and this model may serve as a useful tool to delineate the pathophysiologic effects of MRGPRX2 *in vivo*. In addition to pseudoallergic reactions, MRGPRX2 participates in CU and likely contributes to the pathogenesis of itch and asthma. Thus, the humanized mouse model could potentially be used to screen mAbs and small-molecule inhibitors of MRGPRX2 against MC-mediated allergic and inflammatory disorders.

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