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Roles of MAS-related G protein coupled receptor-X2 (MRGPRX2) on mast cell-mediated host defense, pseudoallergic drug reactions and chronic inflammatory diseases

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Abstract

Mast cells (MCs), which are granulated tissue-resident cells of hematopoietic lineage, contribute to vascular homeostasis, innate/adaptive immunity and wound healing. MCs are, however, 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 are the most common targets of drug therapy. Antimicrobial host defense peptides (HDPs), neuropeptides (NPs), major basic protein (MBP), eosinophil peroxidase (EPO) and many FDA approved peptidergic drugs activate human MCs via 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 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 HDPs that kill microbes directly and activate MCs via MRGPRX2 could serve as novel GPCR targets to modulate host defense against microbial infection. Furthermore, monoclonal antibodies or small molecule inhibitors of MRGPRX2 could be developed for the treatment of MC-dependent allergic and inflammatory disorders.

Keywords

G protein coupled receptor; MRGPRX2; mast cells; host defense peptides; neuropeptides; drug-induced pseudoallergy; chronic urticaria; asthma

Introduction

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 via the cross-

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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 two types based on the protease content of their secretory granules.[1] In humans, most MCs that are found in connective tissues such as the skin contain tryptase, chymase, carboxypeptidase and cathepsin and are known as MC_{TC}. In contrast, majority of MCs that are found in lung and gut express only tryptase and are known as MC_T.[2–4] MCs also differ in their responses to endogenous and exogenous stimuli that promote degranulation. Thus, while both human MC types are activated via the 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 MCs (CTMCs) resembles MC_{TC} while mucosal MCs (MMCs) resemble MC_T.[1, 7] Thus, murine CTMCs are found in the skin and MMC 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 via that activation of MC_{TC} in humans and CTMC in mice.[11–15]

GPCRs represent the largest family of seven transmembrane domain receptors that couple via 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 ~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 and colleagues carried out a comparative analysis of the transcriptome of dorsal root ganglia (DRG) of wild type mice and neurogenin-1 deficient mice, which fails to develop a subclass of nociceptive neurons.[18] This analysis led to the identification of multiple unknown transcripts, including an entire new subfamily of GPCRs related to the MAS1 oncogene and hence the encoding genes are called mas-related gene (MRG) and the receptors MAS-related GPCRs.[18, 19]

The MRG family comprises 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 the subfamily X is specific to human, macaque and rhesus monkey.[23] In human, there are four MRGX genes, MRGPR X1 – X4; 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 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 HDPs and NPs and activate human MC_{TC} via 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 via which peptide ligands activate MRGPRX2 and the mechanism of its regulation.

MRGPRX2 as a novel GPCR for NPs 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 anphylatoxins C3a and C5a.[8, 32–36] Although basic peptides such as MC degranulating peptide (MCDP), NPs and the synthetic histamine releaser compound 48/80 have long been known to activate MC_{TC} via 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 SP.[37–39] Accordingly, a 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 via two pathways, one involving NK1R and the other via the 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, VIP, MCDP and compound 48/80 activate MCs via this receptor. Human cord blood-derived MCs can be cultured under conditions that promote their differentiation into MC_{TC} or MC_T. Using quantitative PCR, it was found that the copy number of MRGPRX2 in *in vitro*-differentiated MC_{TC} is 17,565 per 5 ng 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.[26,24] Interestingly, MC_{TC} express MRGPRX1 and MRGPRX2 but not MRGPRX3 and MRGPRX4.[25, 44, 45] To identify candidate ligands for MRGPRX2, peptide and chemical libraries were screened using a reporter gene assay in PC12 cells transiently transfected with cDNA encoding 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 using transfected rat basophilic (RBL-2H3) cells.[30] In addition, Fijisawa et al.,[26] showed that MRGPRX2 is present in both plasma membrane and at intracellular sites in human skin MC_{TC}. Furthermore, lentiviral small hairpin RNA (shRNA)-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 utilizes MRGPRX2 to promote G protein-dependent MC degranulation.

MrgprB3 as the rat ortholog of human MRGPRX2

Most original studies on the effects of NPs and HDPs on MC activation were performed with rat peritoneal MCs (PMCs).[46–51] Unlike the human genome, the rat genome possesses one each of the MrgprA, MrgprC, MrgprD, MrgprE, MrgprF, and MrgprH genes and six

MrgprB genes.[20, 19] RT-PCR analysis demonstrated that rat peritoneal MCs (PMCs) express high levels of the MrgprB3 and MrgprB8 and low levels of the MrgprB1, MrgprB2, MrgprB6, and MrgprB9 genes. MCDP and SP caused an increase 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 gene. These findings suggest that while NPs activate human MC_{TC} via MRGPRX2 they activate rat PMCs via 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 reverse transcriptase-PCR (RT-PCR) in mouse PMCs, McNeil et al.,[52] showed that these cells express messenger RNA for MrgprB2 and no other MRG GPCRs. Generation of a transgenic mouse with td-Tomato reporter under the control of MrgprB2 promoter demonstrated that the expression of this receptor is restricted to CTMC in the skin, gut and trachea.[52] The same group also generated mice with a 4 base pair deletion in the *MrgprB2* coding region, resulting in a frame shift mutation and early termination of the receptor (shortly after the first transmembrane domain). These MrgprB2 mutant mice have no defect in MC number and respond normally to IgE/FcεRI activation. However, PMCs from these mice show 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 as these receptors are expressed in MC_{TC}/CTMC and are activated by basic ligands such as NPs and compound 48/80. Surprisingly, MRGPRX2 and MrgprB2 differ substantially with respect to the concentration of the agonists required for their activation. Thus, EC_{50} values (concentration required to give 50% response) of most ligands for MrgprB2 are significantly higher than those for MRGPRX2.[25, 52] For example, while SP activates MrgprB2 with an EC_{50} value of 54 μM, it activates MRGPRX2 with EC_{50} of 152 nM. This difference is reflected in only ~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 ~34% and ~47%, respectively. Although the GPCR superfamily comprises of about 800 human proteins, crystal structure of only four of these proteins have been resolved.[53] Based on these studies, it has been proposed that modules within GPCR's extracellular (EC) and transmembrane extracellular regions (TM-EC) domains contribute to agonist binding whereas intracellular (IC) 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 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 while human β-defensins (hBDs) are produced primarily by epithelial cells.[55, 56] Of the four members of the hBD family (hBD1–4) that have been characterized in detail; hBD1 is expressed constitutively while 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 to be the primary function of these peptides and, 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 (TLR-4) on their surface, which may enhance ability of MCs to detect invading pathogens.[14, 61] MC degranulation plays an important role in host defense by causing increased vascular permeability and by initiating the recruitment of neutrophil to the sites of infection.[58, 62–66] We have recently shown that hBDs, which are derived from epithelial cells, activate human MCs via MRGPRX2.[29] LL-37 is produced from activated MCs and neutrophils and it's antimicrobial activity reflects, at least in part, the activation of MCs via MRGPRX2.[28, 31, 57, 67, 68] LL-37 causes the release of the Th2 cytokines IL-4 and IL-5 as well as the proinflammatory cytokine IL-β and may contribute to the development of both innate and adaptive immunity. Interestingly, lipopolysaccharide (LPS) generated from Gram-negative bacteria inhibits LL-37-induced Th2 cytokine, but not IL-β release.[61] These findings raise the interesting possibility that LL-37 co-existing with bacterial infection switches MC function towards innate immunity.[61]

Possible roles of MRGPRX2 on 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 via 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 via their degranulation and release of LL-37.

Moreover, LL-37 protects skin from necrotic skin infections and promotes healing.[79] It is therefore likely that MRGPRX2 expressed in MCs contributes not only 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 may also reflect the activation of leukocytes and epithelial cells. Thus, LL-37 induces human neutrophil and monocyte chemotaxis via the activation of formyl-peptide receptor 2 (FPR2), purinergic receptor P2X7 and chemokine receptor CXCR2.[80, 81,82] Furthermore, hBD3 promote monocyte chemotaxis via CCR2.[83] hBD3 and LL-37 induce keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines via the transactivation of EGFR, 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 (GAPDH) and sequestosome-1 (SQSTM1).[86, 87] This leads to the stimulation of multiple signaling pathways that are important in innate immunity, including p38, extracellular related kinases 1 and 2 (ERK1/2, also called MAPK3 and MAPK1, respectively), stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK), nuclear factor-κB (NF-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 via MRGPRX2, which orchestrate 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 FDA approved peptidergic drugs are known to cause psuedoallergic drug reactions in humans. For example, icatibant, a bradykinin B_2 receptor antagonist, which is used for the treatment of hereditary angioedema, causes injection site erythema and swelling in nearly every patient.[89] Neuromuscular blocking drugs (NMBDs) such as artacurium, mivacurium, tubacurarine and rocurinium, 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, fluoroquinolone family of antibiotics such as ciprofloxacin and levofloxacin activate MCs to cause histamine release and are associated with allergic reactions.[92–95] These three classes of drugs promote Ca^{2+} mobilization in HEK293 cells stably expressing MRGPRX2.[52] Furthermore, they stimulate degranulation, PGD_2 and TNF-α release in the human MC line, LAD2 and these responses are attenuated in cells with 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 mutant mice display reduced paw edema in response to icatibant and atracurium and show significantly blunted systemic anaphylaxis response to the fluoroquinolone antibiotics such as ciprofloxcin, when compared to wild-type mice.[52] Based on these findings, it has been proposed that human MRGPRX2 is responsible for pseudo-allergic drug reactions and that MrgprB2 may serve as a model to develop 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, while ciprofloxacin and levofloxacin activate MrgprB2 with EC_{50} values of ~126 μg/ml and 807 μg/ml, respectively, the same drugs activate MRGPRX2 with EC_{50} values of ~6.8 μg/ml and 23 μg/ml. These findings suggest that there are important species-specific differences between human MRGPRX2 and mouse MrgprB2 and that MrgprB2 mutant mice may 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 NPs contributes to neurogenic inflammation, pain and itch.[15, 98, 100–102] Tryptase released from MCs cleaves and activates proteinase activated receptor-2 (PAR-2) on primary afferent neurons, which promotes Ca^{2+} influx resulting in the release of pre-stored NPs such as CGRP and SP.[100] MC-derived histamine and cysteinyl leukotrienes also interact with their specific GPCRs on sensory afferent neurons to cause the release of NPs.[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 NPs released from sensory nerve endings and from activated MCs can provide positive feedback mechanism for further MC activation and the release of NPs.[100, 104–106] The recent demonstration that NPs activate human MC_{TC} via 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 via the Ca^{2+}-mediated axon potential that travels orthodromically along the axon to the central nervous system (CNS) (Fig. 2).

The effects of MCs on neurogenic inflammation can occur following their activation by both IgE 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, which is associated with inflammation, vascular dysfunction, severe lifelong pain and significant morbidity.[108, 109] Serum level of SP is elevated SCA patients when compared with healthy controls and increase further during painful crises.[110] Because NPs play an important role in cutaneous neurogenic inflammation via MC-dependent mechanisms it has been proposed that MC activation and SP release contribute to the pro-inflammatory milieu and pain in 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 from MCs act on MCs themselves, thus promoting a vicious cycle of MC activation.[112] SP activates murine and human MCs via MrgprB2 and MRGPRX2, respectively.[25, 52] This raises the interesting possibility MRGPRX2 could serve as a novel target for the modulation of neurogenic inflammation and pain that are associated with both allergic and non-allergic 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 via the expression of PAR-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 develop spontaneous atopic dermatitis (AD)-like lesion with severe pruritus.[117] Furthermore, in a mouse model of AD, the expression of the IL-31 receptor (IL-3-RA) is increased and a monoclonal antibody 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, IL-3-RA is expressed on DRG neurons, and their activation leads to the release of NPs.[121] Until recently, T cells were thought to be a major source of IL-31.[121,101] However, Niyonsaba et al.,[122] found that MCs express IL-31 and its expression is elevated 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 the release of other pruritogenic mediators such as histamine, PGE_2 and SP from MCs.[122] Given that HDPs activate MCs via MRGPRX2 suggest that this receptor could be targeted for the treatment of symptoms associated with itch.

Role of MRGPRX2 on chronic inflammatory diseases

Chronic urticaria (CU) is characterized by the presence of hives for at least six 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, generation of lipid-derived mediators and cytokines. The remaining ~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 to healthy control subjects.[125–127] The number of MCs in the skin and their histamine content do not differ between the two 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 via MRGPRX2, it raises the interesting possibility that MRGPRX2 expression could be upregulated in CU and that its activation by NPs contributes to the pathogenesis of CU.

Fujisawa et al.,[26] recently showed that MCs in CU patients express MRGPRX2 at higher level than the healthy subjects. Furthermore, SP causes degranulation and PGD_2 generation in human skin MCs and that these responses are significantly reduced in MRGPRX2-silenced MCs. These findings suggest that increased expression of MRGPRX2 in skin MCs render 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 (TSLP) have 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 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 via 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 asthma via their close interaction with airway smooth muscle (ASM) 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, while 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 number in both submucosa and smooth muscle but severe asthma is dominated by the presence of MC_{TC} in the airway and increased levels of PGD_2.[139, 140] The level of SP is elevated in the lung of asthmatics when compared normal lung.[141, 142] Furthermore, SP causes degranulation of MCs obtained from bronchoalveolar lavage.[143] These findings suggest that as for CU, MRGPRX2 could participate in the pathogenesis of asthma.

Respiratory infection by rhinoviruses causes increased asthma severity in both children and adults and is associated with MC degranulation and the recruitment of eosinophils and neutrophils 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 the production of hBDs in bronchial epithelial cells[148, 150], which activate MCs via 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 it also promotes their chemotaxis and induces the Th2 cytokines IL-4 and IL-5.[28, 61] These findings suggest that MRGPRX2 may be involved in asthma exacerbation by rhinovirus infection.

Signaling and regulation of MRGPRX2 in human MCs

Most studies on the ability of NPs 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 NPs and HDPs cause MC chemotaxis, degranulation and cytokine generation via signaling pathways that involve the activation of pertussis toxin (PTx)-sensitive G proteins. While 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, Orai) and 2-aminoethoxydiphenyl borate (2-APB, a dual inhibitor of inositol 1,4,5-triphosphate receptor and Orai-1/Orai-2) 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 the expression of the potent pruritic cytokine IL-31 via phosphatidylinositol 3-kinase (PI3K) and MAP kinases p38, JNK and ERK in human MCs. Furthermore, treatment of MCs with PTx or inhibitors of MAP kinases resulted in the substantial inhibition of hBD/LL-37-induced IL-31.[122]

Until the discovery of MRGPRX2 cationic amphipathic peptides (HDPs, NPs 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 from other GPCRs in MCs is that it is expressed both on the plasma membrane and in intracellular sites.[26] Although the exact intracellular site for MRGPRX2 has not been determined it appears to co-localize with tryptase, indicating its possible expression in MC granules.[26] However, the mechanism via which amphipathic peptide ligands interact with granule associated MRGPRX2 is unknown. Increased Ca^{2+} mobilization through Orai-1 and Orai-2 is essential for antigen-induced MC degranulation.[154, 155] It is interesting to note that while Orai-1 is found in the plasma membrane, Orai-2 is mainly localized in MC granules.[156] 2-APB, which attenuates MRGPRX2-mediated Ca^{2+} mobilization and degranulation, inhibits both Orai-1 and Orai-2.[29, 157] These findings suggest that MRGPRX2 utilizes plasma membrane Orai-1 and granule-associated Orai-2 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. Following C3a stimulation, the receptor undergoes rapid phosphorylation, desensitization and internalization.[159–161] Furthermore, silencing the expression of G protein coupled receptor kinase – 2 or 3 (GRK2 or GRK) 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 that are expressed in MCs with regards 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 infllamation. Thus, resistance to desensitization may allow the receptor to respond to multiple ligands simultaneously.

Conclusion and future directions

MRGPRX2 is a non-canonical GPCR that is expressed on human MCs and is 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 may 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 may 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's host defense and wound-healing properties. Therefore, HDP-mediated activation of MCs via MRGPRX2 and the resulting inflammatory responses in the context of cutaneous microbial infection likely outweighs the risks of developing adverse reactions.

Based on the 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 NPs and certain peptidergic drugs for MRGPRX2 and MrgprB2, these mice may not be suitable to screen drugs for use in humans.[52] Recently, a number of humanized mouse models have been developed via the engraftment of human hematopoietic stem cells in immune deficient mice.[164,165, 166] These mice develop human MCs in the skin, intestine and lung. Furthermore, human MCs that are generated in these mice express human FcεRI and are responsive to human anti-IgE/IgE and serum from Japanese cedar pollinosis patients for strong passive cutaneous anaphylaxis *in vivo*.[166] We have shown that human MCs that develop in humanized mice express MRGPRX2 (unpublished) and this model may serve as a tool to delineate the pathophysiologic effects of MRGPRX2 *in vivo.* In addition to pseudoallergic allergic reactions, MRGPRX2 participates in chronic urticaria and likely contributes to the pathogenesis of itch and asthma. Thus, the humanized mouse model could potentially be used to screen monoclonal antibodies and small molecule inhibitors of MRGPRX2 against MC-mediated allergic and inflammatory disorders.

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List of Abbreviation

2-APB	2-aminoethoxydiphenyl borate
AD	Atopic dermatitis
CU	Chronic urticaria
CGRP	Calcitonin gene-related peptide
CTMC	Connective tissue mast cell
DRG	Dorsal root ganglia
EGFR	Epidermal growth factor receptor

ERK1/2	Extracellular related kinases 1 and 2
EPO	Eosinophil peroxidase
FcεRI	High affinity IgE receptor
GRK	G protein coupled receptor kinase
GPCR	G protein coupled receptor
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HDP	Host defense peptide
hBD	human β-defensin
IL-31RA	IL-31 receptor
MBP	Major basic protein
MC	Mast cell
MC_T	Tryptase-expressing MC
MC_{TC}	Tryptase and chymase-expressing MC
MrgprB2	Mas-related G protein coupled receptor-B2
MRGPRX2 (MrgX2)	Mas-related G protein coupled receptor-X2
MMC	Mucosal mast cell
NP	Neuropeptide
NMBD	Neuromuscular blocking drug
NK1R	Neurokinin-1 receptor
PMC	Peritoneal mast cell
PAR-2	Proteinase activated receptor-2
PI3K	Phosphatidylinositol 3-kinase
PGD_2	Prostaglandin D_2
SP	Substance P
SQSTM-1	Sequestosome-1
SAPK	Stress-activated protein kinases
TLR	Toll-like receptor
VIP	Vasoactive intestinal peptide

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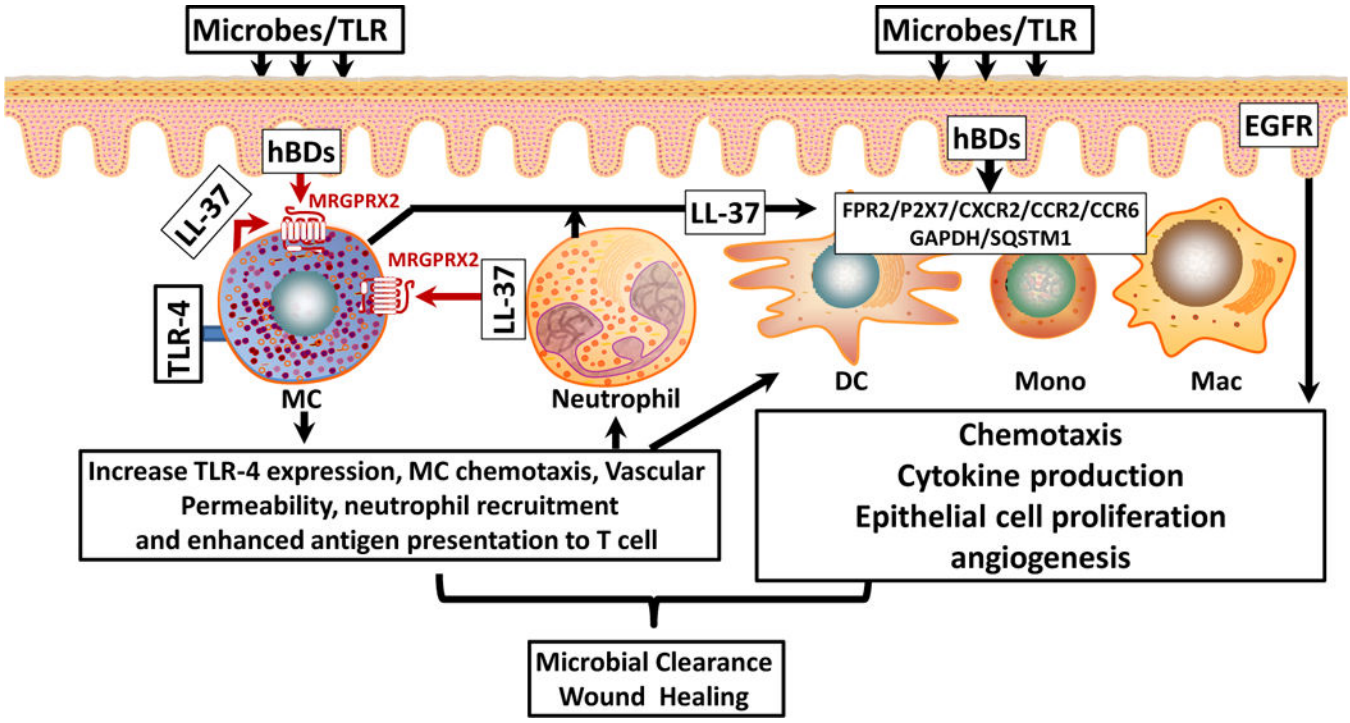

Figure 1. Activation of MRGPRX2 on MCs by HDPs may orchestrate microbial clearance and promote wound healing

Activation of epithelial cells following microbial infection leads to the generation of hBDs which cause MC chemotaxis and degranulation via MRGPRX2. MC-derived mediators cause increased vascular permeability and promote neutrophil recruitment. LL-37 released from neutrophils also activates MRGPRX2, which enhances 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 neutrophil, respectively, also promote keratinocyte migration and pro-inflammatory cytokines production via the transactivation of EGFR receptor. These HDPs also activate other cell surface GPCRs and intracellular receptors (GAPDH/SQSTM1) on monocytes (Mono), and macrophages (Mac) to induce growth factor and cytokine release to promote microbial clearance and wound healing.

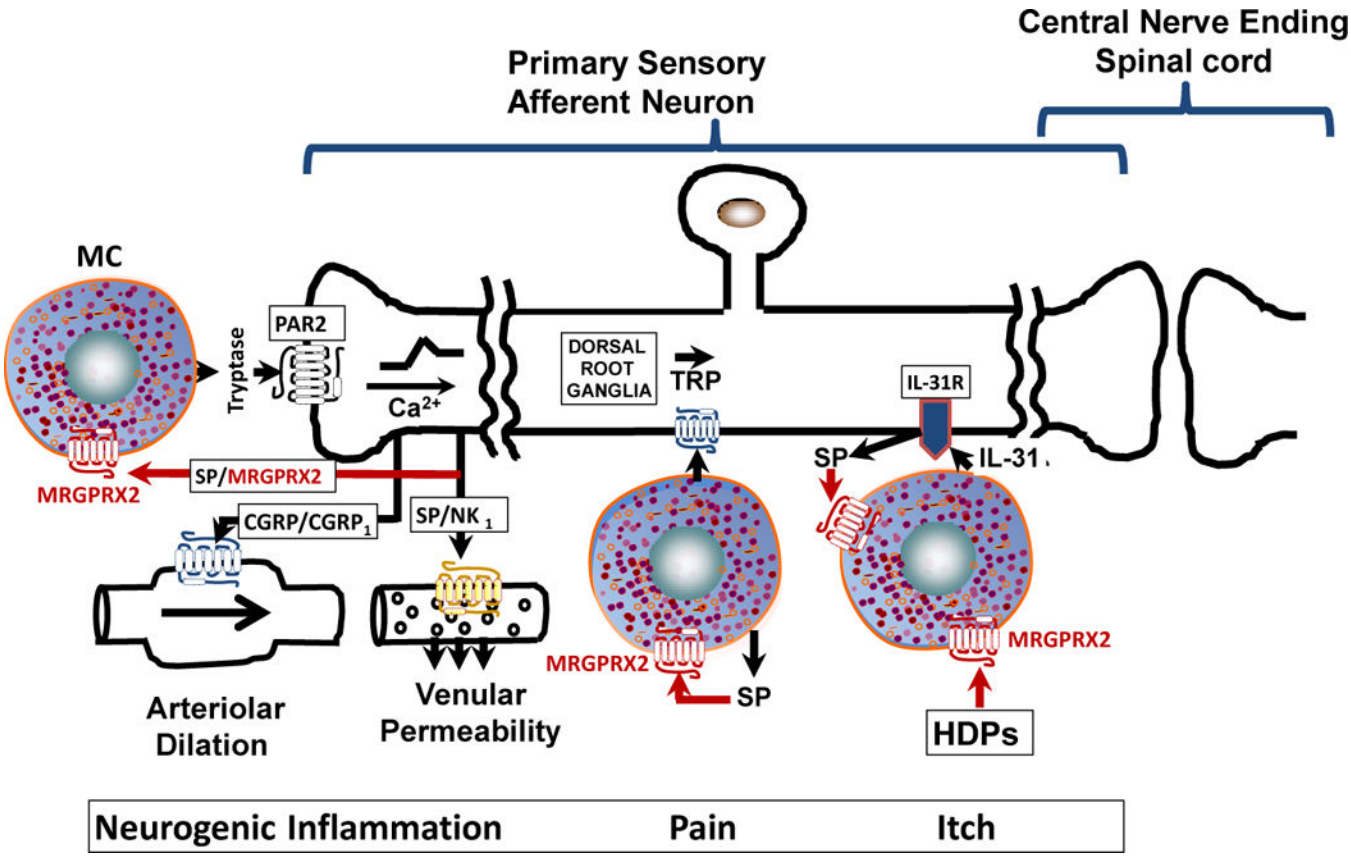

Figure 2. Potential roles for MC-MRGPRX2 on neurogenic inflammation, pain and itch

For neurogenic inflammation and pain, tryptase released from degranulated MCs activates protease activated receptor-2 (PAR-2) 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 from activated MCs also acts on MCs themselves, thus promoting a vicious cycle of MC activation via 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 via MRGPRX2, which results in the release of IL-31. Activation DRG neuron via IL-31R leads to the generation of SP, which further activates MCs via MRGPRX2 to promote the itch response.

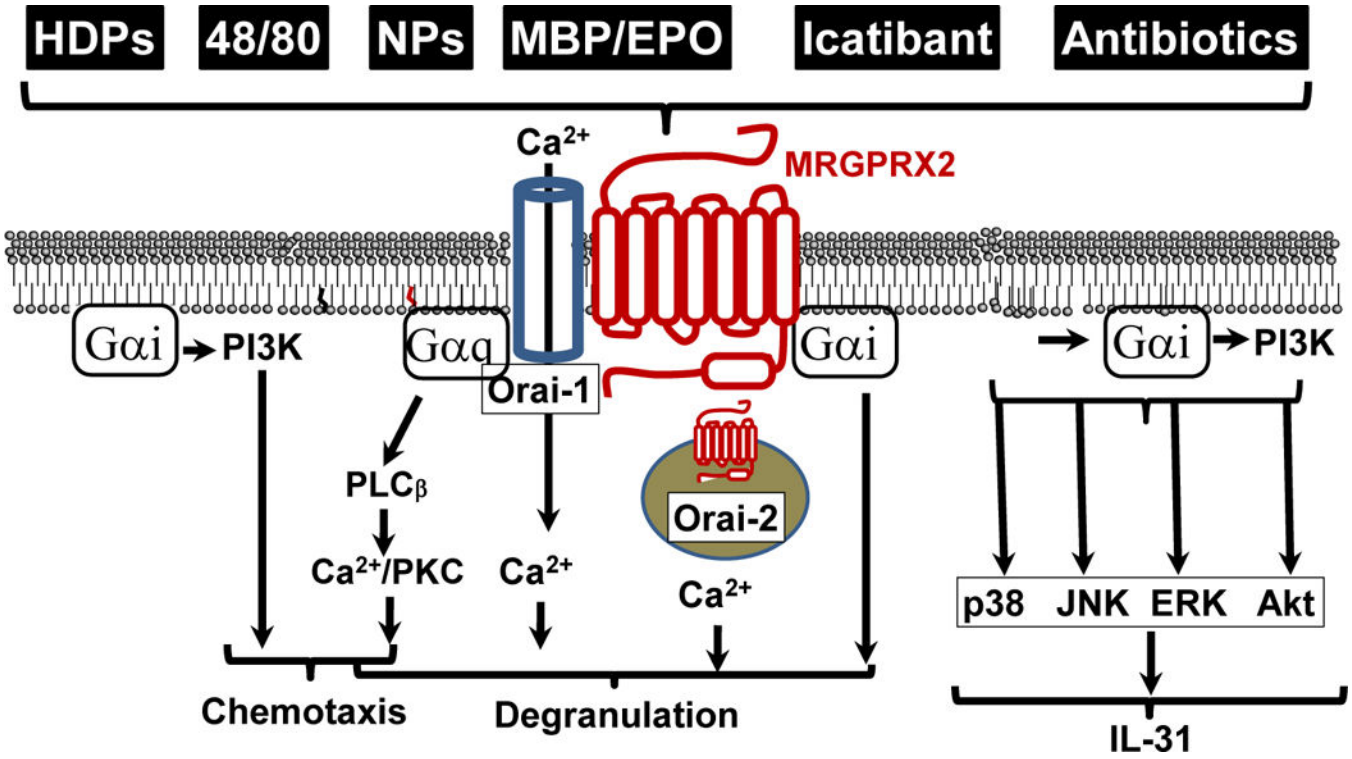

Figure 3. MRGPRX2 signaling in MCs

The ligands for MRGPRX2 activate pertussis toxin-dependent Gαi and pertussis toxin-independent Gαq pathways for MC responses. MCs express MRGPRX2 on the cell surface as well as intracellular sites. The extracellular receptor probably uses the Orai-1 Ca^{2+} channel and the pertussis toxin-independent Gαq pathway for MC degranulation whereas the intracellular receptor may use the Orai-2 Ca^{2+} channel to mediate MC responses. Activation of MRGPRX2 also results in Gαi-dependent production of the pruritogenic cytokine IL-31 via the MAP kinase and Akt-dependent pathways.