Current perspectives

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;■■■:■■■-■■■.)

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

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

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

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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, 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