Review article

Mas-related G protein–coupled receptor X2 and its activators in dermatologic allergies

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The Mas-related G protein–coupled receptor X2 (MRGPRX2) is a multiligand receptor responding to various exogenous and endogenous stimuli. Being highly expressed on skin mast cells, MRGPRX2 triggers their degranulation and release of proinflammatory mediators, and it promotes multicellular signaling cascades, such as itch induction and transmission in sensory neurons. The expression of MRGPRX2 by skin mast cells and the levels of the MRGPRX2 agonists (eg, substance P, major basic protein, eosinophil peroxidase) are upregulated in the serum and/or skin of patients with inflammatory and pruritic skin diseases, such as chronic spontaneous urticaria or atopic dermatitis. Therefore, MRGPRX2 and its agonists might be potential biomarkers for the progression of cutaneous inflammatory diseases and the response to treatment. In addition, they may represent promising targets for prevention and treatment of signs and symptoms in patients with skin diseases or drug reactions. To assess this possibility, this review explores the role and relevance of MRGPRX2 and its activators in cutaneous inflammatory disorders and chronic pruritus. (J Allergy Clin Immunol 2021;147:456-69.)

Key words: *MRGPRX2, MRGX2, agonists, chronic spontaneous urticaria, atopic dermatitis, substance P, neuropeptides, eosinophil granule proteins, antimicrobial peptides, pruritus, itch*

Abbreviations used

AD:	Atopic dermatitis
AMP:	Antimicrobial peptide
BAM22:	Bovine adrenal medulla 22
C48/80:	Compound 48/80
CSU:	Chronic spontaneous urticaria
ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
EPO:	Eosinophil peroxidase
FcεRI:	High-affinity IgE receptor
GPCR:	G protein–coupled receptor
hBD-2:	Human β-defensin-2
hBD-3:	Human β-defensin-3
HC:	Healthy control
MBP-1:	Major basic protein-1
MBP-2:	Major basic protein-2
MC:	Mast cell
MC_T:	Mast cell expressing tryptase but not chymase
MC_{TC}:	Mast cell expressing tryptase and chymase
MRGPR:	Mas-related G protein–coupled receptor
NMBA:	Neuromuscular blocking agent
PAF:	Platelet-activating factor
PAMP:	Proadrenomedullin N-terminal peptide
PTx:	Pertussis toxin
SCF:	Stem cell factor
SP:	Substance P
VIP:	Vasoactive intestinal peptide

First described in 2001, the family of Mas-related G protein–coupled receptors (MRGPRs [formerly MRGs]) comprises

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approximately 50 members in mice[1] and 8 members in humans.[2] It is a family of 7-transmembrane domain receptors that regulate cell proliferation, development, metabolism, survival, and neuronal signal transmission.[3] On the basis of sequence homology, the family of MRGPRs can be divided into several subfamilies, namely, MRGPRA to MRGPRG and MRGPRX. Of these, the subfamilies MRGPR-A, MRGPR-B, and MRGPR-C are uniquely conserved in rodents, whereas the subfamily MRGPRX is characteristic among primates and humans. In humans, 4 MRGPRX genes, *MRGPRX1, MRGPRX2, MRGPRX3,* and *MRGPRX4*, have been described.[1-3]

MRGPRX2 encodes for MRGPRX2 (also known as MRGX2), a 37 kDa G protein–coupled receptor (GPCR) that consists of 330 amino acids.[2,4] Mrgprb2 and Mrgprb3 are mouse and rat orthologues of MRGPRX2, respectively.[5] As MRGPRX2, these orthologues are expressed on mast cells (MCs). On the neuronal level, expression of MRGPRX2 overlaps with expression of murine Mrgpra1 and Mrgpra4, as well as with expression of 1 rat Mrgpr.[5,6] However, MRGPRX2 shares only 45% to 65% amino acid sequence identity with the mouse and rat MRGPR.[7]

Interestingly, the human MRGPRX2 is activated by various exogenous and endogenous ligands with diverse chemical structures. It has been suggested to modulate both inflammatory responses and pseudoallergic drug reactions.

EXPRESSION OF MRGPRX2 ON MCs

A comprehensive single-molecule cDNA sequencing study that included cap analysis of gene expression across 975 human samples was performed. It revealed a major expression of MRGPRX2 on MCs, suggesting MRGPRX2 as a marker for skin-derived human MCs.[8] In confirmation, quantitative mass spectrometry–based proteome analysis showed a unique proteome signature of MCs among immune lineages, in which MRGPRX2 was highly expressed by human skin MCs.[9]

MCs are key effector cells of the innate and adaptive immune system that regulate host defense mechanisms. In tissues exposed to the environment, MCs are present in high quantity. In skin, MCs account for up to 10% of all dermal immune cells.[10] Most MCs found in human skin are connective tissue MCs expressing tryptase and chymase (MC_{TC}s), whereas most lung and gut MCs express tryptase but not chymase (MC_Ts).[3,11] Interestingly, the copy number of *MRGPRX2* transcripts in connective tissue–like human cord blood–derived MCs was remarkably higher than in mucosal-like MCs.[7] In line with this, skin MC_{TC}s but not lung MC_Ts degranulated in response to basic compounds, such as compound 48/80 (C48/80) and substance P (SP) via MRGPRX2.[7,11,12] Furthermore, PCR and microarray data showed that MRGPRX2 is expressed at high levels in human skin and synovial MC_{TC}s but at low levels in lung MC_Ts.[13] Notably, expression of MRGPRX2 decreased in primary human skin MCs with time of cultivation, decreasing approximately 10-fold within 4 to 5 weeks in culture.[8] In addition, maintenance of human skin MCs in culture with stem cell factor (SCF) and IL-4 downregulated the expression of MRGPRX2 as well as MRGPRX2-triggered degranulation.[14] LAD2 MCs and $CD34^+$ cell–derived primary human MCs are known to express functional MRGPRX2 but not the immature human MC line HMC-1.[15]

In addition to occurring in MCs of the connective tissue, expression of MRGPRX2 has been described in small-diameter neurons of the dorsal root ganglion,[16] keratinocytes,[17] thymocytes, and T-lymphocytes,[18] as well as in blood basophils and eosinophils.[19]

HOW IS MRGPRX2 ACTIVATED?

Characteristically, MRGPRX2 is activated by ligands with cationic properties[20] that are endogenous and exogenous compounds with diverse chemical features ranging from peptides[21,22] and proteins[23] to cysteine proteases[24] and opioids[25] (eg, antimicrobial host defense peptides, neuropeptides, eosinophil granule proteins, and many US Food and Drug Administration–approved peptidergic drugs). Tatemoto et al screened peptide and chemical libraries by using a reporter gene assay in PC12 cells transiently transfected with cDNA encoding MRGPRX2. They found that all of the known basic secretagogues tested activate MRGPRX2, a finding that has been confirmed in other studies.[7,26-28] Thus, in contrast to the activation pattern of other GPCRs, MRGRX2 is activated by a broad range of ligands.[29,30]

Unlike other GPCRs (eg, C3aR), MRGPRX2 may be resistant to phosphorylation, internalization, and desensitization following activation by certain, albeit not all, agonists.[15,31] The latter fact may explain the responsiveness of MRGPRX2 to multiple ligands.[3] A single amino acid of the receptor was identified to be critical for binding and activation by its ligands, including pruritogens such as SP.[6] Recently, naturally occurring loss-of-function missense variants of MRGPRX2 were described. These showed impaired responses to SP, hemokinin-1, human β-defensin-3 (hBD-3), and icatibant.[4] Furthermore, Ayudhya et al have reported gain-of-function variants of MRGPRX2.[32]

Activation of MRGPRX2 triggers G protein–coupled signaling cascades (Fig 1). The synthetic polymer C48/80, the basic secretagogue mastoparan, and antimicrobial proteins (AMPs) were able to activate MRGPRX2 and elicit the pertussis toxin (PTx)-sensitive $G_{\alpha i}$ pathway, specifically $G_{\alpha i2}$ and $G_{\alpha i3}$.[15,27,33] This induced phospholipase C signaling,[34] calcium release, and degranulation in MCs.[15,27,33] In addition, AMPs were shown to induce phosphorylation of the mitogen-activated protein kinases p38, extracellular signal-regulated protein kinase (ERK)1/2, and c-Jun N-terminal kinases,[21,35,36] which are required for AMP-induced IL-31 release.[21] Codeine induced MRGPRX2 activation in LAD2 cells triggered by phosphorylation of ERK and JNK, but not p38.[37]

Inhibitory studies were performed using the ion La^{3+}, which blocks calcium release–activated calcium channels on the endoplasmic reticulum and plasma membrane. Their results suggest that MRGPRX2 additionally signals via the PTx-insensitive $G_{\alpha q}$ protein.[15,27] Indeed, calcium mobilization in human MCs or MRGPRX2-transfected RBL-2H3 cells was not attenuated by PTx, but it required direct inhibition of calcium release–activated calcium channels. Furthermore, codeine-triggered degranulation could also be suppressed by a phosphoinositide 3-kinase inhibitor, whereas it was only partially dependent on extracellular calcium.[37]

Besides activation of $G_{\alpha i}$ protein– and $G_{\alpha q}$ protein–coupled signaling, recent studies further suggest a MRGPRX2-mediated activation of β-arrestin.[31] Interestingly, the MRGPRX2 ligand C48/80 induced robust β-arrestin activation and receptor internalization, whereas the MRGPRX2 ligands icatibant and the angiogenic host defense protein Ag-30/5C did not, indicating ligand-dependent internalization mechanisms potentially mediated via a differential activation of β-arrestin.[31]

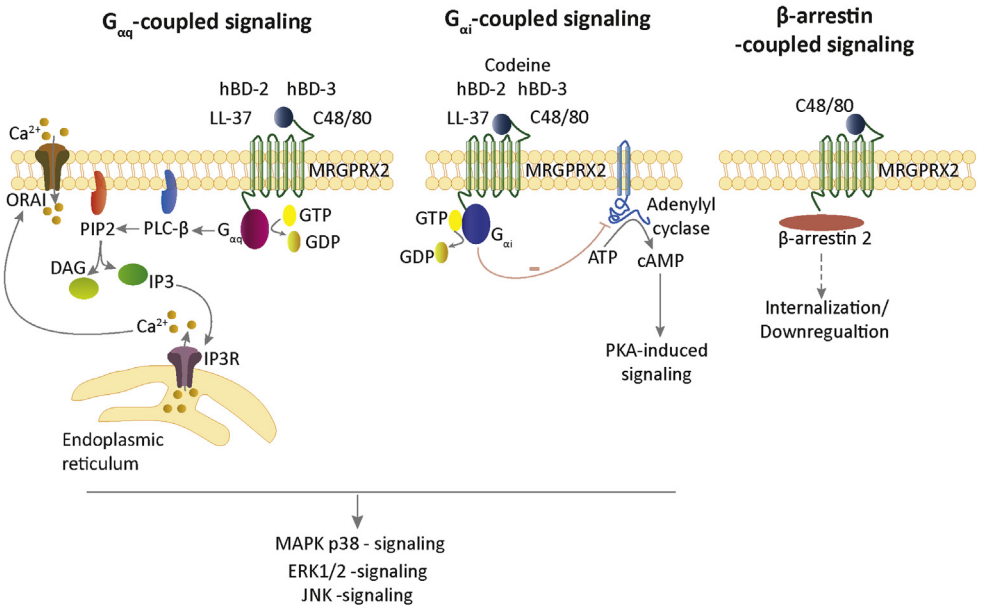

FIG 1. Signaling pathways triggered after MRGPRX2 activation.

REGULATION OF MRGPRX2 BY EXTRACELLULAR CUES

MCs are highly plastic cells, and their phenotype and functional programs are subject to constant adaptation by stimuli from the immediate micromilieu. Microenvironmental factors thereby constitute another level by which responsiveness to MRGPRX2 ligands can be regulated (as a result of altered MRGPRX2 expression, signaling, or both). Several mediators have been demonstrated over recent decades to regulate responsiveness to high-affinity IgE receptor (FcεRI) aggregation,[38,39] whereas little is known about the regulation of MRGPRX2 expression.

Notwithstanding, SCF was shown to attenuate MC degranulation elicited via MRGPRX2.[40] The negative effect of SCF was also maintained after chronic exposure, probably as a result of receptor downregulation. The typical T_H2 cytokine IL-4 also interfered with MRGPRX2 expression and function while promoting allergic degranulation.[14] Additionally, retinoic acid attenuated the pseudoallergic route during prolonged treatment, a response likewise associated with downregulation of MRGPRX2 expression.[41,42]

Although the signaling route triggered by activation of the FcεRI more typically shows an inverse pattern of regulation *vis-à-vis* the MRGPRX2-initiated pathway, this is not a strict rule. With IL-33, for instance, both routes were attenuated, albeit to different degrees, with the effects on allergic degranulation being rather subtle,[43] whereas MRGPRX2 function was basically eradicated, as was receptor expression in IL-33–cultured cells.[42]

Because MC-supportive factors are typically increased in MC-associated diseases such as atopic dermatitis (AD) and urticaria, the clinical significance of MRGPRX2 modulation by extracellular cues is uncertain at present but raises the interesting possibility that MRGPRX2 may act as a sensor of MC-supportive surroundings. Both SCF and IL-33 are enhanced in cutaneous inflammatory diseases,[44,45] and they reduce MRGPRX2 function. Therefore, MCs may be equipped with a mechanism to protect themselves and the surrounding tissue from overt stimulation in inflammatory disorders by dampening MRGPRX2, possibly as part of a negative feedback.

CELLULAR EFFECTS OF MRGPRX2 ACTIVATION AND ITS ROLE IN HEALTH AND DISEASE

Activation of MCs via MRGPRX2 has been described to trigger degranulation, chemotaxis, and cytokine release.[3] For example, SP and other endogenous MRGPRX2 ligands are able to degranulate MCs (Fig 2, Table I[46-62]). Furthermore, AMPs, which are released following activation of epithelial cells, cause MC chemotaxis and degranulation via MRGPRX2,[3,15] which modulates immune responses, orchestrates microbial clearance, and promotes wound healing.[7,15]

Notably, there is a large interindividual variability in the responsiveness to MRGPRX2 ligands, as shown in skin MCs *ex vivo*.[40] Degranulation is largely equipotent between FcεRI aggregation and MRGPRX2 triggering on a population (yet not individual) basis.[40] However, FcεRI-driven allergic secretion did not correlate with the MRGPRX2-driven process.[40]

Activation of MCs via MRGPRX2 may contribute to neurogenic inflammation, pain, itch, and pruritic skin diseases, including chronic spontaneous urticaria (CSU) and AD.[3] For example, increased numbers of MCs and marked degranulation have been found in chronic lesional skin of patients with AD.[63] Furthermore, the release of histamine and other inflammatory mediators by skin MCs promotes acute and chronic urticaria and may contribute to allergic contact dermatitis.[64] Increased numbers of MRGPRX2-positive cells have been reported in the skin of patients with CSU.[12] In complementary studies, patients with CSU responded with augmented wheal reactions to intradermal application of the MRGPRX2 agonists SP and vasoactive intestinal peptide (VIP).[65,66] Moreover, activation of MRGPRX2 has been suggested to lead to $G_{\alpha i}$-dependent production of the

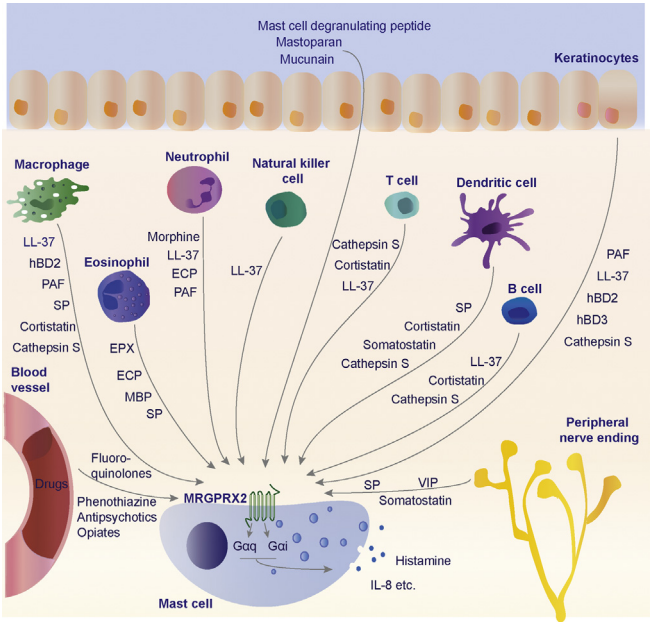

FIG 2. MRGPRX2-mediated MC activation in the human skin. Secretion of MRGPRX2-agonists from various dermal and immune cells and MRGPRX2-mediated MC degranulation. References are to be found in Table I.

pruritogenic cytokine IL-31.[3] Finally, MRGPRX2-associated MC activation may result in IgE-independent pseudoallergic reactions including, injection site reactions to opioids and other cationic drugs.[26]

Interestingly, recent studies suggested a substantial MRGPRX2/Mrgprb2-dependent interaction of MCs and nociceptive sensory neurons modulating the development of allergic skin inflammation[59] and contributing to neurogenic inflammation and pain.[67] Thereby, the neuronal release of SP activates MRGPRX2 and its murine homolog Mrgprb2 on MCs, triggering the secretion of proinflammatory cytokines, whereas MCs can address sensory neurons by the release of distinct signaling molecules (Fig 2, Table I), possibly promoting allergic contact dermatitis.[59,67,68] Deletion of *Mrgprb2* or *Tac1,* which encodes the precursor of SP, reduced the clinical scores of house dust mite–triggered allergic reactions in mice.[59] Also, Mrgprb2 critically influenced immune cell recruitment and inflammation-induced hypersensitivity in a murine model of postoperative inflammatory pain.[67] Together with the notion that Mrgprb2-dependent activation of MCs causes a distinct degranulation pattern with granule morphology and released secretagogues that is different from IgE-dependent degranulation,[68,69] these studies underline the mechanistic role of the murine homolog of MRGPRX2 in the development of MC-mediated skin diseases.

In summary, the notion that MRGPRX2 contributes to the pathogenesis of pruritic skin diseases is supported by (1) the functional expression of MRGPRX2 on skin MCs, (2) its responsiveness to multiple agonists, (3) the role of the murine homolog of MRGPRX2 in allergic conditions in mice, and (4) the upregulation of MRGPRX2 in the skin of patients with skin diseases (eg, CSU). This review explores this possibility by presenting and discussing the most important and interesting ligands of MRGPRX2 and evaluation of their relevance in pruritic skin conditions.

THE ROLE OF MRGPRX2 LIGANDS IN CHRONIC PRURITIC SKIN DISEASES

Neuropeptides and MRGPXR2

Neuropeptides such as SP are critically involved in the crosstalk of nerves and MCs, which is a key element of the bidirectional interaction between the immune and the nervous system. This crosstalk contributes to neurogenic inflammation, pain, and pruritus, which are important clinical features of many skin diseases.[70] After activation and degranulation, MCs release inflammatory, algogenic and pruritogenic mediators, which bind to specific nociceptors on sensory nerve fibers and activate them. Activated nerve fibers, in turn, secrete SP and other neuropeptides with inflammatory and vasoactive properties, which again activate MCs. Activated MCs can also produce neuropeptides, which may trigger autocrine signaling, modulating MC activation.[71]

SP and many other neuropeptides, including cortistatin, somatostatin, VIP, and the proadrenomedullin N-terminal peptides (PAMPs) PAMP-20 and PAMP-12, are MRGPRX2 agonists. They increase, via MRGPRX2 activation, the intracellular calcium concentration in MCs and degranulate them in a dose-dependent manner (Table II[72-102]).

The MRGPRX2-agonist SP. SP, a member of the tachykinin family, exerts its effects via MRGPRX2 and its conventional receptor, the tachykinin receptor 1 (NK-1).[12,103] SP is produced by neuronal and nonneuronal cells, including immune cells. Importantly, it contributes to dermal antimicrobial host defense, neurogenic inflammation, itch, and pain.[104]

Does SP play a role in chronic inflammatory skin diseases? Blood levels of SP in patients with CSU were increased in 3 of 4 studies.[105-108] Also, SP levels were reported to correlate with CSU activity[105] and to be linked to absolute blood basophil counts.[106] SP induced wheal and flare reactions after intradermal injection or skin prick testing. These reactions are suppressed by antihistamines, indicating that SP activates MCs in the skin to release histamine.[82] Interestingly, intradermal injections of SP resulted in significantly larger and longer-lasting wheal responses in patients with CSU than in healthy controls (HCs).[65]

Blood SP levels are also increased in patients with AD, in whom they correlate with disease activity and pruritus intensity. In addition, Kim et al reported that SP increases the production of TNF-α, a proinflammatory cytokine, in PBMCs of patients with AD.[109] SP gene expression and immunoreactivity are increased in the lesional skin of patients with AD.[110-112] In contrast, many studies reported reduced wheal and/or flare responses to SP after intradermal injection and/or skin prick testing in patients with AD.[81,113] This may be because of tachyphylaxis of MCs and blood vessels or their abnormal sensitivity to SP activation.[113]

Furthermore, blood levels and skin expression of SP were found to be increased in patients with cold urticaria, psoriasis, and chronic prurigo.[105,114,115] In bullous pemphigoid, itch severity was described to correlate with SP expression as assessed by immunofluorescence staining.[116] The clinical data are summarized in Table III.[117-137]

Supplementary to the strongly indicative data in humans, recent murine studies have reinforced the mechanistic role of Mrgprb2 and SP in the development of cutaneous inflammatory conditions. In a murine model of allergic inflammation triggered by house dust mite and *Staphylococcus aureus*, primary sensory neurons were shown to release SP, which provoked MC

TABLE I. Expression of MRGPRX2 agonists by cell types present in the human skin

Agonist	Expressing cells in the skin	References
hBD-2	Keratinocytes, macrophages	46
hBD-3	Keratinocytes	46
Cathepsin S	Keratinocytes, dendritic cells, B cells, T cells	47-49
ECP	Eosinophils, neutrophils	50
EPO	Eosinophils	51
LL-37	Neutrophils, macrophages, keratinocytes, natural killer cells, T cells, B cells	52
MBP	Eosinophils	53
Morphine	Neutrophils	54
PAF	Keratinocytes, macrophages, neutrophils	55,56
Somatostatin	Dendritic cells, nerve fibers	57,58
SP	Nerve fibers, macrophages, eosinophils	59-61
VIP	Nerve fibers	62

degranulation via Mrgprb2 and drove disease progression.[59] The activation of murine MCs by SP was furthermore shown to regulate recruitment of innate immune cells. These results may be transferable to humans, as SP-induced activation of LAD2 equally triggered the release of proinflammatory and immunomodulatory chemokines and cytokines.[67]

Neuropeptide activation of MRGPRX2 beyond SP. MRGPRX2 binding and activation is a common feature of neuropeptides (Table II), which are thought to contribute to skin diseases. These include VIP, which in addition to MRGPRX2, exerts its effects via the VIP receptors type 1 and 2 (VPAC-1 and VPAC-2), chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), and pituitary adenylate cyclase–activating polypeptide receptor 1 (PAC1) expressed on MCs, eosinophils, and other immune cells.[138]

Patients with CSU exhibit increased wheal reactions to intradermal injections of VIP,[66] as well as moderately, albeit not significantly, increased blood levels of VIP.[107] Blood levels of VIP are also increased in patients with AD, and they correlate with itch intensity in 1 study but not in others.[114,126,127] Similar to SP, responses to VIP skin prick testing and intradermal injections are reduced in patients with AD[81,113,127] (summarized in Table III).

The role and relevance of other neuropeptide MRGPRX2 agonists, such as cortistatin, somatostatin, bovine adrenal medulla 22 docosapeptide (BAM22), neuropeptide FF, and PAMP-20, in the pathogenesis of chronic inflammatory skin diseases are less well characterized.[22] Cortistatin and somatostatin are products of different genes but have structural similarity. Apart from MRGPRX2, they both activate somatostatin receptors (SSTR1 to SSTR5).[139] In 1 study, cortistatin blood levels and skin expression were lower in patients with psoriasis than in healthy subjects.[140] Furthermore, cortistatin can suppress keratinocyte growth *in vitro* in a dose-dependent manner. Somatostatin expression was detected in the skin of patients with eczema, urticaria pigmentosa, and psoriasis, as well as in HCs.[112] A recent murine study described the PAMP-triggered release of tryptase from murine MCs, whereas there was only a minor release of histamine. This possibly has implications on cutaneous pruritic diseases resistant to treatment with antihistamines, especially as dermal PAMP level was upregulated in patients with allergic contact dermatitis.[68]

Several questions need to be addressed by future research. How important is the activation of MRGPRX2 by neuropeptides in skin diseases compared with that of other neuropeptide receptors (eg, NK-1 for SP)? Are BAM22, neuropeptide FF, and PAMP present in the skin and blood of patients with skin diseases? Can they induce wheal and flare responses after intradermal injection or skin prick testing? How relevant are they for the development of cutaneous disorders?

Activation of MRGPRX2 by eosinophil granule proteins

Peptides and proteins that activate MRGPRX2 are rich in the amino acids proline, phenylalanine, tryptophan, and arginine/lysine, which are responsible for their hydrophobic and cationic properties.[84] Granules of human eosinophils contain various cationic proteins, including major basic protein-1 (MBP-1) and major basic protein-2 (MBP-2), eosinophil peroxidase (EPO [also referred to as EPX]), and the ribonuclease A superfamily members eosinophil cationic protein (ECP) and the eosinophil-derived neurotoxin (EDN).[141] Because of their biochemical properties, these peptides are highly cytotoxic, and they kill parasites as well as bacteria by disrupting the integrity of their lipid bilayer.[141-143] In addition, these peptides are involved in viral defense and activation of effector cells. For example, MBP promotes superoxide anion production and IL-8 release from neutrophils,[144] EPO promotes the generation of reactive oxidants and radicals,[145] and ECP and EDN possess a potent antiviral activity.[146]

MBP, EPO, and ECP have been described to induce histamine release from human MCs (Table II).[23,85] However, their target receptor remained undefined until the discovery of MRGPRX2 on human MCs.[7] Recent studies have provided direct evidence that binding of MBP, EPO, and ECP to MRGPRX2 leads to MC activation (ie, calcium influx and histamine release) in the absence of antigens.[12,23] In contrast, EDN was unable to induce MRGPRX2-dependent calcium influx or histamine release from MCs.[12] This can be attributed to its lower content of basic amino acids as compared with MBP, EPO, or ECP,[12] as strong cationic proteins more likely trigger MRGPRX2 activation.[12]

Eosinophil infiltration in the skin is a key feature of several chronic pruritic skin diseases such as CSU.[92,147] For example, MBP and EPO levels are increased in lesions of patients with chronic urticaria,[12,147] and ECP serum levels and disease activity are linked in this disease.[130] Patients with AD exhibit elevated serum levels of MBP, EDN, and ECP, which correlate with disease activity (Table III). Interestingly, these proteins are detected in the skin of patients with AD even in the absence of eosinophils.[148] Eosinophil granule proteins persist in the skin and may drive sustained and disease-promoting activation of MRGPRX2.

TABLE II. Characteristic of endogenous and exogenous agonists of MRGPRX2

		Activation of MRGPRX2/ Mrgprb2-expressing cells		Cytokine release and/or degranulation of MCs		Wheal and flare reaction	
Class	**Agonist**	**Human**	**Murine**	**Human**	**Murine/rat**	**Intradermal injection**	**Skin prick testing**
Endogenous agonists							
Neuropeptides	PAMP-20 and PAMP-12	Yes[22]	Yes[26]	Yes (LAD2[72])	Yes (peritoneal[73])	Yes[74]	—
	SP	Yes[7,75]	Yes[26]	Yes (skin,[12,13,76], synovial,[75] cord,[7] LAD2[77]); no (lung, gut, adenoids, heart[78])	Yes (bone marrow[79], peritoneal[80])	Yes[66,80,81]	Yes[81,82]
	VIP	Yes[7]	—	Yes (cord[7], skin[76], LAD2[77])	Yes (peritoneal[80])	Yes[66,80,81]	Yes[81]
	Cortistatin	Yes[83]	Yes[26]	Yes (cord[7], LAD2[84])	Yes (peritoneal[26])	—	—
	Somatostatin	Yes[7]	—	Cord[7], skin[76]	Yes (peritoneal[80])	Yes[80]	—
	Neuropeptide FF	Yes[83]	—	—	—	—	—
	BAM22	Yes[83]	—*	—	—	—	—
Eosinophil granule proteins	ECP	Yes[23]	—	Yes (heart[85], cord blood–derived[23])	—	—	—
	EPO	Yes[12]	—	Yes (skin MC[12])	—	—	—
	EDN	No[12]	—	No[12,85]	—	—	—
	MBP	Yes[23]	—	Yes (heart[85], cord blood-derived[23,86], lung[86], skin[12])	—	—	—
AMPs	LL-37	Yes[15]	—	Yes (LAD2, CD34$^+$-derived[87])	—	—	—
	hBD-2	Yes[27]	—	Yes (LAD2, CD34$^+$-derived[27])	No (BMMCs[27])	—	—
	hBD-3	Yes[27]	—	Yes (LAD2, CD34$^+$-derived[27])	No (BMMCs, peritoneal[27])	—	—
Proteases	Cathepsin S	—	—†	Yes (LAD2[24])	—	—	—
Opioids	Morphine	Yes[25]	—	Yes (LAD2[25], skin[78]); no (lung, gut, adenoids, heart[78])	—	Yes[88]	Yes[89]
	Codeine	Yes[25]	—	Yes (LAD2, CD34$^+$-derived[37])	—	Yes[88]	Yes[90]
Phospholipid	PAF	—	—	Yes (lung, peripheral blood-derived[91]); no (skin[91])	—	—	—
Protein fragment	Chaperonin-10	Yes[29]	—	Yes (CD34$^+$-derived[29])	—	—	—
Exogenous agonists							
Protease	Mucunain	Yes[24]	—	Yes (LAD2[24])	—	—	No[92]
Synthetic Polymer	C48/80	Yes[28]	Yes[26,‡]	Yes (LAD2, CD34$^+$-derived, skin[28,78]); no (HMC-1, lung, gut, adenoids, heart[28,78])	Yes (peritoneal[93])	Yes[66]	Yes[94]
Drugs	Fluoroquinolone antibiotics	Yes[26]	Yes[26]	Yes (LAD2[26])	Yes (peritoneal[26])	Yes[95]	Yes[95]
	Phenothiazine antipsychotics	Yes[96]	Yes[96]	Yes (LAD2[96])	—	—	—
	NMBAs	Yes[26,97]	Yes[26,97,98]	Yes (LAD2[26,97,98])	Yes (peritoneal[26,97,98])	Yes[99]	Yes[100]
	Hormone and kinin receptor (ant)agonists	Yes[4,26]	Yes[26]	Yes (LAD2[26,31])	Yes (peritoneal[26])	Yes[101]	—
	Natural remedies	Yes[25,93]	Yes[93]	Yes (LAD2[93,102])	Yes (peritoneal[93])	—	—

Em dash indicates no data.
*BAM22 is able to activate rat MrgprC.
†Cathepsin S was shown to activate murine Mrgprc11.
‡Mrgprb2-KO MCs show deficient activation.[26,93]

AMPs as MRGPRX2 ligands

Antimicrobial peptides (AMPs) are key innate defense molecules that strengthen epithelial barrier function,[149] modulate immune responses[150] and interact with bacteria inducing membrane damage.[151] The human AMPs LL-37 and β-defensins are secreted by various cell types and tissues, including neutrophils, keratinocytes, and MCs.[151] LL-37, as well as human β-defensin (hBD)-2 (DEFB4) and hBD-3 (DEFB103), activate and degranulate MCs at low micromolar concentrations, with subsequent release of proinflammatory IL-8 and pruritogenic IL-31.[21] This effect of AMPs on MCs is mediated via MRGPRX2, as has been shown in LAD2 and CD34$^+$ cell–derived primary MCs by

TABLE III. Evidence for a possible role of MRGPRX2 agonists in selected skin diseases

Parameter	CSU	AD	Other skin diseases
SP			
Serum levels	↑[105-107] or no difference[108]; correlation with disease activity[105]	↑[114,117,118]; correlation with intensity of pruritus[114,118] and disease activity[117]	↑ in patients with cold urticaria,[105] psoriasis,[114] and prurigo[115]
Wheal and flare reaction after i.d. injections and/or skin prick testing	↑ In patients with CSU vs in HCs[65]; no difference[66]	↓ in patients with AD vs in HCs[81,113]; ↑ in patients with AD vs in HCs[119]	No difference between patients with psoriasis and HCs[120]
Presence/expression in the skin	—	↑[110-112] or ↓[121]	↑ in patients with cold urticaria (vs in HCs),[122] psoriasis,[110] lesional nummular eczema skin,[111] prurigo nodularis,[115,123,124] bullous pemphigoid,[116] and chronic itch; no difference in patients with eczema, psoriasis, or axillary hyperhidrosis vs in controls[125]; no correlation with itch in patients with prurigo nodularis[123]
PAMP			
Presence/expression in the skin	—	—	↑ in patients with allergic contact dermatitis[68]
VIP			
Serum levels	No difference[107]	↑[114]; correlation with itch intensity[114] or no correlation with itch[126] or severity[127]	↓ in patients with acute urticaria[128]; ↑in patients with dermographism and cold urticaria (vs in HCs)[122]
Wheal and flare reaction after i.d. injections and/or skin prick testing	↑ in patients with CSU vs in HCs[66]	↓ in patients with AD vs in HCs[81,113,127]	—
Presence/expression in the skin	—	↑ in patients with lesional vs nonlesional skin[129] or no difference[111]; no difference vs HCs[112]	↑ in patients with cold urticaria, eczema, and psoriasis (vs in HCs)[122,125]; no difference in lesional vs nonlesionsal skin of patients with nummular eczema and prurigo nodularis (vs in HCs)[111,124]
Somatostatin			
Serum levels	—	—	↑ in acute urticaria (vs HCs)[128]
Presence/expression in the skin	—	No difference in patients with AD vs in HCs[112]	No difference in patients with eczema, psoriasis, or axillary hyperhidrosis vs in controls[125]; ↑ in lesional skin of patients with urticaria pigmentosa[58]; ↑ in patients with urticaria pigmentosa (vs in HCs)[58]
ECP			
Serum levels	↑ [130]	↑[131,132]; correlation with disease activity[131,132]	—
hBD-2			
Serum levels	—	↑[133]; correlation with disease activity[133]	—
Presence/expression in the skin	—	↑[134]	—
LL-37			
Serum levels	—	Correlation with disease activity[135]	—
Presence/expression in the skin	—	↑[136]	—
Cathepsin S			
Presence/expression in the skin	—	—	↑ in patients with seborrheic dermatitis,[137] psoriasis, and actinic keratosis[49]

i.d., Intradermal.
Upward arrow indicates upregulated or increased levels or larger reaction; downward arrow indicates downregulated or decreased levels or reduced reaction, and em dash indicates no data.

using knockdown approaches[15,27] (Table II). After binding to MRGPRX2, LL-37 is internalized, where it is colocalized with MRGPRX2 in the perinuclear region.[87]

AMPs are thought to be involved in the pathogenesis of AD, CSU, and other cutaneous allergic and inflammatory diseases by promoting inflammatory responses via the activation of MCs (Table III). AMP protein levels, but not mRNA, are increased in the lesional skin of patients with AD.[136,152-155] Furthermore, the expression of LL-37 and hBD-2 has been reported to moderately correlate with bacterial colony numbers[152] and disease severity in patients with AD quantified with use of the Scoring Atopic

Dermatitis (SCORAD) index.[133,135,153] As a result, LL-37 and hBD-2 have been proposed to serve as biomarkers for AD severity, although AMP expression in the skin of patients with AD does not appear to be linked directly to the degree of skin inflammation as evaluated by dermal infiltrates and epidermal $CD3^+$ T cells.[136]

Several synthetic and humanized AMPs showed antimicrobial activity and induced MRGPRX2-dependent degranulation of LAD2 cells.[31,156] Interestingly, mutated hBD-3 in which all cysteine residues were replaced by serine residues and a shortened LL-37 (comprising residues 17-29) induced MRGPRX2-dependent degranulation of LAD2 cells and lacked the lipopolysaccharide-induced blockage of degranulation.[156] Thus, compared with naturally occurring AMPs, these synthetic AMPs potentially exhibit augmented antimicrobial efficiency.

Activation of MRGPRX2 by proteases

The cysteine endopeptidase cathepsin S activates MRGPRX2, possibly via hydrolysis of its N-terminus,[157] and it degranulates MCs[24] (Table II). Cathepsin S also hydrolyses the N-terminus of the protease-activated receptors (PARs) PAR 2 and PAR 4, which unmasks the peptide sequence SLIGKV, activates PAR2, and evokes itch.[158,159] Mouse studies suggest that both, MRGPRX2 and PAR2-mediated effects contribute to cathepsin S-induced itch.[157,160] Cathepsin S has been suggested to contribute to the pathogenesis of chronic inflammatory and pruritic skin diseases. First, its overexpression induces a spontaneous AD-like disorder in mice.[161] Second, cathepsin S expression is strongly increased in the dermis of patients with AD, actinic keratosis, and psoriasis.[49] Moreover, increased expression of cathepsin S correlates with pruritus in dandruff/seborrheic dermatitis[137] (Table III).

MRGPRX2 is further activated by mucunain, a cysteine protease of the tropical legume *mucuna pruriens*.[162] Mucunain also activates PAR2 and PAR4. When mucunain is applied intradermally with use of cowhage spicules, it induces strong itch but does not cause a wheal,[163,164] although it has been reported to degranulate LAD2 MCs *in vitro*.[6,24,162] In patients with AD, mucunain causes increased itch sensations, stronger hyperkinesis,[165] and prolonged itch duration compared with in HCs.[166] Taken together with an enhanced wheal formation in response to histamine, the latter predicts AD with a sensitivity of 91% and a specificity of 94%.[166]

Opioids, opiates, and MRGPRX2

Opioids (ie, natural and synthetic substances that act on opioid receptors) and opiates (ie, drugs derived from opium) both degranulate MCs, especially rat peritoneal and human skin MCs.[88,167] Opiates are used as positive controls in skin prick testing and intradermal tests,[89,90] and opiate therapy can cause urticaria and pruritus.[168] Opioids and opiates have long been known to degranulate MCs[37,89] and recent overexpression and knockdown strategies hinted at MRGPRX2 as the preferential receptor[25,169] (Table II). A recent study demonstrated that human skin MCs degranulate in response to morphine and SP whereas lung or heart MCs do not,[13] corroborating the preferential expression of MRGPRX2 on skin MCs.[12]

Whether endogenous opioids contribute to chronic inflammatory skin diseases, such as AD or urticaria, is currently unknown. A positive correlation between β-endorphin levels and AD severity has been reported in several studies.[170,171] κ-Opioid receptor agonists as well as μ-opioid receptor antagonists are potential therapeutics in the treatment of AD itch,[172] and this action is likely unrelated to MRGPRX2 but is mediated by the targeted opioid receptors alone. Because some deregulation of the opioid system (including the canonic opioid receptors) is apparently involved in AD pathology, a proper interpretation of the relative contribution of MRGPRX2 versus opioid receptors to the connections between endogenous opioids and AD will require more definitive evidence (eg, from Mrgprb2-deficient versus opioid receptor–deficient mice).

In contrast to in AD, β-endorphin levels were found to be decreased in acute spontaneous urticaria.[128]

Drugs that activate MRGPRX2

Several classes of commonly used drugs are known to activate MRGPRX2 (Table II). These include fluoroquinolone antibiotics, phenothiazines, neuromuscular blocking agents (NMBAs), hormone receptor (ant)agonists, and natural remedies.

Fluoroquinolone antibiotics. The fluoroquinolones ciprofloxacin, moxifloxacin, norfloxacin, and lomefloxacin are broad-spectrum antibiotics that inhibit prokaryotic gyrase enzymes. In 2015, McNeil et al demonstrated activation of MRGPRX2 by these fluoroquinolones *in vitro*, as measured by calcium mobilization assays with use of a MRGPRX2-overexpressing cell culture model and by β-hexosaminidase release of human LAD2.[26] Han et al confirmed these findings,[102] whereas Lansu et al questioned them.[25] Hypersensitivity reactions (such as urticaria or anaphylaxis) after fluoroquinolone medication are common,[95,173,174] as is pruritus.[175,176] However, a few studies reported no pruritus as an adverse event in fluoroquinolone treatment.[177,178] This might be due to differences in dose, application type, or study design.

Phenothiazines. Phenothiazines, in particular the world's first antipsychotic, chlorpromazine, have a wide field of action (eg, antipsychotic, sedative, anesthetic); a broad range of molecular interactions (eg, anticholinergic, antihistaminergic, antiadrenergic); and thus, a vast variety of adverse effects such as extrapyramidal symptoms,[179] hepatotoxicity,[180] and weight gain.[181] The phenothiazine family members chlorpromazine, thioridazine, and trifluoperazine activate MRGPRX2, as shown by calcium mobilization and degranulation assays.[96] They also interact with various receptors, such as dopamine receptors.[182]

NMBAs. The NMBA atracurium and its isomer cisatracurium induce intracellular calcium influx and MC degranulation via MRGPRX2 *in vitro*.[26,98] Other NMBAs such as tubocurarine and mivacurium also likely activate MRGPRX2, as they have been shown to be agonists for the murine MRGPRX2 homolog Mrgprb2.[26] All 3 substances provoke wheal and flare responses following intradermal injection in humans.[99] Additionally, atracurium, which is used as a paralytic NMBA, is associated with perioperative hypersensitivity reactions.[183] In 2018, Navinés-Ferrer et al. demonstrated a direct link between NMBAs, MRGPRX2, and hypersensitivity reactions by using sera from patients who experienced anaphylactic reactions during anesthesia. These sera, but not control sera, induced MC degranulation, which was reduced following MRGPRX2 knockdown.[169]

Hormone and kinin receptor (ant)agonists. Several hormone or kinin receptor agonists and antagonists bind to MRGPRX2. These include cetrorelix (a gonadotropin-releasing hormone receptor antagonist), icatibant (a bradykinin B_2 receptor

antagonist), the gonadotropin-releasing hormone receptor agonist leuprorelin (also known as leuprolide), and sermorelin (a growth hormone–releasing hormone receptor agonist). All of them induced intracellular calcium influx in MRGPRX2-overexpressing cells.[26] For icatibant, Alkanfari et al additionally provided evidence for MRGPRX2-dependent MC degranulation in stably transfected RBL cells.[4] Subcutaneous injections of icatibant, which is used for the treatment of swelling attacks by patients with hereditary angioedema, induced MC-mediated urticarial injection site reactions[184] that were attenuated by H1 antihistamines.[101]

Natural remedies. Many natural remedies, especially those used in traditional Chinese medicine, are ligands of MRGPRX2 and are therefore associated with anaphylactic or pseudoallergic reactions. These include praeruptorin A, schizandrin A, baicalin, the alkaloid sinomenine, the flavone glucuronides apigenin and luteolin, and salvianolic acid and its isomers.[25,93,102,185] On the other hand, natural remedies are proposed to also act as MRGPRX2 antagonists (eg, quercetin[186] and saikosaponin A[187]).

Additional MRGPRX2 activators

PAF. The role of platelet-activating factor (PAF) in the pathogenesis of anaphylaxis is well described in both murine models and human subjects.[188] It is a phospholipid (1-O-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) released by various human cells, such as platelets, MCs, macrophages, keratinocytes, and basophils,[55,56,189] and it is involved in the regulation of immune responses. PAF can directly induce human MC degranulation (Table II), and it potentiates IgE-dependent mediator release. Marked heterogeneity of human MCs in the lung and skin has been observed with regard to mRNA expression of both the PAF receptor and MRGPRX2 receptors.[91] This finding indicates a role of MRGPRX2 in PAF-mediated MC activation. Recently, serum PAF levels were found to be increased and linked to disease activity in patients with CSU.[190]

Chaperonin-10. Certain endogenous protein and enzyme fragments, including chaperonin-10 fragment, have been shown to act as bioactive peptides and MRGPRX2-activating ligands, inducing degranulation of MCs (Table II). Chaperonin-10 is the N-terminal fragment of proteins found abundantly in the body. In a study utilizing porcine brain and intestinal extracts, chaperonin-10 demonstrated potent degranulating effects in human MCs that are comparable to those of other better-known basic secretagogues (eg, SP). Identification of these MC-activating protein fragments provides future opportunities in study of the role of MRGPRX2 in various inflammatory disorders.[29,191]

C48/80. C48/80, which is a synthetic polymer produced by condensation of N-methyl-p-methoxy phenylethylamine with formaldehyde, has been known to activate human and rodent MCs since the early 1970s.[192] When applied dermally, C48/80 induces MC degranulation and histamine release, triggering acute pruritus, protein extravasation, wheal and flare reactions, and increased heat radiation.[94] In 2011, Kashem et al showed that C48/80 degranulates differentiated, mature MCs as well as LAD2 and $CD34^+$ cell–derived MCs but not cells of the immature MC line HMC-1.[28] As the latter lacks MRGPRX2 expression, MRGPRX2 was suggested to be a receptor for C48/80 (Table II). Indeed, C48/80 triggers transient calcium influx and degranulation in MRGPRX2-overexpressing RBL-1 cells.[28]

Studies of anaphylactic reactions during cutaneous diseases showed that patients with CSU exhibited responses to C48/80 treatment similar to those exhibited by HCs.[66] Compared with the reaction of patients with CSU toward C48/80, the reaction of patients with AD to C48/80 treatment has aroused more controversy. Although several studies have suggested no aberrant C48/80 reaction in patients with AD,[193,194] others have observed a reduced extravasation response in patients with AD.[195] Interestingly, C48/80-induced pruritus was abolished in HCs by application of the histamine receptor H1 inhibitor cetirizine, whereas H1 blockage did not affect pruritus in patients with AD.[195] These observations suggest that further MC mediators are involved in C48/80-induced pruritus.

Because of the potent histamine-releasing effects of C48/80, the substance has been widely used to study anaphylaxis in humans and mouse models. Besides having well-studied effects on human MCs via MRGPRX2, C48/80 also activates the human MRGPRX1,[28] potentially triggering further MRGPRX2-independent signaling cascades.

LIMITATIONS, CONTROVERSIES, AND PERSPECTIVES

In summary, the current state of research implies an important role of MRGPRX2 agonists in pruritic skin diseases. MRGPRX2 and several of the endogenous MRGPRX2 agonists, including SP, MBP, EP, LL-37, and hBD-2, are upregulated in serum and/or skin of patients with pruritic skin diseases such as AD and CSU. This suggests a link between MRGPRX2 signaling and the progression of cutaneous diseases.

Although the summarized data provide strong evidence for the contribution of MRGPRX2 and its agonists in dermatologic diseases, the exact role of MRGPRX2 in development of these diseases remains to be clarified. Elevated levels of MRGPRX2 agonists in the skin of patients with cutaneous allergies suggest that MCs are activated via MRGPRX2; as of now, however, possible negative feedback mechanisms have not been taken into account. MRGPRX2 internalization may affect availability of the receptor in skin diseases. Whether MRGPRX2 activation triggers receptor internalization is still controversially discussed, since C48/80 provoked β-arrestin-mediated receptor internalization, but LL-37 and AG-30/5C did not.[15,31] In this context, it is likely that the variety of MRGPRX2 agonists have different effects on MRGPRX2 availability, MC activation, released signaling molecules, and immune cell recruitment. Until now, the role of MRGPRX2 agonists in skin diseases has been analyzed only individually; however, it is very likely that allergic conditions are provoked by a "cocktail" of MRGPRX2 agonists that may complement or reverse each other's effects.

Importantly, MRGPRX2 and its agonists might be potential biomarkers for cutaneous diseases and/or responses to treatment, as has recently been proposed for allergic asthma.[196] Moreover, MRGPRX2 might represent a promising target for the treatment of symptoms in patients with CSU, AD, and other skin diseases or drug reactions. For example, MRGPRX2 antagonists have recently been identified and have been able to completely inhibit the degranulation of human cord blood–derived MCs[197] and lessen C48/80-induced local allergic inflammation in mice.[198]

Targeting of MRGPRX2 further enables aimed MC depletion as recently described[9] and may thus introduce new treatment options for patients with dermatologic allergies.

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