3-23-2021

Beyond somatosensation: Mrgprs in mucosal tissues

Juan M Inclan-Rico

Brian S Kim

Ishmail Abdus-Saboor

Neuroscience Letters 748 (2021) 135689

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Minireviews

Beyond somatosensation: Mrgprs in mucosal tissues

Juan M. Inclan-Rico [a,b], Brian S. Kim [c,d,e,f,*], Ishmail Abdus-Saboor [b,**]

[a] Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA
[b] Department of Biology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, USA
[c] Division of Dermatology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
[d] Department of Anesthesiology, Washington University School of Medicine, St. Louis, MO, USA
[e] Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA
[f] Center for the Study of Itch and Sensory Disorders, Washington University School of Medicine, St. Louis, MO, USA

ARTICLE INFO

Keywords:
Mrgprs
GI tract
Airways
Sensory neurons
Mast cells

ABSTRACT

Mas-related G coupled receptors (Mrgprs) are a superfamily of receptors expressed in sensory neurons that are known to transmit somatic sensations from the skin to the central nervous system. Interestingly, Mrgprs have recently been implicated in sensory and motor functions of mucosal-associated neuronal circuits. The gastrointestinal and pulmonary tracts are constantly exposed to noxious stimuli. Therefore, it is likely that neuronal Mrgpr signaling pathways in mucosal tissues, akin to their family members expressed in the skin, might relay messages that alert the host when mucosal tissues are affected by damaging signals. Further, Mrgprs have been proposed to mediate the cross-talk between sensory neurons and immune cells that promotes host-protective functions at barrier sites. Although the mechanisms by which Mrgprs are activated in mucosal tissues are not completely understood, these exciting studies implicate Mrgprs as potential therapeutic targets for conditions affecting the intestinal and airway mucosa. This review will highlight the central role of Mrgpr signaling pathways in the regulation of homeostasis at mucosal tissues.

1. Mrgprs recognized role in somatosensation

Mas-related G protein coupled receptors (Mrgprs) comprise a large family of seven transmembrane-domain receptors expressed in sensory neurons of the dorsal root (DRG) and trigeminal ganglia (TG) that have been traditionally studied in the context of itch, pain, touch, and temperature [1,2]. From the time of their discovery until recent times, much excitement has been generated about this unique family, whose expression predominates in sensory neurons that govern aversive behaviors. This has raised the intriguing possibility that members of this family could make potent therapeutic targets, with limited off-target effects due to their relatively restrictive expression patterns in the periphery outside of the brain. Based on their sequence, the Mrgpr family is subdivided into nine separate subfamilies, with eight of these (A–H) expressed in mice and one (X) exclusively in humans [2]. Further, expression of Mrgpr family members has been detected in other vertebrates including rats, gerbils, *Canis lupus, Gallus gallus* and *Bos taurus* [2,3]. The study of Mrgprs has been challenging, in part due to the differences in the number of receptors expressed by mice and humans. Specifically, mice express at least 27 protein-coding Mrgpr family members classified into 8 subfamilies (A–H), whereas humans express only eight Mrgpr proteins and only four of them exhibit clear sequence homology to their murine counterparts [2,3]. Nonetheless, two of the human-specific receptors that belong to the MRGPRX family (MRPGRX1 and MRGPRX2) exhibit functional similarities with murine Mrgprs. For example, human MRGPRX1 mediates itch response to the pruritogens chloroquine and BAM8-22 that stimulate the murine MrgprA3 and MrgprC11, respectively [4,5]. Despite differences in the numbers of Mrgprs between mice and humans, such studies suggest that murine studies may inform the importance of these receptors in sensory processes in humans.

The large number of genes in the *MrgprA* and *MrgprC* clusters is thought to be due to insertion of an L1 retro-transposon into the 3′ end of the *MrgprA* gene as well as unequal crossover events, resulting in the presence of protein-coding sequences of MrgprA-C subfamilies in highly repetitive regions of genomic DNA [6]. Therefore, targeting members of the MrgprA-C subfamilies has been challenging. Further complicating these studies, compensatory effects of subfamily members with similar

* Corresponding author at: Division of Dermatology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA.
** Corresponding author.
E-mail addresses: briankim@wustl.edu (B.S. Kim), ishmail@sas.upenn.edu (I. Abdus-Saboor).

https://doi.org/10.1016/j.neulet.2021.135689
Received 15 October 2020; Received in revised form 11 January 2021; Accepted 19 January 2021
Available online 11 February 2021

sequence identity have already provided confounding results [1]. To overcome these challenges, Liu and colleagues generated a mouse model that lacks a cluster of *Mrgpr* genes (*Cluster*$^{-/-}$) that include the sequences of 12 protein-coding family members including *Mrgpra1, Mrgpra3,* and *Mrgprc11* [4], which have been extensively studied for the role of Mrgprs in itch response to pruritogens.

Pain and itch are distinct unpleasant sensations that evoke unique behavioral responses such as "withdrawal" to avoid a noxious stimulus or "scratching" to alleviate removal of a pruritic substance. As mentioned earlier, landmark studies of Mrgpr functions, especially with the use of *Cluster*$^{-/-}$ mice, have provided critical insight into the neuronal circuits that transmit itch [4]. For example, while *Cluster*$^{-/-}$ mice had intact acute pain responses to mechanical and thermal stimuli as well as histamine-mediated pruritus, they proved to be resistant to chloroquine-induced itch [4]. Further, DRG neurons from *Cluster*$^{-/-}$ mice failed to exhibit calcium (Ca^{2+}) responses or firing of action potentials (APs) when stimulated with chloroquine. Heterologous expression of MrgprA3 in HEK293 cells defined this receptor as the primary sensor for chloroquine [4]. Human MRGPRX1 also mediated similar responsiveness to chloroquine, suggesting that this receptor is a functional ortholog of murine MrgprA3. Subsequent studies have demonstrated the relative specificity of MrgprA3 as an itch-specific receptor [7], suggesting that it may label a subset of sensory afferents that code exclusively for itch behavior but not pain responsiveness. Nonetheless, a recent study showed that while metabotropic chemogenetic stimulation of MrgprA3^{+} neurons induced by chloroquine evokes itch behavior, fast ionotropic optogenetic activation of the same neurons resulted in pain responses [8]. Thus, additional studies are required to determine the extent to which MrgprA3 defines the pruriceptive identity of specific sensory neurons.

Cluster$^{-/-}$ mice, in which *Mrgprc11* is also ablated, were shown to be unresponsive to BAM8-22 injection, the ligand for MrgprC11 [4]. Similar to MrgprA3, heterologous expression of murine MrgprC11 or human MRGPRX1 in HEK293 cells conferred responsiveness to BAM8-22, suggesting that both murine MrgprA3 and MrgprC11 exhibit some degree of functional homology with human MRGPRX1 [4]. Further, a recent study revealed that MrgprC11 is expressed by a subset of itch-sensing skin neurons with extensive axonal branching that are also enriched for several pruritogenic receptors and soluble mediators including MrgprA3, histamine H1 receptor, Nppb and NMB [9]; which suggests that MrgprC11 may label different types of itch-inducing sensory afferents. Cysteine proteases including protease-activated receptor 2 (PAR2) and cathepsin S have also been reported to induce pruritus by activating MrgprC11 [10,11]. While the N-terminal tethered peptide SLIGRL, derived from PAR2, was identified as the ligand for MrgprC11, cathepsin S directly stimulates MrgprC11 by cleaving its N-terminal site, suggesting differential activation of MrgprC11 by different cysteine proteases [11]. Interestingly, SLIGRL was found to activate human MRGPRX2 but not MRGPRX1, highlighting the complex relation of murine and human Mrgprs.

In contrast to the lack of human homologs to many members of the MrgprA-C subfamilies, the subfamilies MrgprD-G are comprised of only one receptor each that is conserved across rodents and humans. In particular, MrgprD is expressed in skin-innervating non-peptidergic DRG neurons and mediates itch behavior and firing of action potentials evoked by the amino acid β-alanine [12,13]. Interestingly, MrgprD-deficient DRG neurons have also been reported to show lower sensitivity to mechanical and thermal noxious stimuli, suggesting that MrgprD may participate in broader nociception beyond itch [14]. Indeed, genetic ablation of MrgprD+ neurons reduced responsiveness to noxious mechanical stimuli but did not affect their thermal sensitivity [15]. Further, an optogenetic approach in which channelrhodopsin (ChR2) expression is induced by the *Mrgprd* gene (MrgprD-ChR2+ mice [16]) demonstrated that photostimulation of MrgprD+ nociceptors triggered "pain-like" paw withdrawal responses in mice with chronic inflammation [17]. Interestingly, MrgprD+ neurons were found to be responsive to extracellular ATP and use this mechanism to respond to tissue injury [18].

Contrary to MrgprA-D family members, expression of MrgprE and MrgprF, and their human homologs, is not restricted to DRG neurons. While MrgprE is expressed in the spinal cord, sciatic nerve, and different areas of the brain including cerebral cortex, medulla, and cerebellum [19,20]; MrgprF expression was detected in uterus, gut, vas deferens, brain, and aorta [21]. Likewise, human MRGPRE was reported to be enriched in the pons and medulla regions of the brain, whereas MRGPRF expression is localized mainly in the cerebellum [22]. However, the precise functions and ligands of MrgprE and MrgprF remain poorly characterized. MrgprE-deficient mice have not exhibited major alterations in nociception [23] and the effects of targeting MrgprF have not been characterized. Nonetheless, these findings demonstrate that Mrgprs can be expressed in visceral tissues that are innerved by sensory neurons like the skin. As stated above, skin Mrgprs are mostly recognized as crucial receptors for somatosensation [1,2], therefore, it is plausible that their visceral counterparts participate in the sensing of damaging signals in visceral tissues, particularly those exposed constantly to noxious stimuli such as the gastrointestinal (GI) tract and airways. However, the functions of Mrgprs expressed in mucosal sites remain to be defined. Therefore, we will focus on the reported contributions of this large superfamily of receptors in visceral-associated neurons and other non-neuronal cell populations.

2. Contributions of Mrgprs in mucosal-associated neurons

2.1. Functions of Mrgprs in enteric neurons

Interoception refers to the process of sensing and relaying messages from endogenous signals within the body. In recent times, with the emergence of murine genetic tools, the neuronal circuits that govern interoception are being identified with molecular resolution [24–26]. In response to these sensations, the host elicits different mechanisms designed to maintain homeostasis including reflexes (baroreflex), motivational drivers (hunger and thirst) or bodily sensations (visceral pain). Despite the critical relevance of the neuronal circuits that mediate interoception, our understanding of these pathways is greatly limited. The GI tract is innervated by autonomic neurons that provide both sympathetic and parasympathetic input to regulate a variety of physiologic functions. Additionally, a vast network of neurons referred to as the enteric nervous system (ENS), operates independently of the central nervous system to regulate GI motility as well as secretory and absorption functions. Despite being capable of functioning independently, the ENS can be regulated by autonomic input from the vagus nerve and prevertebral ganglia. Additionally, the cell bodies of colonic vagal afferents are located within the nodose and jugular ganglia, while the cell bodies of spinal splanchnic and pelvic afferents are located in the DRG of the thoracolumbar and lumbosacral spinal segments. Although these spinal afferents have been proposed to relay high-threshold sensations including pain and discomfort, the molecular and cellular pathways that relay those messages remain poorly defined.

In contrast to the well-described role of Mrgprs in somatosensation, particularly itch and pain responses, the contributions of these receptors in interoceptive processes are unclear. Emerging studies have started to address whether Mrgprs participate in the functions of neurons innervating visceral organs. For example, MrgprA3 and MrgprC11 were found to be expressed by discrete subsets of thoracolumbar DRG neurons that innervate the colon [27,28]. Interestingly, while MrgprA3+ and MrgprC11+ neurons in skin-innervating DRG have been shown to nearly overlap [16], MrgprA3 and MrgprC11 in colonic neurons exhibited partial co-expression [27], which may suggest different functions compared to their skin counterparts. Retrograde tracing demonstrated that MrgprA3+ and MrgprC11+ neuronal bodies are housed in thoracolumbar and lumbosacral DRG and MrgprA3+ and MrgprC11+ neurons also co-expressed the cation channel proteins TRPV1 and TRPA1

[27,28]. Given the well-defined role of TRP channels in mediating somatosensation, these findings suggest that these neurons may operate as sensory afferents. Further, *ex vivo* stimulation of colonic afferents with chloroquine or BAM8-22 induced mechanical hypersensitivity and Ca^{2+} responses. A portion of chloroquine- and BAM8-22-responsive colonic neurons also reacted to AITC and capsaicin further suggesting that MrgprA3- and MrgprC11-expressing colonic neurons overlap with TRPA1+ and TRPV1+ sensory afferents [27]. Critically, intracolonic administration of chloroquine or BAM8-22 enhanced the visceromotor response to colorectal distention in wildtype mice but not in *Cluster*$^{-/-}$ mice, confirming that stimulation of MrgprA3 or MrgprC11 elevates visceral hypersensitivity [28]. Moreover, intracolonic stimulation of MrgprA3+ and MrgprC11+ sensory afferents decreases the locomotor activity and increases grooming behavior without triggering itch [27]. In addition to these exciting results, MrgprA3-expresing neurons were surprisingly reported to innervate the meninges [29], however, whether these afferents have a sensory function in this internal tissue remains to be defined. Thus, although traditionally classified as itch-specific receptors, emerging studies on the role of Mrgprs in neurons innervating visceral organs suggests unique functions in regulating their physiology (Fig. 1).

Chronic visceral pain is a debilitating symptom in disease states such as irritable bowel syndrome (IBS) or inflammatory bowel disease (IBD). Therefore, the contributions of MrgprA3 and MrgprC11 were evaluated in an experimental model of trinitrobenzenesulphonic acid (TNBS)-induced colitis. In this model, mice exhibit chronic mechanical hypersensitivity in colonic neurons despite inflammation being resolved in the acute phase of treatment. Strikingly, colonic afferents of TNBS-treated mice exhibited increased mechanical hypersensitivity that was further exacerbated by treatment with MrgprA3 and MrgprC11 agonists [27]. However, whether *Cluster*$^{-/-}$ mice are resistant to the mechanical hypersensitivity induced by TNBS was not evaluated. Importantly, TNBS-induced colitis resulted in elevated MrgprC11 expression and increased the proportion of MrgprA3 and MrgprC11 co-expression. Translating these murine studies, human thoracolumbar DRG neurons were also found to co-express the homolog MRGPRX1 along with TRPV1 and responded to chloroquine and BAM8-22 [27,28]. These responses were enhanced when DRG neurons were exposed to an inflammatory cocktail containing histamine, PGE2, serotonin, bradykinin [27]. Taken together, these studies reveal a previously unappreciated role of MrgprA3 and MrgprC11 in the context of inflammation-induced intestinal motility, suggesting that Mrgprs may operate in autonomic mechanical functions of the GI tract. However, whether colonic neurons sense endogenous Mrgpr ligands to exacerbate visceral hypersensitivity remains unclear.

Similar to MrgprA3 and MrgprC11, retrograde labelling of colonic sensory afferents revealed MrgprD expression in a discrete neuronal subset [30]. Further, MrgprD was required by colonic neurons to respond to 5-oxo-eicosatetraenoic acid (5-oxoETE), a polyunsaturated fatty acid enriched in biopsies from IBS patients. Moreover, heterologous expression of MrgprD conferred 5-oxoETE responsiveness in HEK293 cells and MrgprD-deficient mice were resistant to 5-oxoETE-induced visceral hypersensitivity after colorectal distension [30]. Remarkably, MrgprD expression was also found in human DRGs that also exhibit calcium responses to 5-oxoETE [30], suggesting that murine and human MrgprD may be functional homologs. Although MrgprD expression was also originally reported outside of DRG neurons in lamina propria macrophages and lymphocytes [31], analysis of

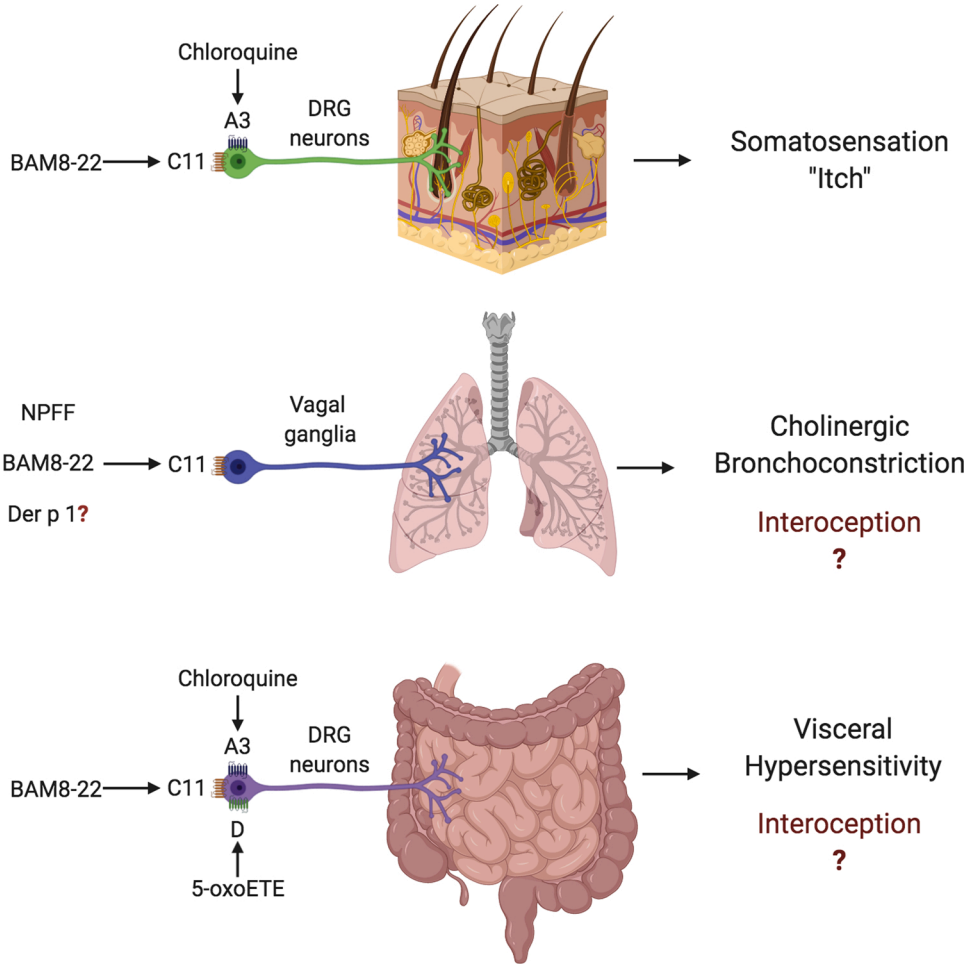

Fig. 1. Mrgpr signaling in visceral-associated neurons. MrgprA3 and MrgprC11 expressed in skin-innervating dorsal root ganglia (DRG) sensory neurons are well recognized to participate in somatosensation processes such as itch, pain, and temperature. However, a growing body of evidence has identified the expression of Mrgpr family members in vagal and DRG sensory afferents innervating mucosal tissues like the lungs and GI tract. Although activation of these signaling pathways by exogenous (chloroquine and BAM8-22) or endogenous (NPFF and 5-oxoETE) ligands induces autonomic functions including bronchoconstriction and visceral hypersensitive, whether Mrgprs relay messages that inform on the internal state of the body (interoception) remains unclear. Images created using Biorender.com.

MrgprD-deficient mice indicate that such expression is unlikely [32], highlighting the need to perform retrograde tracing approaches to confirm the sensory nature of Mrgpr-expressing neurons in visceral organs. Similarly, MRGPRD mRNA expression is reported in human intestinal tissue [33], however, further studies are required to determine the specific cellular compartment that expresses MrgprD and its function on such cells. MrgprE and MrgprF were found to be expressed in the ileum of naïve mice, which was reduced in mice undergoing TNBS-induced colitis [34]. Further characterization of MrgprE and MrgprF expression revealed that both receptors were present in three main enteric neuronal compartments: (1) nerve fibers of the tunica muscularis and lamina propria as well as in (2) neuronal somata and fibers of the myenteric and (3) submucosal enteric plexus. To assess whether MrgprE and MrgprF expression is affected by inflammatory stimuli, TNBS-induced colitis and parasitic infection with *Schistosoma mansoni* experimental models were evaluated. In the lamina propria, intestinal inflammation provoked by *S. mansoni* infection resulted in elevated numbers of MrgprE+ and MrgprF+ neurons whereas MrgprE- and MrgprF-expressing neurons were severely reduced in mice subjected to TNBS-induced colitis [34], suggesting complexity on the effects of intestinal inflammation regarding Mrgpr enteric neuron expression. Based on the co-expression patterns of MrgprE + and MrgprF + neurons in the myenteric plexus with calretinin (CRT), calbindin (CB), and neuronal nitric oxidase synthase (nNOS), MrgprE and MrgprF were proposed to be expressed in sensory neurons as well as motor and interneurons [34]. Nonetheless, further retrograde labeling is required to confirm this possibility. Importantly, the co-expression of CRT, CB and nNOS within MrgprE + and MrgprF + neurons was significantly reduced in mice with TNBS-induced intestinal inflammation but not in *S. mansoni* infected mice. Similarly, MrgprE + and MrgprF + neurons of the submucosal plexus expressed CRT and the neuropeptide vasoactive intestinal peptide (VIP), suggesting that these afferents are secretomotor or vasodilator neurons. In the submucosal plexus, CRT and VIP co-expression with MrgprE and MrgprF was significantly reduced in *S. mansoni*-infected and TNBS-treated mice. Of note, poor co-expression of MrgprE and MrgprF was detected in both the myenteric and submucosal plexus, suggesting MrgprE and MrgprF may label discrete subsets of enteric neurons [34]. Thus, although originally known for their expression on somatosensory neurons, the functional identity of MrgprD, and particularly MrgprE and MrgprF, in terms of their potential sensory, motor, and peptidergic properties requires further investigation.

2.2. MrgprC11 participates in airway hyperresponsiveness

Given the emerging studies with regard to previously unappreciated roles of Mrgprs in the GI tract, whether Mrgpr signaling operates in other mucosal tissues, such as the lungs, is also an exciting field of inquiry. Similar to the GI tract, the lungs and upper airways are innervated by sensory afferents derived from the vagus nerve and DRG [24,35]. In addition to other ion channels recently identified to regulate airway hyperresponsiveness including TRPV1 and TRPA1 [36–38], MrgprC11 was proposed to regulate the activity of the lungs in stressing conditions such as asthma. MrgprC11 is expressed in TRPV1-expressing small diameter jugular sensory neurons of the vagal ganglia [35]. Retrograde tracing further demonstrated that jugular sensory neurons that innervate the airways are MrgprC11+, whereas none of the airway-innervating DRG neurons were positive for MrgprC11. These findings are intriguing considering that MrgprC11+ neurons from the DRG have traditionally been known to innervate the skin and mediate itch sensation [4]. As expected, *ex vivo* BAM8-22 induces Ca^{2+} responses and firing of action potentials of MrgprC11+ vagal jugular neurons from wildtype but not *Cluster*$^{-/-}$ mice. Further, administration of BAM8-22 directly into the airways elicits robust Ca^{2+} responses of vagal sensory neurons, reinforcing the hypothesis that MrgprC11 labels a subset of jugular sensory neurons that innervate the airways [35]. Thus, although originally identified as a marker for somatosensory neurons in the skin, and more recently as potential receptors on visceral afferents in the gut, studies in the lung ascribe Mrgpr expression and function in vagal sensory neurons. Nonetheless, it remains to be determined whether other Mrgpr family members, such as MrgprA3, are expressed in the airway-innervating afferents and what are the noxious signals that stimulate Mrgprs in the lungs.

Airway stimulation of MrgprC11+ afferents with BAM8-22 increased the amplitude of the respiratory waveform and elevated the respiratory rate of wildtype mice, an effect absent in BAM8-22-treated *Cluster*$^{-/-}$ mice [35]. Moreover, blocking of the muscarinic acetylcholine receptor (mAChR) also prevented the respiratory effects of BAM8-22 treatment, confirming that MrgprC11-expressing afferents relay messages to the vagus nerve. Importantly, BAM8-22 treatment increased lung resistance that was inhibited by mAChR blocker and surgical vagotomy, suggesting that MrgprC11 signaling activates the parasympathetic vagus nerve and induces cholinergic bronchoconstriction [35]. To evaluate whether MrgprC11 signaling contributes to bronchoconstriction during pathologic conditions, control and *Cluster*$^{-/-}$ mice were subjected to an anaphylactic experimental model induced by ovoalbumin (OVA) sensitization. Remarkably, while OVA induced airway hyperresponsiveness in sensitized wildtype mice, *Cluster*$^{-/-}$ mice were resistant to this anaphylactic response [35]. Further, treatment with the MrgprC11 agonist, Neuropeptide FF, also induced bronchoconstriction in a Mrgpr-cluster-dependent manner. Moreover, *Cluster*$^{-/-}$ mice exhibited reduced airway hyperresponsiveness induced by influenza A virus (IFA) compared to wildtype animals despite presenting with similar immune cell infiltrates, suggesting that MrgprC11 signaling induces bronchoconstriction independently of lung inflammation [35]. In support of this possibility, BAM8-22 pre-treatment increased the airway responsiveness elicited by methacholine, which directly acts on smooth muscles and vagal nerves to induce bronchoconstriction in the absence of inflammation, an effect that was abolished in *Cluster*$^{-/-}$ mice or by surgical vagotomy. Altogether, these elegant studies indicate that in addition to the sensory functions that MrgprC11 may perform in the airways, it also participates in autonomic reflexes induced by the vagus nerve (Fig. 1).

Interestingly, treatment with the MrgprC11 agonists SLIGRL, which is derived from PAR2, or BAM8-22 was reported to reduce the immune cell infiltrate into the airways of IFA-infected mice [39,40]. However, this immunoregulatory effect was also observed in PAR2$^{-/-}$ or *Cluster*$^{-/-}$ mice [39,40], suggesting that SLIGRL may function independently of MrgprC11 stimulation in this context and requires further investigation. Notwithstanding this, the pharmacological potential of activating MrgprC11 to regulate airway hyperresponsiveness and its ability to act as a substrate for pathogens remains unclear. The cysteine protease Der p 1 is an allergen derived from house dust mites that has been reported to act on PAR2 receptors to promote airway inflammation. Stimulation with Der p 1 induced calcium responses in HELA cells transfected with mMrgprC11 or hMRGPRX1 associated with IL-6 secretion [41]. However, it is unclear if Der p 1 or other cysteine proteases directly act on MrgprC11-expressing neurons to induce bronchoconstriction. Taken together, these studies uncover a previously unappreciated role of MrgprC11+ sensory neurons in the regulation of lung function and airway responsiveness, urging the study of other Mrgprs originally identified in skin sensory afferents.

3. Mrgprs mediate cross-talk between sensory neurons and mast cells

Neuroimmunology is a rapidly emerging field, in part due to the seminal discovery that the stimulation of the vagus nerve triggers a cholinergic anti-inflammatory reflex that protects against lethal endotoxaemia [42]. Similarly, barrier sites that are densely innervated by sensory and autonomic nerves are also populated by a myriad of tissue-resident immune cells, offering the ideal location for sensory neurons and immune cells to communicate during homeostatic and

inflammatory conditions. Critically, recent studies have positioned Mrgprs at the center of this cross-talk between neurons and immune cells. In an interesting aberration of the aforementioned studies, murine MrgprB2 and its human functional homolog MRGPRX2, are expressed outside of the nervous system, with high enrichment in mast cell (MC) populations [43–45]. MCs are granulocytic innate immune cells that populate connective tissues and barrier sites such as the skin, lungs and GI tract. Although MCs are mostly recognized for their critical contributions to allergic inflammation and anaphylaxis, they play critical host-protective functions in response to a myriad of pathogens including bacteria, fungi and helminth parasites. Upon activation, MCs are well-known to trigger robust inflammatory responses by releasing their secretory granules containing several bioactive and immunomodulatory molecules including lysosomal enzymes (β-hexosaminidase), biogenic amines (histamine and serotonin), proteases (Mcpt1, tryptase and chymase), bioactive lipids (prostaglandins and leukotrienes), cytokines (TNFα and IL-4), chemokines and antimicrobial peptides. MC activation and degranulation is traditionally known to occur by binding of microbial or allergen-derived antigens to surface-bound immunoglobulin E (IgE) and crosslinking of the high-affinity IgE receptor, Fc epsilon RI (FcεRI). However, emerging studies indicate that MrgprB2/X2 activation can drive MC degranulation independently of the actions of IgE-mediated antigen stimulation [43–45]. Specifically, while IgE-induced stimulation of human MCs promotes sustained elevation of intracellular Ca^{2+} and formation of large heterogeneously shaped granules, MRGPRX2 activation led to rapid calcium responses and secretion of small spherical granules [46]. Accordingly, MrgprB2 stimulation of murine MCs provoked rapid systemic anaphylactic responses, while IgE-mediated MC degranulation resulted in slower and prolonged vascular leakage [46], suggesting differential effector responses. Of note, MrgprB2/X2 stimulation in MCs triggered robust Ca^{2+}-dependent responses similar to other Mrgpr family members expressed in sensory neurons, suggesting that Mrgpr signaling operates by a shared downstream mechanism in ontogenically distinct cells.

A unique feature of MrgprB2/X2 is that it is activated by a multitude of exogenous and endogenous molecules. Drug-induced anaphylactic reactions are severe life-threatening hypersensitivity reactions where the central role of MCs is well-documented [47]. Several FDA-approved peptidergic drugs have cationic properties suggesting they can activate MCs by stimulation of MrgprB2/X2. Indeed, MrgprB2- or MRGPRX2-expressing HEK293 cells presented Ca^{2+} responses induced by a myriad of cationic peptidergic drugs including icatibant and leuprolide [43]. Moreover, stimulation with cationic drugs promotes histamine release and local vascular leakage in an MrgprB2-dependent fashion. Small molecule drugs that have been reported to induce pseudo-allergic reactions including neuromuscular blocking drugs and fluoroquinolone antibiotics also induce MC activation that is dependent on MrgprB2 expression [43]. In addition to pharmacological agents, the endogenous neuropeptide cortistatin-14 was identified as a ligand of MRGPRX2 that causes human MC degranulation [43,48]. Moreover, cutaneous injection of high dose Sinomenine, a natural alkaloid with therapeutic properties for rheumatoid arthritis, causes vascular leakage that is reversed in MC-deficient or MrgprB2$^{-/-}$ mice [49]. Further, MrgprB2 expression was required by Sinomenine to cause Ca^{2+} signaling in murine MCs. Sinomenine also induces degranulation of human MCs and chemokine secretion dependent on MRGPRX2 expression [49]. Similarly, mastoparan, a naturally-occurring cationic peptide, causes MrgprB2/X2-dependent MC degranulation that promotes their secretion of cytokines and chemokines, including TNFα and IL-8 [43,50]. In line with these findings, mastoparan promoted skin wound healing and neutrophil recruitment in a cutaneous infection model with *Staphylococcus aureus* in an MC-dependent manner [50]. However, additional studies using MC-specific MrgprB2$^{-/-}$ mouse models are required to characterize the signaling pathways activated by these pharmacological agents. Nonetheless, these exciting results suggest that, while detrimental in anaphylactic conditions, MrgprB2-induced activation of MCs contributes to efficient innate antibacterial responses. In support of this hypothesis, the anti-microbial peptide LL-37 was reported to trigger degranulation and activation of human MCs that was impaired by MRGPRX2 silencing [51]. Further, human β-defensins (hBDs), which are cationic antimicrobial peptides secreted by epithelial cells, induced the degranulation of human MCs associated with robust intracellular Ca^{2+} mobilization that was impaired by partial silencing of MRGPRX2 expression [52]. Collectively, these exciting studies demonstrate the complex roles of MrgprB2/X2 signaling in response to both exogenous and endogenous signals, as well as in allergic pathology and protective antibacterial responses.

Beyond revealing new modes of MC regulation, MrgprB2 expression appears to vary depending on the anatomic location of MCs. For example, both peritoneal and bone-marrow derived MCs have been reported to express MrgprB2 [43], while MRGPRX2 is expressed in human skin and synovial MCs, but not lung MCs [45,53,54]. The heterogeneity of MrgprB2/MRGPRX2 expression across different populations of MCs suggests diversity in their functions as well. In addition to cationic small molecule drugs and antibiotics, a number of basic secretagogues have been described to stimulate MCs by directly acting on MrgprB2/X2 including substance P (SP), proadrenomedullin amino-terminal 20 peptide, fragment 9–20 (PAMP(9–20)), and compound 48/80 [43,44,55,56]. Following ligand binding, MrgprB2/X2 activation promotes dissociation of $G_{\alpha q}$ subunit to elicit intracellular Ca^{2+} flux that activates PLC signaling [57]. Taken together, MrgprB2/X2 signaling is now recognized as a central activation pathway of MCs in response to a variety of stimulants and tissue-derived signals that may confer a diversity of functions across numerous organs.

MCs and peripheral sensory neurons reside in close proximity at barrier surfaces such as the skin, lungs, and intestine [58,59]. Based on this anatomic relationship, MCs have been proposed to communicate to sensory neurons through the release of a variety of factors including monoamines, neuropeptides, and cytokines [60]. Along these lines, MrgprB2 has been recently highlighted to mediate MC-neuron interactions in the context of pain, itch and allergic inflammation. MrgprB2$^{-/-}$ mice have reduced pain sensitivity associated with diminished local immune cell infiltrate and chemokine secretion in a model of post-operative pain [61]. Further, nerve injury in this neurogenic model was reported to be associated with their expression of SP, a ligand for MrgprB2 [54]. While blocking of SP recapitulated pain resistance observed in MrgprB2$^{-/-}$ mice, SP treatment induced immune cell recruitment in wildtype but not MrgprB2$^{-/-}$ mice independently of neurokinin-1 receptor (NK1-R) expression, the classical receptor of SP.

As mentioned above, Mrgpr family members are well known to contribute to itch perception on sensory neurons, and a recent study indicates that MrgprB2+ MCs also contributes to pruritogenic responses. PAMP(9–20) or compound 48/80 induced non-histaminergic itch that was impaired in MrgprB2-deficient animals or mice lacking mast cells [62]. However, IgE-induced histaminergic itch was not altered by the lack of MrgprB2 expression. Critically, MrgprB2-mediated activation of MCs induced their release of tryptase and low levels of serotonin whereas IgE-induced MC activation preferentially promoted the release of histamine and higher levels of serotonin. Injection with MrgprB2 agonists more efficiently activated a broad array of MrgprD+, MrgprA3/C11+, and 5-HT1F+ (serotonin receptor) itch-specific neurons, while IgE activation triggered histamine-sensitive neurons, indicating that MCs activate distinct populations of sensory neurons in response to MrgprB2. Importantly, MrgprB2$^{-/-}$ mice exhibit reduced itch behavior in experimental models of allergic contact dermatitis (ACD) associated with diminished immune cell infiltrates [62]. Moreover, adrenomedullin, the endogenous PAMP9-20 precursor, was enriched in skin explants from ACD patients, suggesting that MRGPRX2-mediated mechanism may contribute to the chronic pruritus characteristic of ACD. In slight contrast, in a house dust mite model of allergic dermatitis, SP-deficient mice exhibit reduced skin inflammation and milder pathology [59]. Skin-innervating sensory neurons were

found to be highly enriched for SP expression and proteases derived from house dust mite extracts can directly stimulate DRG neurons to release SP [59], thereby stimulating MrgprB2 on MCs. Sensory neurons and SP were required for allergen-induced MC degranulation which resulted in clusters of dermal MCs in close proximity to sensory neurons [59]. Collectively, these studies highlight how MrgprB2 may represent a key regulator of MC-neuronal neuroimmune interactions in the skin and potentially other barrier surfaces (Fig. 2). Importantly, other Mrgpr family members were shown to be highly enriched in other immune cell populations such as MrgprA6 in basophils [63], urging the study of this critical signaling pathway in innate and adaptive immune cells.

In addition to MrgprB2, MC populations were reported to express other Mrgpr family members including MrgprE, MrgprA4 and low levels MrgprC11 [63,64]. Importantly, stimulation with neuropeptide AF (NPAF), an MrgprA4 reported ligand, induced oscillatory calcium signaling in wildtype MCs but not in those derived from *Cluster*$^{-/-}$ mice [64]. Moreover, partial degranulation as well as Mcpt1 release were induced following stimulation of MCs with NPAF. NPAF and its precursor pro-NPFF belong to the family of RFamide-related peptides expressed in several regions of the CNS [65]. Interestingly, RFamide expression was detected in enteroendocrine cells in the ileum and colon as well as in nerve fibers of the submucosal and myenteric plexus that also co-expressed tyrosine hydroxylase suggesting these fibers are extrinsic afferents of sympathetic origin [65]. In line with these studies, MC populations expressed RF-amide peptides that were secreted upon degranulation induced by IgE-crosslinking [66]. Moreover, stimulation of MrgprC11-expressing cells with NPFF or IgE-activated MCs promoted Ca^{2+} responses. Although these results propose that MCs communicate with sensory neurons by their secretion of RF-amide peptides that activate MrgprC11 (Fig. 2) [66], additional MC- and neuron-specific gene ablation approaches are required to confirm this hypothesis. Furthermore, a recent study has shown that activation of skin sensory neurons by the proteolytic action of allergens such as papain results in their release of substance P, which in turn promotes the migration and activation of dendritic cells in a MrgprA1-dependent manner [67]. Collectively, these exciting studies may reveal a novel circuit in which Mrgpr family members and their ligands represent an essential mechanism that mediates neuroimmune cross-talk at barrier sites.

4. Concluding remarks

Mrgpr family members are well recognized to mediate a variety of somatosensory responses including itch and pain. However, emerging recognition of their expression outside of the DRG, including vagal neurons that innervate visceral organs, suggests other sensory functions that communicate the internal state of the host. However, it remains to be investigated whether Mrgprs in such neuronal populations may have other physiologic properties beyond sensation to regulate organ function. Notwithstanding this, identifying the endogenous ligands and cellular interactions that elicit such neurosensory and neurophysiologic responses presents an exciting area of future inquiry.

Another unique feature of the Mrgpr family is that MrgprB2/X2 are preferentially expressed on MCs. Given that MCs reside in close proximity to peripheral neurons across numerous barrier surfaces including the skin, airway, gut, and bladder; how MCs and their associated neurons are coordinately regulated by various Mrgpr ligands remains a major open question in neuroimmunology. Although originally described mostly in the context of somatosensation, such interactions hold high promise for the discovery of a variety of unique sensory and physiologic processes well beyond even mucosal immunology.

CRediT authorship contribution statement

Juan M. Inclan-Rico: Conceptualization, Writing - original draft. **Brian S. Kim:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition. **Ishmail Abdus-Saboor:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition.

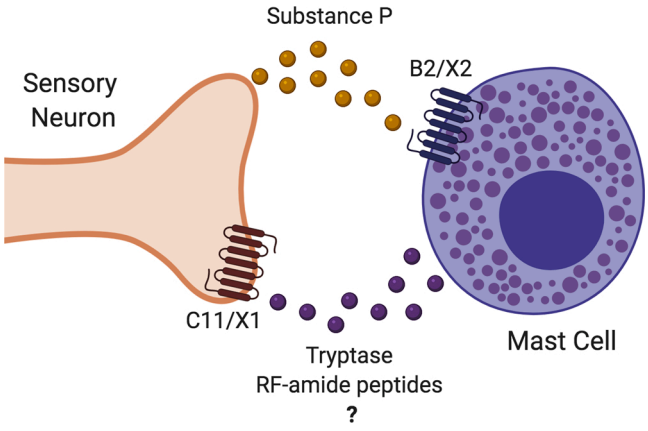

Fig. 2. Mrgprs facilitate cross-talk between sensory neurons and mast cells. Mucosal tissues provide an ideal context for interactions between sensory neurons and immune cells such as mast cells. Mrgpr family members are now recognized to mediate the interactions between immune cells and neurons. While substance P released by sensory neurons activates MrgprB2/X2 expressed in mast cells, their degranulation of tryptase or RF-amide peptides was proposed to signal back to sensory neurons by stimulation of MrgprC11/X1-expresseing neuronal fibers. Although further studies are required to confirm this possibility, these exciting findings suggest that Mrgprs are central mediators of neuroimmune cross-talk in visceral organs. Images created using Biorender.com.

Declaration of Competing Interest

B.S.K. has served as a consultant for AbbVie, ABRAX Japan, Almirall, Cara Therapeutics, Maruho, Menlo Therapeutics, Pfizer, and Third Rock Ventures. He has also participated on the advisory board for Almirall, Boehringer Ingelheim, Cara Therapeutics, Kiniksa Pharmaceuticals, Menlo Therapeutics, Regeneron Pharmaceuticals, Sanofi Genzyme, and Trevi Therapeutics. He is also Founder, Chief Scientific Officer, and stockholder of Nuogen Pharma, Inc. He is stockholder of Locus Biosciences. All other authors declare that they have no relevant conflicts of interest.

Acknowledgements

J.I-R. and I.A-S. are supported by startup funds from the University of Pennsylvania and by a grant from the National Institutes of Health (NIH/NIDCR, R00-DE026807). B.S.K. is supported by National Institutes of Health (NIH/NIAMS, R01-AR070116).

References

[1] X. Dong, S. Han, M.J. Zylka, M.I. Simon, D.J. Anderson, A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons, Cell 106 (2001) 619–632, https://doi.org/10.1016/s0092-8674(01)00483-4.
[2] J. Meixiong, X. Dong, Mas-related g protein–coupled receptors and the biology of itch sensation, Annu. Rev. Genet. 51 (2017) 103–121, https://doi.org/10.1146/annurev-genet-120116-024723.
[3] H.J. Solinski, T. Gudermann, A. Breit, Pharmacology and signaling of MAS-related G protein–coupled receptors, Pharmacol. Rev. 66 (2014) 570, https://doi.org/10.1124/pr.113.008425.
[4] Q. Liu, et al., Sensory neuron-specific GPCR mrgprs are itch receptors mediating chloroquine-induced pruritus, Cell 139 (2009) 1353–1365, https://doi.org/10.1016/j.cell.2009.11.034.
[5] P.M. Lembo, et al., Proenkephalin A gene products activate a new family of sensory neuron–specific GPCRs, Nat. Neurosci. 5 (2002) 201–209, https://doi.org/10.1038/nn815.
[6] M.J. Zylka, X. Dong, A.L. Southwell, D.J. Anderson, Atypical expansion in mice of the sensory neuron-specific Mrg G protein-coupled receptor family, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 10043–10048, https://doi.org/10.1073/pnas.1732949100.

[7] L. Han, et al., A subpopulation of nociceptors specifically linked to itch, Nat. Neurosci. 16 (2013) 174–182, https://doi.org/10.1038/nn.3289.

[8] B. Sharif, A.R. Ase, A. Ribeiro-da-Silva, P. Séguéla, Differential coding of itch and pain by a subpopulation of primary afferent neurons, Neuron 106 (2020) 940–951, https://doi.org/10.1016/j.neuron.2020.03.021, e944.

[9] Y. Xing, et al., Visualizing the itch-sensing skin arbors, J. Invest. Dermatol. (2020), https://doi.org/10.1016/j.jid.2020.08.030.

[10] Q. Liu, et al., The distinct roles of two GPCRs, MrgprC11 and PAR2, in itch and Hyperalgesia, Sci. Signal. 4 (2011) ra45, https://doi.org/10.1126/scisignal.2001925.

[11] V.B. Reddy, et al., Redefining the concept of protease-activated receptors: cathepsin S evokes itch via activation of Mrgprs, Nat. Commun. 6 (2015) 7864, https://doi.org/10.1038/ncomms8864.

[12] Q. Liu, et al., Mechanisms of itch evoked by β-alanine, J. Neurosci. 32 (2012) 14532–14537, https://doi.org/10.1523/jneurosci.3509-12.2012.

[13] M.J. Zylka, F.L. Rice, D.J. Anderson, Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to *Mrgprd*, Neuron 45 (2005) 17–25, https://doi.org/10.1016/j.neuron.2004.12.015.

[14] K.K. Rau, et al., Mrgprd enhances excitability in specific populations of cutaneous murine polymodal nociceptors, J. Neurosci. 29 (2009) 8612, https://doi.org/10.1523/JNEUROSCI.1057-09.2009.

[15] D.J. Cavanaugh, et al., Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 9075–9080, https://doi.org/10.1073/pnas.0901507106.

[16] W. Olson, et al., Sparse genetic tracing reveals regionally specific functional organization of mammalian nociceptors, eLife 6 (2017), e29507, https://doi.org/10.7554/eLife.29507.

[17] I. Abdus-Saboor, et al., Development of a mouse pain scale using sub-second behavioral mapping and statistical modeling, Cell Rep. 28 (2019) 1623–1634, https://doi.org/10.1016/j.celrep.2019.07.017, e1624.

[18] G. Dussor, M.J. Zylka, D.J. Anderson, E.W. McCleskey, Cutaneous sensory neurons expressing the Mrgprd receptor sense extracellular ATP and are putative nociceptors, J. Neurophysiol. 99 (2008) 1581–1589, https://doi.org/10.1152/jn.01396.2007.

[19] L. Zhang, et al., Cloning and expression of MRG receptors in macaque, mouse, and human, Mol. Brain Res. 133 (2005) 187–197, https://doi.org/10.1016/j.molbrainres.2004.10.007.

[20] S. Milasta, et al., Interactions between the mas-related receptors MrgD and MrgE alter signalling and trafficking of MrgD, Mol. Pharmacol. 69 (2006) 479, https://doi.org/10.1124/mol.105.018788.

[21] P.C. Ross, et al., RTA, a candidate G protein-coupled receptor: cloning, sequencing, and tissue distribution, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 3052–3056, https://doi.org/10.1073/pnas.87.8.3052.

[22] E. Sjöstedt, et al., An atlas of the protein-coding genes in the human, pig, and mouse brain, Science 367 (2020), eaay5947, https://doi.org/10.1126/science.aay5947.

[23] P.J. Cox, et al., The effect of deletion of the orphan G - protein coupled receptor (GPCR) gene MrgE on pain-like behaviours in mice, Mol. Pain 4 (2008), https://doi.org/10.1186/1744-8069-4-2, 2-2.

[24] S.L. Prescott, B.D. Umans, E.K. Williams, R.D. Brust, S.D. Liberles, An airway protection program revealed by sweeping genetic control of vagal afferents, Cell 181 (2020) 574–589, https://doi.org/10.1016/j.cell.2020.03.004, e514.

[25] W.Z. Zeng, et al., PIEZOs mediate neuronal sensing of blood pressure and the baroreceptor reflex, Science 362 (2018) 464–467, https://doi.org/10.1126/science.aau6324.

[26] S.M. Sternson, Exploring internal state-coding across the rodent brain, Curr. Opin. Neurobiol. 65 (2020) 20–26, https://doi.org/10.1016/j.conb.2020.08.009.

[27] J. Castro, et al., Activation of pruritogenic TGR5, MrgprA3, and MrgprC11 on colon-innervating afferents induces visceral hypersensitivity, JCI Insight 4 (2019), e131712, https://doi.org/10.1172/jci.insight.131712.

[28] S. Van Remoortel, et al., Mas-related G protein-coupled receptor C11 (Mrgprc11) induces visceral hypersensitivity in the mouse colon: A novel target in gut nociception? Neurogastroenterol. Motil. 31 (2019), e13623 https://doi.org/10.1111/nmo.13623.

[29] L.J. von Buchholtz, R.M. Lam, J.J. Emrick, A.T. Chesler, N.J.P. Ryba, Assigning transcriptomic class in the trigeminal ganglion using multiplex in situ hybridization and machine learning, Pain 161 (2020).

[30] T. Bautzova, et al., 5-oxoETE triggers nociception in constipation-predominant irritable bowel syndrome through MAS-related G protein-coupled receptor D, Sci. Signal. 11 (2018), eaal2171, https://doi.org/10.1126/scisignal.aal2171.

[31] C. Zhou, et al., Expression and localization of MrgprD in mouse intestinal tract, Cell Tissue Res. 377 (2019) 259–268, https://doi.org/10.1007/s00441-019-03017-7.

[32] S. Van Remoortel, J.P. Timmermans, Presence of MrgprD within the gastrointestinal wall: reality or fake? Cell Tissue Res. 378 (2019) 555–558, https://doi.org/10.1007/s00441-019-03097-5.

[33] M. Uhlén, et al., Tissue-based map of the human proteome, Science 347 (2015), 1260419, https://doi.org/10.1126/science.1260419.

[34] L.R. Avula, et al., The effect of inflammation on the expression and distribution of the MAS-related gene receptors MrgE and MrgF in the murine ileum, Histochem. Cell Biol. 136 (2011) 569–585, https://doi.org/10.1007/s00418-011-0862-7.

[35] L. Han, et al., Mrgprs on vagal sensory neurons contribute to bronchoconstriction and airway hyper-responsiveness, Nat. Neurosci. 21 (2018) 324–328, https://doi.org/10.1038/s41593-018-0074-8.

[36] A.I. Caceres, et al., A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 9099–9104, https://doi.org/10.1073/pnas.0900591106.

[37] S. Talbot, et al., Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation, Neuron 87 (2015) 341–354, https://doi.org/10.1016/j.neuron.2015.06.007.

[38] D. Tränkner, N. Hahne, K. Sugino, M.A. Hoon, C. Zuker, Population of sensory neurons essential for asthmatic hyperreactivity of inflamed airways, Proc. Natl. Acad. Sci. 111 (2014) 11515–11520, https://doi.org/10.1073/pnas.1411032111.

[39] A.Y. Chang, et al., Investigating the role of MRGPRC11 and capsaicin-sensitive afferent nerves in the anti-influenza effects exerted by SLIGRL-amide in murine airways, Respir. Res. 17 (2016) 62, https://doi.org/10.1186/s12931-016-0378-8.

[40] R.J. Betts, T.S. Mann, P.J. Henry, Inhibitory influence of the hexapeptidic sequence SLIGRL on influenza a virus infection in mice, J. Pharmacol. Exp. Ther. 343 (2012) 725, https://doi.org/10.1124/jpet.112.196485.

[41] V.B. Reddy, E.A. Lerner, Activation of mas-related G-protein-coupled receptors by the house dust mite cysteine protease Der p1 provides a new mechanism linking allergy and inflammation, J. Biol. Chem. 292 (2017) 17399–17406, https://doi.org/10.1074/jbc.M117.787887.

[42] K.J. Tracey, The inflammatory reflex, Nature 420 (2002) 853–859, https://doi.org/10.1038/nature01321.

[43] B.D. McNeil, et al., Identification of a mast-cell-specific receptor crucial for pseudo-allergic drug reactions, Nature 519 (2015) 237–241, https://doi.org/10.1038/nature14022.

[44] K. Tatemoto, et al., Immunoglobulin E-independent activation of mast cell is mediated by Mrg receptors, Biochem. Biophys. Res. Commun. 349 (2006) 1322–1328, https://doi.org/10.1016/j.bbrc.2006.08.177.

[45] M. Babina, S. Guhl, M. Artuc, T. Zuberbier, Allergic FcεRI- and pseudo-allergic MRGPRX2-triggered mast cell activation routes are independent and inversely regulated by SCF, Allergy 73 (2018) 256–260, https://doi.org/10.1111/all.13301.

[46] N. Gaudenzio, et al., Different activation signals induce distinct mast cell degranulation strategies, J. Clin. Invest. 126 (2016) 3981–3998, https://doi.org/10.1172/JCI85538.

[47] M.I. Montañez, et al., Epidemiology, mechanisms, and diagnosis of drug-induced anaphylaxis, Front. Immunol. 8 (2017), https://doi.org/10.3389/fimmu.2017.00614, 614-614.

[48] N. Robas, E. Mead, M. Fidock, MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion, J. Biol. Chem. 278 (2003) 44400–44404, https://doi.org/10.1074/jbc.M302456200.

[49] R. Liu, et al., MRGPRX2 is essential for sinomenine hydrochloride induced anaphylactoid reactions, Biochem. Pharmacol. 146 (2017) 214–223, https://doi.org/10.1016/j.bcp.2017.09.017.

[50] M. Arifuzzaman, et al., MRGPR-mediated activation of local mast cells clears cutaneous bacterial infection and protects against reinfection, Sci. Adv. 5 (2019), eaav0216, https://doi.org/10.1126/sciadv.aav0216.

[51] H. Subramanian, K. Gupta, Q. Guo, R. Price, H. Ali, Mas-related gene X2 (MrgX2) is a novel G protein-coupled receptor for the antimicrobial peptide LL-37 in human mast cells: resistance to receptor phosphorylation, desensitization, and internalization, J. Biol. Chem. 286 (2011) 44739–44749, https://doi.org/10.1074/jbc.M111.277152.

[52] H. Subramanian, et al., β-Defensins activate human mast cells via Mas-related gene X2, J. Immunol. (Baltimore, MD.: 1950) 191 (2013) 345–352, https://doi.org/10.4049/jimmunol.1300023.

[53] D. Fujisawa, et al., Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria, J. Allergy Clin. Immunol. 134 (2014) 622–633, https://doi.org/10.1016/j.jaci.2014.05.004, e629.

[54] G. Varricchi, et al., Heterogeneity of human mast cells with respect to MRGPRX2 receptor expression and function, Front. Cell. Neurosci. 13 (2019) 299.

[55] V.B. Reddy, T.A. Graham, E. Azimi, E.A. Lerner, A single amino acid in MRGPRX2 necessary for binding and activation by pruritogens, J. Allergy Clin. Immunol. 140 (2017) 1726–1728, https://doi.org/10.1016/j.jaci.2017.05.046.

[56] S.W. Kashem, et al., G protein coupled receptor specificity for C3a and compound 48/80-induced degranulation in human mast cells: roles of Mas-related genes MrgX1 and MrgX2, Eur. J. Pharmacol. 668 (2011) 299–304, https://doi.org/10.1016/j.ejphar.2011.06.027.

[57] M. Kamohara, et al., Identification of MrgX2 as a human G-protein-coupled receptor for proadrenomedullin N-terminal peptides, Biochem. Biophys. Res. Commun. 330 (2005) 1146–1152, https://doi.org/10.1016/j.bbrc.2005.03.088.

[58] S.A. van Diest, O.I. Stanisor, G.E. Boeckxstaens, W.J. de Jonge, R.M. van den Wijngaard, Relevance of mast cell–nerve interactions in intestinal nociception, Biochimica et Biophysica Acta (BBA) – Mol. Basis Dis. 1822 (2012) 74–84, https://doi.org/10.1016/j.bbadis.2011.03.019.

[59] N. Serhan, et al., House dust mites activate nociceptor-mast cell clusters to drive type 2 skin inflammation, Nat. Immunol. 20 (2019) 1435–1443, https://doi.org/10.1038/s41590-019-0493-z.

[60] A.M. Trier, M.R. Mack, B.S. Kim, The neuroimmune Axis in skin sensation, inflammation, and immunity, J. Immunol. 202 (2019) 2829, https://doi.org/10.4049/jimmunol.1801473.

[61] D.P. Green, N. Limjunyawong, N. Gour, P. Pundir, X. Dong, A mast-cell-specific receptor mediates neurogenic inflammation and pain, Neuron 101 (2019) 412–420, https://doi.org/10.1016/j.neuron.2019.01.012, e413.

[62] J. Meixiong, et al., Activation of mast-cell-Expressed mas-related G-Protein-Coupled receptors drives non-histaminergic itch, Immunity 50 (2019) 1163–1171, https://doi.org/10.1016/j.immuni.2019.03.013, e1165.

[63] D.F. Dwyer, et al., Expression profiling of constitutive mast cells reveals a unique identity within the immune system, Nat. Immunol. 17 (2016) 878–887, https://doi.org/10.1038/ni.3445.
[64] N. Abdellah, et al., Neuropeptide AF induces piecemeal degranulation in murine mucosal mast cells: a new mediator in neuro-immune communication in the intestinal Lamina propria? Anatom. Rec. (Hoboken, N.J.: 2007) 301 (2018) 1103–1114, https://doi.org/10.1002/ar.23780.
[65] P. Panula, A.A. Aarnisalo, K. Wasowicz, Neuropeptide FF, a mammalian neuropeptide with multiple functions, Prog. Neurobiol. 48 (1996) 461–487, https://doi.org/10.1016/0301-0082(96)00001-9.
[66] M.-G. Lee, et al., Agonists of the Mas-Related gene (Mrgs) orphan receptors as novel mediators of mast cell-sensory nerve interactions, J. Immunol. 180 (2008) 2251, https://doi.org/10.4049/jimmunol.180.4.2251.
[67] C. Perner, et al., Substance P release by sensory neurons triggers dendritic cell migration and initiates the Type-2 immune response to allergens, Immunity 53 (2020) 1063–1077, https://doi.org/10.1016/j.immuni.2020.10.001, e1067.