Mas-related G protein-coupled receptors (Mrgprs) – Key regulators of neuroimmune interactions

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Abstract

Interplay between physiological systems in the body plays a prominent role in health and disease. At the cellular level, such interplay is orchestrated through the binding of specific ligands to their receptors expressed on cell surface. G protein-coupled receptors (GPCR) are seven-transmembrane domain receptors that initiate various cellular responses and regulate homeostasis. In this review, we focus on particular GPCRs named Mas-related G protein-coupled receptors (Mrgprs) mainly expressed by sensory neurons and specialized immune cells. We describe the different subfamilies of Mrgprs and their specific ligands, as well as recent advances in the field that illustrate the role played by these receptors in neuro-immune biological processes, including itch, pain and inflammation in diverse organs.

Introduction

Recent advances in the field of neuro-immunology successfully uncovered various circuits responsible for itch sensation, pain transmission and inflammatory reactions. Mas-related G protein-coupled receptors (Mrgprs) are key receptors involved in the regulation of such biological processes. First described more than 25 years ago, the Mrgpr family now comprises more than 50 members in humans and rodents. Many Mrgprs are still considered as orphan receptors, with no identified endogenous or synthetic ligands. However, important advances in molecular biology helped to deorphanize many of them expressed by primary sensory neurons. Most Mrgprs are associated with nociception and itch transmission, through their binding to various itch-inducing or pain-associated substances: such as β–alanine, chloroquine (CQ), BAM(8-22) or substance P (SP) (Z. Li et al., 2017; Q. Liu et al., 2009, 2012; Meixiong, Vasavda, et al., 2019; Qu et al., 2014; Sanjel et al., 2019, 2019; Van Remoortel et al., 2019; C. Wang et al., 2019). Recently, a member of the Mrgpr family was found to be expressed on mast cells, a specialized innate immune cell involved in conveying non-histaminergic and histaminergic itch, nociception, inflammation and host protection (McNeil et al., 2014). Importantly, Mrgprs were found to be substantial actors in the communication established between the nervous and immune systems, regulating mechanisms involved in chronic inflammation (D. P. Green et al., 2019; Perner et al., 2020; Pundir et al., 2019; Serhan et al., 2019). In this review, we describe the diversity of Mrgprs and identified ligands, as well as the upshots of Mrgpr-mediated activation of sensory neurons and immune cells, in the contexts of itch, pain and tissue inflammatory processes.

Mrgprs: evolution, subfamilies, expression pattern and main functions

Mrgprs are GPCRs encoded by the *Mrgpr* gene family. This family was characterized more than 30 years ago with the description of the *Mas* gene encoding for oncogene-like MAS receptors (Young et al., 1986). Rapid advances in molecular biology, cloning strategies and bioinformatics helped decipher the *Mrgpr* gene family that now gathers more than 50 members in rodents and humans (Dong et al., 2001a; Lembo et al., 2002; Takeda et al., 2002; Wittenberger et al., 2001; Lin Zhang et al., 2005). All the receptors have been relabeled according to their functions and structures and were reassigned to several subfamilies: human receptors include eight subfamilies, MRGPRX 1 to 4, and MRGPR D to G, whereas rodent receptors include 7 subfamilies, Mrgpr A to G. *Mrgpr* gene clusters are all regrouped on chromosome (CR)11 in human (with the exception of *Mas1l* and *Mas* genes that are located on CR6) and CR7 in mouse (with the exception of *Mas H* gene located on CR17). After the identification of all coding sequences of these genes, the murine Mrgpr family now comprises the MrgprA subfamily gathering 14 members, the MrgprB and MrgprC families regrouping together 27 members, and the other families with unique members, the single-gene families, Mrgpr D, E, F and G. As for human receptors, MRGPRX 1-4 can be paired to the mouse MrgprA and B family members, while the other receptors (D to G) have clear defined identical murine orthologs (Bader et al., 2014).

Originally, Mrgprs expression pattern was described in dorsal root ganglia (DRG) and trigeminal ganglia (TG) by transcriptomic studies that revealed their expression in nociceptive and pruriceptive neurons of the above-cited ganglia, and more specifically in isolectin B4-positive small-diameter somatosensory afferents neurons (Dong et al., 2001a; Y. Liu et al., 2008; Zylka et al., 2003). Some Mrgprs are since considered as the prevailing markers of specific sensory neuron subsets in mice. Indeed, large-scale single-cell RNA sequencing identified subsets of sensory neurons characterized by the expression of specific Mrgprs: the non-peptidergic (NP) population 1 mostly expressed MrgprD, while the NP2 expressed

MrgprA3 & MrgprC11 (Usoskin et al., 2015). However, other works have shown that Mrgpr expression is not limited to sensory neurons and could also be found in other tissues, like the heart (MrgprH) (Wittenberger et al., 2001), genitals (MrgprB3, MrgprB8 and MrgprD) (Y.-H. Huang et al., 2013; Shinohara et al., 2004), cerebellum (MrgprE and MrgprF) (Ross et al., 1990), cerebral cortex and hippocampus (MrgprE), smooth muscle-containing tissues (MrgprF, drastically upregulated during monocyte-to-macrophage differentiation) (Hohenhaus et al., 2013; Ross et al., 1990) and in the enteric nervous system (MrgprD) (Avula et al., 2011; Zhou et al., 2019), the latter still being controversial (Van Remoortel & Timmermans, 2019).

Beside highly-innervated tissues, immune cells are also listed as Mrgpr-expressing cells (Dwyer et al., 2016). In particular, MRGPRX2 was found to be expressed at the surface of human cutaneous mast cells, and the ortholog MrgprB2 by murine skin, peritoneal, esophagus and tracheal mast cells (Fujisawa et al., 2014; McNeil et al., 2014; Subramanian et al., 2016; Subramanian, Gupta, et al., 2011a; Subramanian, Kashem, et al., 2011).

Diversified expression patterns imply a wide selection of ligands and consequently different functions for these receptors and for the resulting signaling. Divergent ligand specificities have been delineated for some Mrgprs, nevertheless many of them are left orphaned until today. Known ligands (listed in **Table 1**) include proteins and peptides, as well as non-peptidic ligands, such as lipids and some metabolites (e.g. alamandine, angiotensin(1-7), opioid precursor proenkephalin-derived peptides such as Bovine Adrenal Medulla [BAM]22, proopiomelanocortin [melanocyte-stimulating hormone] and many others) (S.-K. Han et al., 2002; Karnik et al., 2017; Kashem et al., 2011; Lautner et al., 2013; Lembo et al., 2002; Santos et al., 2003). The following sections describe the role of Mrgprs in sensory neurons and immune cells biological functions.

Role of Mrgprs in itch and pain

Implication of Mrgprs in the itch pathway.

Mrgprs were first identified on specialized sensory neurons that encode itch (pruriceptors) and pain (nociceptors) (Dong et al., 2001a; Usoskin et al., 2015). Both of these neuronal subtypes have their cell bodies residing in the DRG and the TG (Dubin & Patapoutian, 2010; Hunt & Mantyh, 2001). Importantly, activation of Mrgprs expressed at the surface of sensory neurons has been shown to induce both itch and pain sensations.

Itch or pruritus is clinically defined as the subjective and unpleasant sensation of a desire to scratch (Ikoma et al., 2006; LaMotte et al., 2014). The pathophysiological causes of pruritus are still largely unknown, but it is thought to be mainly mediated by molecules known as "pruritogens" particularly efficient at activating pruriceptors. Two distinct chemical itch pathways have been described: the histaminergic (involving mainly Histamine Receptor H1 activation by mast cell-derived histamine) and non-histaminergic (involving other pruritogens and other receptors) (LaMotte et al., 2014). In the periphery, itch is mainly encoded by two specialized populations of sensory neurons, the NP2 population expressing MrgprA3 and the NP3 population expressing Natriuretic Peptide B Precursor (NPPB) (Meixiong et al., 2020; Usoskin et al., 2015). Altogether, these two populations form the so-called pruriceptors. Furthermore, some pruritogens can also activate mixed nociceptive/pruriceptive populations, such as the β-alanin that can trigger the activation of the NP1 MrgprD^{+} population of sensory neurons (Shinohara et al., 2004), or the highly specific mu-opioid receptor agonist [D-Ala^{2}, N-MePhe^{4}, Gly-ol]-enkephalin (DAMGO) that can activate the TRPV1^{+} population of sensory neurons to induce itch (Melo et al., 2018).

Numerous studies have characterized known pruritogens as "Mrgpr agonists", such as the Chloroquine (CQ), BAM(8-22) or the β−alanine (Davidson et al., 2012; Sator et al.,

2003). Importantly, all these pruritogens stimulate the non-histaminergic itch pathway. CQ is an antimalarial drug that has been known for long to induce itch in human and animal models (Ezeamuzie et al., 1991; A. D. Green et al., 2006; Inan & Cowan, 2004; Onigbogi et al., 2000). In mice, loss and gain of function mutations showed that CQ can activate MrgprA3. Accordingly, CQ also activates MRGPRX1 which is the human ortholog of MrgprA3 resulting in non-histaminergic itch (Q. Liu et al., 2009). Nociceptor ablation based on Cre recombinase expression under the control of the *Mrgpra3* promoter resulted in a less severe and inducible itch by most tested pruritogens (L. Han et al., 2013). Other pruritogens that activate Mrgprs also include endogenous peptides like BAM(8-22), a peptide derived from the proopiomelanocortin protein. Indeed, Lembo *et al.* described that BAM(8-22) can activate MrgprC11 (Lembo et al., 2002). *Mrgprc11* mutant mice were shown to have significantly less susceptibility to BAM(8-22)-induced itch. To further validate the crucial role of Mrgpr in mediating the itch response to both CQ and BAM(8-22), it was shown that mice in which the *Mrgpr* gene cluster was deleted (altering up to 12 coding frames, including those coding for MrgprA3 and MrgprC11) did not scratch in response to either molecule (Lembo et al., 2002; Q. Liu et al., 2009). In humans, injections of BAM(8-22) resulted in stinging and burning sensations accompanied by a strong itch, that could eventually be driven by sustained activation of MRGPRX1 (Sikand et al., 2011). The absence of a neurogenic flare and the lack of efficacy of pre-treatment with anti-histaminic provided further complementary evidence of a potential non-histaminergic type of itch. Interestingly, a very recent study showed that MrgprC11 and human MRGPRX1 are the main receptors for two tick salivary peptides, IPDef1 and IRDef2. Accordingly, IPDef1 and IRDef2-mediated activation of MrgprC11/X1-on DRG neuron resulted in non-histaminergic itch via the Transient Receptor Potential Vanilloid (TRPV1), independently of the TRP Ankyrin 1 (TRPA1) (X. Li et al., 2020). These findings round off the clinical observation of the inefficiency of anti-histaminic drugs to treat

tick bites-induced itch. Another well-known pruritogen is β−alanine, that binds and activates MrgprD. β−alanine is a small amino acid present at high concentrations in muscles and skin (Rau et al., 2009), and considered as a potent pruritogen, in human and rodents (Qu et al., 2014; Shinohara et al., 2004). It has been shown that oral or intradermal administration of β−alanine induced itch and scratching behavior in wild type mice, but not in *Mrgprd*$^{-/-}$ mice (Q. Liu et al., 2012). In a model of chemically-induced psoriatic itch, gene expression levels of *Mrgprd* were found to be significantly decreased (Sakai et al., 2016), which might eventually underline a protective mechanism against the development of excessive itch sensation. In a model of contact hypersensitivity induced by haptens, MrgprD was found to become "hypersensitive", since MrgprD^{+} neurons innervating the hapten-challenged skin exhibited spontaneous activity and/or abnormal after-discharges in response to mechanical and heat stimuli, with a greater number of action potentials (Qu et al., 2014). Likewise, spontaneous itch-like (biting or scratching) behaviors were reported in these mice. Interestingly, intradermal injections of β−alanine in human subjects elicited itch, but without wheal and flare, that are two clinical manifestations classically associated with histaminergic itch. These data suggested a possible activation of the non-histaminergic itch pathway by β−alanine (Q. Liu et al., 2012). Very recently, the bilirubin (an endogenous degradation metabolite of hemoglobin) was described as a novel ligand for mouse MrgprA1 and human MRGPRX4 in the pathological context of cholestatic pruritus. A study by Meixiong *et al.* suggested that bilirubin activated sensory neurons through MrgprA1 in a TRP channel-dependent mechanism (Meixiong, Vasavda, et al., 2019). In a mouse model of cholestasis, removing either MrgprA1 or biliverdin reductase (the enzyme that facilitates the conversion of biliverdin to bilirubin) both strongly attenuated cholestatic itch.

Implication of Mrgprs in the pain pathway.

Mrgpr activation has also been shown to mediate pain signaling. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is characterized by hypersensitivities to multiple stimuli that lead to painful sensation once integrated in the brain (M. J. Caterina et al., 2000; Michael J. Caterina et al., 1999; Costigan & Woolf, 2000). The role for Mrgprs in the pain phenotype has not been completely elucidated yet. Majority of the studies conducted focused on the role of MrgprD-expressing nociceptors in mechanical hypersensitivity and neuropathic pain behavior in mouse models rather than on the role of the receptor itself (Qu et al., 2014; C. Wang et al., 2019). A recent study by *Huang et al.* used *Mrgprd*^{DTR} mice, in which the human diphtheria toxin receptor (DTR) is driven from the *Mrgprd* locus, to specifically ablate MrgprD^{+} neurons in adult mice. Basal mechanical thresholds were significantly increased in mice that lack MrgprD^{+} nociceptor (T. Huang et al., 2019). Mrgprs could be involved in the modulation of somatic pain phenotype. Intrathecal injection of the MrgprC agonist [Tyr^{6}] γ2-MSH(6-12) in wild type mice induced acute-like pain behavior (Chang et al., 2009). Interestingly, in a humanized mouse model expressing MRGPRX1, Zhe Li *et al.* showed that intrathecal injection of BAM(8–22) reduced pain behavior in a model of chronic constriction injury. These data strongly suggest that MRGPRX1 could be a potential target for the treatment of chronic pain (Z. Li et al., 2017). Further studies would be pertinent to uncover and thoroughly characterize the role of neuronal Mrgprs in somatic pain.

Mrgprs have also been reported to be important regulators of visceral pain phenotype. Since its discovery in 2002, the expression of MrgprD was thought to be restricted to skin-projecting neurons and its biological function limited to cutaneous somatosensation. However, *Hockley et al.* reported the expression of *Mrgprd,* and of other Mrgprs including *Mrgpra9*, *Mrgpra2b*, *Mrgprc11* (referenced as *Mrgprx1* in the study), *Mrgprb5* and *Mrgpra3* in the soma of sensory neurons that project to the colon (Hockley et al., 2019). Additional

studies using single-cell quantitative PCR (qPCR) (Bautzova et al., 2018; Castro et al., 2019), *Mrgprd*^{EGFP} reporter mice and retrogradely-traced mouse colonic sensory neurons (Bautzova et al., 2018) also confirmed the expression of *Mrgprd* in sensory neurons innervating the colon. In human, mRNA expression of *Mrgprd*, *Mrgpra3* and *Mrgpc11* have been detected and quantified by reverse transcription (RT)-qPCR in spinal DRG well known to innervate the colon (T9-L1). Of note, *Mrgpra3*, *Mrgprc11* and *Mrgprd* were found co-expressed together with *Trpv1* and/or *Trpa1* (Bautzova et al., 2018; Castro et al., 2019). When global sensory neuron populations were assessed, MrgprD and TRPV1 were detected in discrete and almost mutually-exclusive neurons (Cavanaugh et al., 2009; Usoskin et al., 2015; Zylka et al., 2005). In contrast, in colon-projecting sensory neurons, 41% of TRPV1-positive neurons were also reported to express *Mrgprd* (Bautzova et al., 2018). To our knowledge, such co-expression has only been reported in colonic sensory neurons.

Of most relevance to clinical practice, activation of MrgprD signaling in the colon has been shown to participate in the development of pain sensation in the context of irritable bowel syndrome (IBS). IBS is a functional bowel disorder in which recurrent abdominal pain is associated with changes in bowel habit (Enck et al., 2016). The pathophysiology of IBS is still elusive and the underlying mechanisms contributing to afferent sensitization remain incompletely understood (Spiegel et al., 2008). In a recent study, Bautzova *et al.* quantified an increase of the arachidonic acid metabolite 5-oxoETE in biopsies from patients with clinically-established IBS compared to healthy subjects (Bautzova et al., 2018). Low-grade inflammation associated with peripheral sensory nerves hyperactivity has been widely described in IBS, with several fundamental studies implicating pro-inflammatory molecules in the pathophysiology of IBS symptoms (Brierley & Linden, 2014; Enck et al., 2016). The 5-oxoETE has been shown to induce calcium signaling in sensory neurons. In the absence of MrgprD, activation of sensory neurons by 5-oxoETE was significantly decreased, suggesting

that 5-oxoETE might signal in neurons via MrgprD (Bautzova et al., 2018). However, because of its lipid-based nature, 5-oxoETE could not be officially classified as a new MrgprD ligand due to the lack of suitable binding assays.

In the gut, Castro *et al.* hypothesized that the mechanisms of histamine-independent itch involving MrgprA3 and MrgprC11 represent important biological components of visceral sensory neuron sensitization (Castro et al., 2019). They highlighted clear individual roles for MrgprA3 and MrgprC11 in the activation of colonic afferent neurons and induction of mechanical hypersensitivity. In primary cultures of mouse DRG, 10% of colonic sensory neurons were efficiently activated by CQ, allyl isothiocyanate ([AITC], a TRPA1 agonist) and capsaicin (a TRPV1 agonist); and another 10% responded to BAM(8-22), AITC and capsaicin (Castro et al., 2019). Functional interactions between MrgprA3, MrgprC11, TRPA1 and/or TRPV1 have also been proposed in colonic sensory neurons (X. Li et al., 2020; Wilson et al., 2011). In wild type mice, activation of MrgprA3 by CQ and MrgprC11 by BAM(8-22) induced visceral hypersensitivity (Castro et al., 2019; Van Remoortel et al., 2019). As demonstrated in the skin in the context of itch (Q. Liu et al., 2009), the effects of CQ and BAM(8-22) on visceral sensitivity were abrogated in mice that lack a cluster of *Mrgpr* genes. In addition, an itch cocktail combining 3-(2-chlorophenyl)-N-(4-chlorophenyl)-N,5-dimethyl-4-isoxazolecarboxamide (CCDC), BAM(8-22) and CQ, triggered mechanical visceral hypersensitivity, and had no effect in *Trpa1*$^{-/-}$ mice (Castro et al., 2019). After confirming the co-expression of MRGPRX1, TRPV1 and/or TRPA1, human sensory neurons were incubated with inflammatory mediators known to be increased in biopsies of IBS patients (histamine, PGE_2, serotonin and bradykinin). Consequently, the levels of intracellular calcium in response to treatment with CQ were increased in such neurons, however this activation was not observed with BAM(8-22). CQ activates the human MRGPRX1 with 1000-fold less affinity and 2.5-fold less efficiency than BAM(8–22) (Hughes et al., 2009). Taken together, the

described experiments proposed that inflammatory mediators were able to sensitize and increase the expression of Mrgprs in colonic sensory neurons, suggesting an important function of Mrgprs in visceral hypersensitivity.

Role of Mrgprs expression by immune cells during inflammation, host defense, and neuroimmune interactions

The immune system is made up of coordinated sets of cellular elements that participate to many physiological processes (maintenance of tissue homeostasis, tissue remodeling, cell-host relationship, etc.) and responsible for the protection against environmental cues. Being part of the innate or adaptive responses, immune cells orchestrate a large panel of effector mechanisms with distinct biological functions via the release of a large array of cytokines and the engagement of specific surface and intracellular receptors. Exacerbated activation of immune responses can often lead to the development of chronic inflammation, pain, itch, and global disruptions of organs metabolism (Altan-Bonnet & Mukherjee, 2019, 2019; Eisenbarth, 2019; Klose & Artis, 2016; Masopust & Soerens, 2019; Ng et al., 2019).

The expression and the role of Mrgprs on immune cells remain a vast and exciting area of exploration. Transcriptomic data suggested that mast cells are naturally-expressing some Mrgprs at steady state (Dwyer et al., 2016). As active members of the innate immune system, mast cells express a wide range of receptors activated upon binding of specific ligands such as toxins, hormones, immunoglobulins and cytokines. Mast cell activation usually results in the quasi-instantaneous release of granule-associated bioactive mediators (i.e., a phenomenon named “degranulation”) and the sequential secretion of *de novo* molecules over time (lipid mediators, cytokines and chemokines) (Wernersson & Pejler, 2014). Secreted substances include histamine, proteases, lipid mediators and cytokines, all being efficient

immunomodulatory molecules. Among the pleiotropy of receptors expressed on mast cell surface, MrgprB2 (and its human ortholog MRGPRX2) was the first Mrgpr found to be detected outside of the sensory nervous system (Dwyer et al., 2016; Fujisawa et al., 2014; McNeil et al., 2014; Subramanian, Gupta, et al., 2011a; Subramanian, Kashem, et al., 2011). The spatio-temporal dynamics of MrgprB2/X2-mediated mast cell degranulation differs from the canonical FcR-dependent activation and is characterized by a fast and local degranulation with quasi-immediate effects on surrounding tissue and inflammation (Gaudenzio et al., 2016). Activation of mast cell-MrgprB2 (or MRGPRX2) has been recently involved in multiple pathophysiological processes.

MrgprB2 has been reported to be a mast cell receptor for quorum sensing molecules secreted by bacterial populations, including the competence-stimulating peptide 1 (CSP-1). Upon activation of MrgprB2, mast cells exteriorized their granular content which helped to control bacterial infection by preventing bacterial growth and biofilm formation (Pundir et al., 2019). In a recent study, small-molecule host-defense peptides have also been shown to signal through MrgprB2/X2 and promote antifungal and antibacterial activity, presumably via a similar mechanism (Alkanfari et al., 2019). Another report also showed that MrgprB2 could also sense tick salivary defensing peptides, IPDef1 and IRDef2, that were shown to efficiently activate mast cells resulting in acute inflammation (X. Li et al., 2020).

Recently, complementary studies have shed new lights on the key role played by MrgprB2 in regulating bidirectional interactions between mast cells and nociceptors, mainly via the cationic neuropeptide SP. SP belongs to the tachykinin family and is considered as an important element of pain perception (Donkin et al., 2007; Mantyh, 2002; Usoskin et al., 2015). Recent findings by Green *et al.* involved SP binding to MrgprB2 and MRGPRX2 in inflammatory mechanical and thermal hyperalgesia (D. P. Green et al., 2019). SP is enriched in patients who suffer from post-operative pain. Compared to littermate controls, MrgprB2

knock-out mice were significantly less susceptible to develop inflammatory pain, such as in models of postoperative incision or injection of complete Freund's adjuvant. These mice also exhibited less nerve injury and a significant decrease in the recruitment of immune cells to the site of injury (mainly $CD11b^{+}Ly6G^{-}$ myeloid cells). Ablation of $MrgprB2^{+}$ mast cells *using MrgprB2-cre; DTR* mice (in which the human DTR is under the control of *MrgprB2* promotor) showed reductions in the perception of inflammatory pain (D. P. Green et al., 2019). This study indicated that the SP-MrgprB2 axis could promote neurogenic inflammation and be involved in pain mechanisms. Another study by Zhang *et al.* has also documented a participation of MRGPRX2 in the development of morphine-induced granulomas that could in turn cause persistent pain (T. Zhang et al., 2019).

Nociceptors and mast cells can also regulate the development of itch sensation. Mast cells are known to be classically associated with histaminergic itch (Hall et al., 2012; Harvima et al., 2008). However, recent findings have also highlighted the major role played by skin mast cells in histamine-independent itch, through the activation of MrgprB2/X2. The authors found that injection of the MrgprB2 agonist Pro-adrenomedullin peptide 9–20 (PAMP9-20) or the compound 48/80 in mice resulted in histamine-independent itch, as it was not alleviated by histamine receptors antagonists. They further showed that MrgprB2 stimulation was associated with the release of high amounts of tryptase (but less histamine and serotonin) compared to canonical IgE stimulation that resulted in the release of high a mounts of histamine and serotonin (but less tryptase), such differential release of mediators then resulted in the activation different population of neurons. In mouse models of two common skin diseases, allergic contact hypersensitivity (ACD) and atopic dermatitis (AD), mice deficient in MrgprB2 showed significantly less pruritus (Meixiong, Anderson, et al., 2019). Finally, the authors found that the skin of patients suffering from ACD was particularly enriched in PAMP(1-20), underlining the potential translational value of these findings. The effective

communication between Mrgprs-expressing neuronal and immune cells was also highlighted using intravital calcium imaging of mice carrying a genetically-encoded calcium tracer into sensory neurons, as activation of mast cells via MrgprB2 resulted in the further activation of MrgprD$^+$ and MrgprA3$^+$ itch sensory neurons (Meixiong, Anderson, et al., 2019). In another study, Li Zhang, McNeil *et al*. proposed that anti-microbial peptides such as β–defensin (Defb14) could induce itch behavior via MrgprB2. This hypothesis was confirmed in MrgprB2-deficient mice (Li Zhang & McNeil, 2019b). Unexpectedly, the same group observed that β–defensin could also activate MrgprA3/C11 and their ortholog MRGPRX1, suggesting that different Mrgprs could eventually share common ligands.

Recently, Serhan, *et al.* uncovered a new mechanism regulating type 2 (allergic) skin inflammation that is governed by a subpopulation of skin-projecting *Tac1*$^+$ (SP-producing) TRPV1$^+$ nociceptors and MrgprB2$^+$ dermal mast cells (Serhan et al., 2019). SP deficiency (*Tac1*$^{-/-}$) and selective depletion of TRPV1$^+$ nociceptors protected mice from the development of a model of type 2 skin inflammation that shares many pathological features with the human AD. Same observations were made when mice were deficient in mast cells or carried a truncated and non-functional form of MrgprB2. Imaging of isolated *ex vivo* DRG neurons revealed that common domestic allergens found in most AD patients, the house dust mites *D. farinae* and *D. pteronyssinus*, were able to directly activate neurons via their cysteine protease activity. Combining genetic models and intravital imaging, the authors finally showed that TRPV1$^+$ SP-producing nociceptors formed physical and functional clusters with MrgprB2$^+$ mast cells capable to detect allergens and drive the development of AD-like inflammation in the mouse (Serhan et al., 2019).

Another recent study using different models of allergen exposure decrypted a similar mechanism of activation, having SP as the main actor that activated CD301b$^+$ dendritic cells via expression of MrgprA1. SP-activated dendritic cells migrated to the draining lymph node

where they initiated a type 2 immune response (Perner et al., 2020). This study strongly suggests that, albeit expressed on different immune cells, MrgprB2 and MrgprA1 could share the SP as a common ligand in the skin.

Immune cells can also release inflammatory mediators capable to activate Mrgprs, for example the 5-oxoETE that is formed by the oxidation of 5-HETE by the 5-hydroxyeicosanoid dehydrogenase (5-HEDH) (Y. Zhang et al., 1996), a microsomal enzyme that is highly selective for 5(S)-HETE and requires $NADP^+$ as a cofactor (Erlemann et al., 2007). 5-HEDH is found in neutrophils as well as in a variety of other inflammatory and stromal cells, including monocytes (Erlemann et al., 2007), dendritic cells (Stamatiou et al., 2004) and mucosal epithelial cells (Erlemann et al., 2007). However, more studies would be needed to develop a clear picture of the role of immune cell-derived Mrgprs agonists in the regulation of neuroimmune interactions.

Conclusion and perspectives: Mrgprs as potential therapeutic targets to treat inflammation, pain and itch sensations

Mrgprs have emerged as substantial players in the biology of itch and pain, as well as in the onset and development of inflammation in the skin and in visceral organs. Despite the significant progresses made in the past few years on Mrgprs biology, there is still a lot to be discovered about their physiological and pathological functions. Many Mrgprs are still orphan receptors with no identified endogenous or chemical ligands. For the moment, very few agonists or antagonists have been described; the “deorphanisation” of additional Mrgprs is an exciting area of investigation and should bring invaluable informations on the molecular mechanisms that govern neuroimmune crosstalks and their roles in health and diseases.

Because Mrgprs are found to be expressed in both sensory nervous and immune systems, it is interesting to speculate that a sustained co-evolutionary communication has

occurred to promote mutually beneficial biological events. Immune cells and nociceptors are distributed in all tissues, especially those located at the interface between the host and the environment. Studies described in this review showcased an important number of interactions between Mrgprs-expressing cells and other cells involved in the regulation of pain, itch and inflammation in different organs. Both chronic and acute diseases are often associated to various mechanisms that involve GPCRs and notably Mrgprs. Skin diseases such as AD and ACD, as well as visceral disorders (such as IBS) are thought to be accompanied by Mrgprs-driven inflammation. New antagonists for MRGPRD and MRGPRX1 are currently being investigated in pre-clinical trials as novel anti-itch drug targets. In line with these molecules, the design of new MRGPRX2 antagonists could constitute an innovative line of treatment in the context of pain, itch and allergic inflammation. Finally, it is important to keep in mind that translational studies of neuroimmune interactions in human tissues will constitute a major (but nevertheless difficult) challenge in the field, due to the remote anatomical location of sensory neuron cellular bodies in DRG or other related ganglia.

Figures, tables and legends

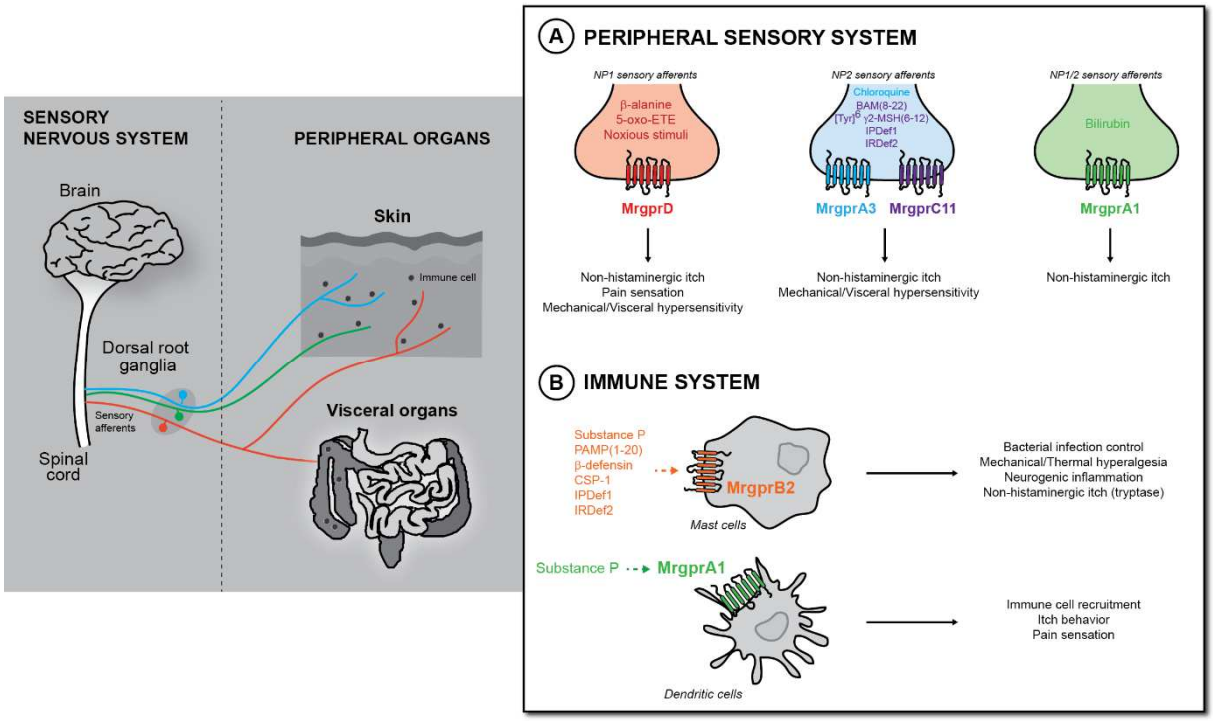

Figure 1. Mrgprs-expressing cells belong to the peripheral sensory and immune systems, and are involved in pruriception, nociception and inflammation. Isolectin B4-positive small-diameter afferents neurons that express Mrgprs innervate the skin and the visceral organs and can be classified into three distinct classes. **A**, MrgprD is mainly expressed by nociceptors belonging to the NP1 subpopulation (**red**) and is activated by β–alanine and 5-oxo-ETE causing non-histaminergic itch, mechanical and visceral hypersensitivity. MrgprA3 and MrgprC11 are Mrgprs expressed by pruriceptors belonging to the non-peptidergic family 2 (NP2, **blue**) and are activated by CQ and BAM(8-22) and [Tyr^6] γ2-MSH(6-12) respectively. Their activation drives itch and may modulate pain. MrgprA1-positive sensory neurons (both NP1 and NP2 population) (**green**) are activated by bilirubin and are strongly linked to pruritus. **B,** Immune cells were also found to express Mrgprs. MrgprB2$^+$ mast cells can be activated by diverse ligands such as PAMP(1-20), β–defensin and SP resulting in non-histaminergic itch and pain behavior. CSP-1 can also bind to MrgprB2 resulting in the control of bacterial infection. Tick-derived salivary protein IPDef1 and IRDef2 also activate MrgprB2 and drive local inflammation. Additionally, binding of SP to mast cell-MrgprB2 or dermal

dendritic cell-MrgprA1 promotes skin inflammation and onset of type 2 immune response. *Abbreviations: Mrgpr: Mas-related G protein-coupled receptor, NP: non-peptidergic, 5-oxo-ETE: 5-oxoeicosatetraenoic acid, BAM(8-22): Bovine Adrenal Medulla, PAMP(1-20): proadrenomedullin peptide 1-20, SP: Substance P, CSP-1 : Competence Stimulating Peptide 1.*

		Ligand		
Receptor	**Human ortholog**	**Endogen**	**Exogen**	**References**
MrgprB2	**MRGPRX2**	Substance P Platelet factor-4 AG-30/5C β-defensin Cathelicidin LL-37 BAM (8-22;13-22;22) Catestatin Cortistatin Hemokinin-1 Kallidin Neuropeptide FF Oxytocin PACAP (6-27) PAMP(9-20) Somatostatin Susbstance P VIP Vasopressin Dynorphin A	Ciprofloxacin Levofloxacin Moxifloxacin Ofloxacin Atracurium Rocuronium Tubocurarine Angiopeptin Cetrorelix Hexarelin Icatibant Leuprolide Octreotide Sermorelin Compound 48/80 Mastoparan CSP-CSP1-CSP2 Entf Streptin-1	(Alkanfari et al., 2019; Dong et al., 2001b; Kamohara et al., 2005; McNeil et al., 2015; Pundir et al., 2019; Robas et al., 2003; Subramanian et al., 2013; Subramanian, Gupta, et al., 2011b; Tatemoto et al., 2006; Gaudenzio et al., 2016; Serhan, et al., 2019)
Mrgprc11	**MRGPRX1**	BAM(8-22) β-defensin SLIGRL [Tyr^6] γ2-MSH(6-12) Neuropeptide FF	IPDef1 IRDef2	(Chang et al., 2009; S.-K. Han et al., 2002; Lembo et al., 2002; X. Li et al., 2020)
Mrgpra3	**MRGPRX1**	β-defensin	Chloroquine	(Q. Liu et al., 2009; Li Zhang & McNeil, 2019a)
Mrgprd	**MRGPRD**	β-alanine Alamandine 5-oxoETE Angiotensin(1-7) GABA	ND	(Bautzova et al., 2018; Karnik et al., 2017; Lautner et al., 2013; Qu et al., 2014; Santos et al., 2003; Shinohara et al., 2004; Uno et al., 2012)
Mrgpra1	**MRGPRX4**	Bilirubin Salusin β Arg-Phe-amide containing neuropeptides (FLRF-amide, FMRF-amide and NPFF)	ND	(Dong et al., 2001c; S.-K. Han et al., 2002; Meixiong, Vasavda, et al., 2019; Z. Wang et al., 2006)
Mrgpra4		Neuropeptide FF ACTH	ND	(Dong et al., 2001c)

Table 1. Human and murine Mas-related G protein-coupled receptor ligands. Table abbreviations: BAM: Bovine Adrenal Medulla, PACAP: Pituitary adenylate cyclase

activating polypeptide, PAMP: proadrenomedullin peptide, CSP: competence-stimulating peptide 1, MSH: Melanocyte-Stimulating Hormone, 5-oxoETE: 5-Oxoeicosatetraenoic Acid, GABA: Gamma aminobutyric acid, ACTH: Adrenocorticotropic hormone, ND: not determined.

Author contributions

All authors participated in writing and/or editing the paper.

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Conflict of interest statement

N.G. is acting as Chief Scientific Officer consultant for Genoskin. He is a member of the advisory board for MaxiVAX. He has served as consultant for Boehringer Ingelheim and Novartis. He has a patent pending for the treatment of type 2 inflammation and mast cell-dependent disorders. N.S., L.B. and N.C. declares no competing interest.

List of abbreviations

5-HEDH 5-Hydroxyeicosanoid Dehydrogenase

5-oxoETE 5-Oxoeicosatetraenoic Acid

ACD Allergic Contact Hypersensitivity

AD	Atopic Dermatitis
AITC	Allyl Isothiocyanate
Ang	Angiotensin
BAM	Bovine Adrenal Medulla
CQ	Chloroquine
CR	Chromosome
CVH	Chronic Visceral Hypersensitivity
CSP-1	Competence-Stimulating Peptide 1
DRG	Dorsal Root Ganglia
DTR	Diphtheria Toxin Receptor
GPCR	G Protein-Coupled Receptor
IBS	Irritable Bowel Disease
IL	Interleukin
Mrgpr	Mas-Related G Protein-Coupled Receptor
MSH	Melanocyte-Stimulating Hormone
NP	Non-Peptidergic
NPFF	Neuropeptide FF
PAMP(1-20)	Proadrenomedullin Peptide 1-20
qPCR	Quantitative PCR
RT	Reverse Transcription
SP	Substance P
TRPA1	Transient Receptor Potential Ankyrin 1
TG	Trigeminal Ganglia
TNBS	Trinitrobenzenesulphonic acid

462 TRPV1 Transient Receptor Potential Vanilloid 1

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