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Neuro-immune interactions and the role of Mas-related G protein-coupled receptors (Mrgprs) in the gastrointestinal tract.

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Abstract:

Over the past decade, the research field dealing with the role of a new family of Rhodopsin A-like G protein-coupled receptors, i.e. the family of Mas-related G protein-coupled receptors (Mrgprs) has expanded enormously. A plethora of recent studies have provided evidence that Mrgprs are key players in itch and pain, as well as in the initiation and modulation of inflammatory/allergic responses in the skin. Over the years, it has become clear that this role is not limited to the skin, but extends to other mucosal surfaces such as the respiratory tract and the gastrointestinal (GI) tract. In the GI tract, Mrgprs have emerged as novel interoceptive sensory pathways linked to health and disease, and are in close functional association with the gut's immune system. This review aims to provide an update of our current knowledge on the expression, distribution and function of members of this Mrgpr family in intrinsic and extrinsic neuro-immune pathways related to the GI system.

1. ***The family of Mas-related G protein-coupled receptors***

More than 20 years ago, a new family of Rhodopsin A-like G protein-coupled receptors (GPCRs) was discovered in the mouse and human genome (Dong et al., 2001; Lembo et al., 2002). The members of this family showed 30–41% sequence homology with the Mas oncogene, described some 15 years earlier (Young et al., 1986), and were accordingly termed Mas-related G protein-coupled receptors (Mrgprs). Mouse and human Mrgprs now comprise more than 50 members and are subdivided into nine separate subfamilies (A-H and X). Subfamilies D to G are evolutionarily conserved between species and thus considered direct orthologues (Castro et al., 2019; Dong et al., 2001; Lembo et al., 2002; Zylka Mark et al., 2003) whereas subfamilies A, B, C, and H are only found in rodents and subfamily X is specific to primates, including humans, macaques and rhesus monkeys (Burstein et al., 2006; Dong et al., 2001; Lembo et al., 2002; Zhang et al., 2005; Zylka Mark et al., 2003). In humans, Mrgpr gene clusters are mainly located on chromosome 11, in mice on chromosome 7 (Bader et al.,

2014). These rodent-specific and human-specific receptors, while divergent in their gene and protein sequences, do share common expression patterns and ligands and are therefore considered functional orthologues. Meanwhile, expression of some Mrgpr members has also been shown in other vertebrate species, such as chickens, cows, dogs and wolves (Hamamura-Yasuno et al., 2020; Inclan-Rico et al., 2020; Meixiong and Dong, 2017; Solinski et al., 2014) and species differences between rodents and other mammals in terms of distribution characteristics of some of the Mrgpr members have been reported (Hamamura-Yasuno et al., 2020). Initially, these receptors were found to be expressed in spinal and trigeminal ganglia and therefore thought to be specifically linked to neural pathways associated with itch and nociception, which is also why they were originally referred to as sensory neuron-specific receptors (Avula et al., 2013; Bader et al., 2014; Solinski et al., 2014). Meanwhile, it has become clear that Mrgprs are also involved in a wide array of human disorders. Additionally, evidence has been provided that these receptors play a hitherto underestimated role in allergic responses and visceral hypersensitivity (Castro et al., 2019; Grundy et al., 2021; Van Remoortel et al., 2019). We now know that Mrgprs, apart from being expressed in sensory neurons, are also present in other cell types, such as mast cells (MCs) and macrophages, as well as in other tissues than skin and intestine, e.g. in airways and in the heart (de Carvalho Santuchi et al., 2019; Han et al., 2018; Hrenak et al., 2016; McNeil et al., 2015; Oliveira et al., 2018). Although our knowledge of Mrgpr/MRGPR signaling pathways and polymorphisms has increased substantially in recent years, we should be aware that there still is a long way to go, since the majority of these receptors are still orphans with no known endogenous or synthetic ligands, which hampers functional testing (Bader et al., 2014).

2. ***Mrgpr expression in the gastrointestinal tract***

Given the obvious similarities between skin, airways and gut in terms of continuous exposure to external harmful stimuli as well as similarities in neurochemical features of afferent pathways, a logical question arises whether Mrgprs are also involved in gut afferent pathways. The gastrointestinal (GI) tract, just like the skin and lungs, is characterized by a delicate balance between neuronal elements and key players of the innate and adaptive immune responses in order to maintain homeostasis. We do know from our earlier studies (Avula et al., 2011; Avula et al., 2013) that several of the Mrgpr subfamilies are indeed present in the

65 intrinsic component of the enteric nervous system (ENS), and also in the extrinsic component
66 as recently demonstrated by our lab (Van Remoortel et al., 2019; Van Remoortel and
67 Timmermans, 2019) and others (Castro et al., 2019)
68
69 ### 2.1. ***Presence of Mrgprs in the enteric nervous system***
70 The first immunohistochemical studies focused on the expression and topographical
71 distribution of murine Mrgpr members at the level of the distal intramural part of the small
72 intestine. Previous studies in our lab investigated the expression of several murine MrgprA
73 subfamily members (MrgprA1, A2, A4, A5 and A7), multiple MrgprB subfamily members
74 (MrgprB1, B2, B4, B5, B8, B10), as well as the subfamily e and f members in the murine
75 terminal ileum (Avula et al., 2011; Avula et al., 2013). qPCR analysis revealed low mRNA
76 expression for most of these members in the healthy intestine, but significantly increased
77 mRNA expression for several *MrgprA* and *MrgprB* subfamily members in inflamed intestine,
78 as seen e.g. in intestinal schistosomiasis or trinitrobenzenesulfonic acid (TNBS)-induced
79 ileitis/colitis. Interestingly, *MrgprE* and *MrgprF* expression was significantly reduced in the
80 TNBS-inflamed ileum. Further immunohistochemical studies using custom-developed
81 antibodies showed that MrgprA4, B2, B8, B10, E and F are expressed, be it moderately, by
82 several enteric neuronal subtypes, i.e. not only sensory neurons, but also in secretomotor and
83 vasodilator neurons, as well as nerve fibers in the *lamina propria* and outer muscle layers
84 (Avula et al., 2011; Avula et al., 2013). Using biophysical and biochemical techniques, we were
85 also able to unambiguously reveal hitherto undiscovered heteromeric interactions between
86 MRGPRE and MRGPRF by Luciferase complementation (LC)-based NanoLuc binary technology,
87 bioluminescence resonance energy transfer (BRET), fluorescence resonance energy transfer
88 (FRET) and co-immunoprecipition (co-IP) (Arora et al., unpublished). In addition, LC, BRET and
89 FRET pointed to heteromeric interactions between MRGPRF and MRGPRD, although this could
90 not be confirmed by co-IP. Conversely, interaction between hMRGPRE and hMRGPRD was
91 evident by co-IP, but was not picked up by LC while the BRET and FRET signals were only
92 marginally above background. Although these heteromeric interactions between MRGPRD
93 and MRGPRE/F could not be confirmed by all assays used, the results are in line with earlier
94 reports on rat MrgprD and MrgprE stating that these heterodimers might modulate the
95 function(s) of MrgprD (Milasta et al., 2006). This latter member of the Mrgpr family has been
96 reported primarily in isolectin B4-positive, small non-myelinated sensory neurons in dorsal

97 root ganglia (DRG) and trigeminal ganglia (Dong et al., 2001; Zylka Mark et al., 2003; Zylka et
98 al., 2005), and has been shown to be co-expressed, at the mRNA level, with *MRGPRX1* in a
99 subpopulation of TRPV1^{+} human DRG neurons (Klein et al., 2021). More recently, some debate
100 has emerged about whether or not MrgprD is expressed not only by extrinsic (nociceptive)
101 visceral pathways but also by intrinsic primary sensory neurons (IPANs) in the intestinal wall.
102 Reason for this discussion were two publications by the Lan lab (Xu et al., 2022; Zhou et al.,
103 2019) who claimed to have revealed the presence of MrgprD in myenteric neurons using two
104 distinct antibodies against MrgprD. The antibody used in the first study (Zhou et al., 2019)
105 clearly turned out to be aspecific, since a similar staining pattern was found in MrgprD
106 knockout mice using the same antibody (Van Remoortel and Timmermans, 2019). The follow-
107 up study of the Lan group (Xu et al., 2022), in which they crossed MrgprD-creERT2 mice with
108 ROSA26/tdTomato reporter mice to obtain MrgprD tdTomato/+ mice and in which they used
109 another commercial antibody, is not convincing either for two main reasons: (1) In the
110 MrgprD-creERT2-tdTomato mouse line, postnatal tamoxifen treatment induces Cre
111 recombinase expression in MrgprD-expressing cells, which in turn results in Cre-mediated
112 removal of a genetic STOP codon from the CAG promoter-driven tdTomato gene sequence
113 located in the ROSA26 locus. The removal of this STOP codon induces strong expression of the
114 fluorescent tdTomato reporter and allows to easily identify MrgprD-expressing cells based on
115 the presence of homogenous tdTomato signal in the cytoplasm. Hence, in case MrgprD would
116 be expressed by myenteric neurons, one would expect a bright and homogenous tdTomato
117 signal in the cytoplasm of enteric neurons. However, while Xu and colleagues convincingly
118 showed this homogenous cytoplasmic and nuclear tdTomato labeling in DRG neurons (in line
119 with the already established presence of MrgprD in extrinsic visceral afferents), the tdTomato
120 signal in the myenteric plexus was very faint and granular, i.e. similar to the tdTomato images
121 seen in a channel with overamplified signal and hence to be considered as aspecific
122 background signalling. (2) The appearance and number of cells of the immunolabeling with a
123 commercial antibody in the DRG does not match with that of the tdTomato labeling in DRGs.
124 Overall, MrgprD expression in the ENS remains debatable at this point although this naturally
125 does not detract from the group's earlier functional finding that MrgprD signaling could be
126 involved in GI motility (Xu et al., 2021); in any case, it does underline the need for further
127 studies investigating whether MrgprD controls GI motility through intrinsic or extrinsic
128 neuronal pathways.

129 Several earlier immunofluorescence and electron microscopical studies in rodent models have
130 provided evidence for the expression of glutamate, its release and reuptake transporters, and
131 its various receptor subtypes in the ENS (Brumovsky et al., 2011; Giaroni et al., 2003; Liu et al.,
132 1997; Seifi and Swinny, 2016; Tong et al., 2001; Tsai, 2005). So far, no direct link has been
133 made with Mrgpr-mediated pathways, but a more recent study demonstrated that
134 endogenous glutamate excites myenteric calbindin-positive interneurons and intrinsic
135 sensory neurons in the mouse colon (Swaminathan et al., 2019). Furthermore, Bautzova and
136 colleagues (Bautzova et al., 2018) recently established that the polyunsaturated fatty acid
137 metabolite, 5-oxoeicosatetraenoic acid (5-oxoETE), can induce MrgprD-mediated somatic and
138 visceral hyperalgesia without inflammation, in this way triggering noxious symptoms in
139 constipated irritable bowel syndrome (IBS) patients.
140

141 ### ***2.2. Presence of Mrgprs in intestinal extrinsic afferents.***

142 #### *2.2.1. Mrgprs in spinal visceral afferents*

143 Pioneering studies from the last decade have unambiguously demonstrated that expression
144 of Mrgprs is not limited to cutaneous somatosensory pathways but that several subfamilies of
145 these receptors also play important roles in viscero-sensory pathways as well. Using *in situ*
146 hybridization and immunohistochemistry, our lab for the first time reported MrgprC11
147 expression in up to 20% of colon-projecting peptidergic thoracolumbar DRG neurons (Van
148 Remoortel et al., 2019). We also demonstrated that intracolonic administration of an
149 MrgprC11 ligand, i.e. BAM(8-22), results in the sensitization of spinal afferents and induces
150 visceral hypersensitivity in healthy mice, an effect that was not seen in a transgenic mouse
151 model lacking functional MrgprC11 expression (Van Remoortel et al., 2019). These findings
152 were independently confirmed a few months later by an elegant study conducted by the
153 Brierley lab, who provided evidence that not only MrgprC11, but also another member, i.e.
154 MrgprA3, plays a very similar role in causing visceral hypersensitivity (Castro et al., 2019).
155 Additionally, Castro and collaborators (Castro et al., 2019) showed functional upregulation of
156 MrgprA3 and MrgprC11 signaling pathways in a mouse model for chronic visceral
157 hypersensitivity, highlighting the potential role for these Mrgprs as drivers of chronic
158 abdominal pain. Furthermore, also MrgprD has been recently linked to visceral nociception.
159 Hockley and colleagues identified, by means of single cell RNA sequencing, a subset of colon-
160 projecting MrgprD-expressing DRG neurons (Hockley et al., 2019). More recently, the

161 Vergnolle/Cenac lab further demonstrated that MrgprD activation in these neurons by the
162 poly-unsaturated fatty acid metabolite 5-oxo-ETE directly causes visceral hypersensitivity,
163 thus pointing to a role for MrgprD in the gut that is similar to that of MrgprA3 and MrgprC11
164 (Bautzova et al., 2018). Interestingly, in the skin innervation, Mrgprs are known to mediate
165 their effects through direct coupling with TRP channels, which raises the question whether
166 the same holds true for Mrgprs in the gut extrinsic innervation (Wilson et al., 2011). A similar
167 downstream coupling of MrgprA3/MrgprC11 with TRP channels was seen in visceral afferents,
168 where Mrgpr-mediated effects on gut pain sensitivity were abolished in mice lacking TRPA1
169 (Castro et al., 2019). As far as MrgprD is concerned, Wang and collaborators (Wang et al.,
170 2019) showed that the MrgprD agonist, β-alanine, has a modulatory effect on TRPA1 channels,
171 in this way eliciting histamine-independent neuropathic itch and pain responses . Although
172 the latter study focused on skin pathways, the many parallels suggest that similar mechanisms
173 might be at play in the gut. Noteworthy is our recent finding that constitutive, basal and β-
174 alanine-mediated activation of MRGPRD induces the release of interleukin-6 (IL-6) and is
175 dependent on the NF-κB signalling pathway (Arora et al., 2021). These findings are also in
176 agreement with other recent data revealing that MRGPRX1/MRGPRD-mediated signalling
177 might be linked to increased production and release of inflammatory cytokines (Reddy and
178 Lerner, 2017). In that study, the authors could show that MRGPRX1-expressing cells, upon
179 activation of the MRGPRX1 receptor, release the pro-inflammatory cytokine IL-6. In another
180 study, De Carvalho Santuchi and colleagues (de Carvalho Santuchi et al., 2019) showed that
181 alamandine-activated mouse MrgprD decreased TNF-α and IL-1β levels in lipopolysaccharide
182 (LPS)-primed macrophages and very recently mouse MrgprD involvement has been suggested
183 in LPS-induced inflammatory pain and in activation of the NF-κB signaling pathway (Lan et al.,
184 2020). Our data therefore lend further support to a role for MrgprD in visceral nociceptive
185 pathways similar to that of MrgprA3 and MrgprC11. Overall, the above findings on mouse
186 Mrgprs and their role in the extrinsic gut innervation strongly suggest a high translational
187 relevance in relation to future treatment strategies for GI disorders with chronic visceral pain
188 as a debilitating feature, such as irritable bowel disease (IBD), comprising ulcerative colitis and
189 Crohn's disease, and irritable bowel syndrome (IBS), all the more so since these two studies
190 of the Timmermans and Brierley labs also confirmed the presence of the human functional
191 orthologue of mouse MrgprA3 and MrgprC11, i.e. MRGPRX1, in human thoracolumbar DRG
192 neurons. Klein and colleagues (Klein et al., 2021) reported a 95% overlap between MRGPRX1

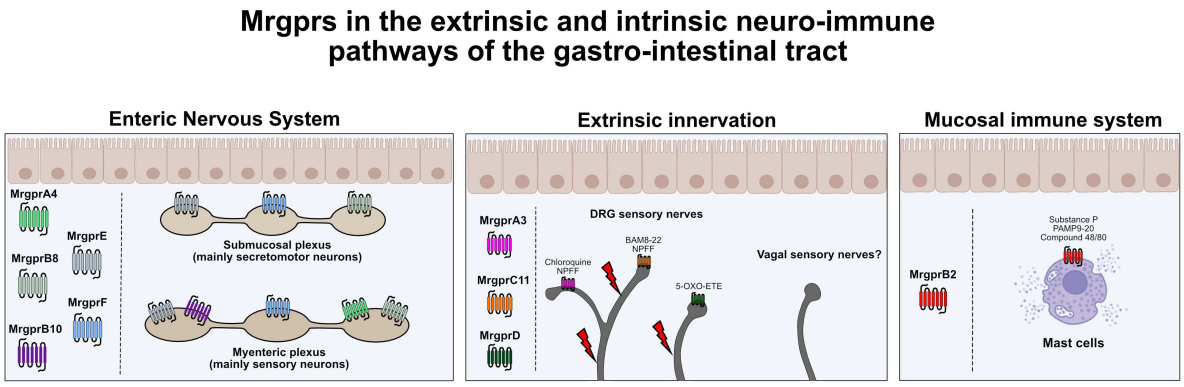
193 and MRGPRD as well as 100% co-expression of the MRGPRX1 neurons with TRPV1 in human
194 DRG. Comparable results were also obtained for macaque DRG, be it that the overlap between
195 MRGPRX1/MRGPRD was somewhat less.
196
197 *2.2.2. Mrgprs in vagal visceral afferents*
198 In contrast to the extensive evidence of Mrgpr involvement in spinal afferent pathways, much
199 less is known about the presence and functional roles of Mrgprs in vagal sensory neurons. Part
200 of the jugular sensory neurons that innervate the mouse airways were found to be MrgprC11-
201 positive, whereas all of the airway-innervating mouse DRG neurons were MrgprC11-negative.
202 These MrgprC11-positive neurons, representing 2% of all vagal sensory neurons, mediate
203 cholinergic bronchoconstriction and play a role in the development of airway
204 hyperresponsiveness in mice (Han et al., 2018). Whether Mrgprs play a prominent role in vagal
205 sensory pathways innervating the GI tract remains to be determined.
206
207 ***2.3 Mrgprs as mediators of the crosstalk between visceral sensory neurons and mast***
208 ***cells***
209
210 MCs participate in a plethora of physiological functions, including blood flow control,
211 homeostasis and anti-microbial defense. Also within the intestinal wall, MCs are in close
212 proximity to components of the ENS. MC mediators can target nerve fibers/neurons leading
213 to overexcitation of sensory pathways and neurotransmitters/neuromodulators released by
214 nerves can act on MCs, leading to the development of neurogenic inflammation (Aguilera-
215 Lizarraga et al., 2022; Green et al., 2019; Pinho-Ribeiro et al., 2017). There is now ample
216 evidence for intense bidirectional communication between enteric neurons and MCs as well
217 as between extrinsic spinal visceral afferents and MCs (Buhner and Schemann, 2012; De Jonge
218 et al., 2004; Van Nassauw et al., 2007; Wood, 2011) and these neuro-immune interactions in
219 the gut have become a highly relevant hot topic in the field of neurogastroenterology. This
220 complex reciprocal crosstalk between enteric neurons and immune cells, which is not limited
221 to MCs but also includes other cell types such as macrophages and innate lymphoid cells, is
222 represented by so-called neuro-immune units. These units are important for maintaining
223 homeostasis in the gut and their dysfunctioning underlies several GI pathologies seen in IBD,
224 IBS, food allergies or parasitic infections (Mischopoulou et al., 2022; Stakenborg et al., 2020;

225 Yashiro et al., 2021). It was not until recently, with the exception of an earlier study by
226 Tatemoto and co-workers (Tatemoto et al., 2006), who pointed to the fact that IgE-
227 independent activation of MCs was mediated by Mrprs - that the neurogastroenterology
228 community started to realize that Mrgprs and their ligands might fulfil an essential mediating
229 role in this neuro-immune crosstalk (Babina et al., 2018; Green et al., 2019; Inclan-Rico et al.,
230 2021; McNeil et al., 2015; Serhan et al., 2021).
231 We showed that Mrgprb10 was expressed *de novo* in mucosal MCs of *Schistosoma mansoni*-
232 infected ileum (Avula et al., 2013) and that *in vitro* mucosal-type bone marrow-derived mast
233 cells (BMDMCs) were found to express *Mrgpra1* and *Mrgpra4* mRNA, but these findings have
234 not been confirmed *in vivo* (Abdellah et al., 2018) . Plum and colleagues (2020) demonstrated
235 MRGPRX2 expression in human gut MCs. Furthermore, adrenomedullin, a precursor of the
236 MRGPRX2 ligand PAMP20, appears to be upregulated in ulcerative colitis patients, indicating
237 a possible role for this receptor in driving aberrant MC activity (Chen et al., 2021). More
238 recently, a growing body of evidence hints at an important role of Mrgprb2/MRGPRX2 in
239 connective tissue MCs. Activation of Mrgprb2/X2 by substances, such as the neuropeptide
240 substance P and antimicrobial peptides (e.g. β-defensins), an avenue that we are currently
241 exploring in various GI models, results in MC degranulation but not exclusively through the
242 classical IgE-mediated pathway as already shown for itch responses. Recently, it has also been
243 reported that *MRGPRX2* mRNA levels are significantly increased in mucosal biopsies of a
244 subset of immune-activated IBS patients (Aguilera-Lizarraga et al., 2019). With regard to the
245 mouse counterpart of MRGPRX2, i.e. Mrgprb2, we are currently using transgenic mouse lines
246 to investigate the expression and role of Mrgprb2 as a novel IgE-independent MC activation
247 pathway in healthy and pathological conditions.
248 In addition to the direct role of Mrgpr signaling in MCs affecting their activity, Zhang and
249 colleagues (Zhang et al., 2021) provided pioneering evidence that Mrgprs might also have an
250 indirect impact on MC functionality. In particular, they showed that MrgprD-mediated
251 signaling in skin sensory neurons leads to glutamine release by these sensory neurons, which
252 directly suppresses MC hyperresponsiveness and inflammatory responses.
253
254 ***3. Mrgprs: novel targets in chronic abdominal pain disorders?***
255 Similar to the established pro-nociceptive role of Mrgprs in the skin, evidence is accumulating
256 that the Mrgpr-mediated signaling seen in gut viscero-sensory pathways probably represents

257 the 'visceral counterpart' of the itch- and pain-related pathways found in the skin. In this
258 respect, Mrgpr-mediated signaling might act as a novel irritant-sensing mechanism in the
259 bowel, which upon activation impacts gut sensory pathways that are designed to expel
260 (potentially) harmful stimuli from the body. As such, it could contribute to the sensory
261 disturbances that are commonly found in GI disorders. This hypothesis is strengthened by the
262 significant increase in gut afferent nerve signaling, seen in a post-inflammatory chronic
263 abdominal pain mouse model that is characterized by gut afferent nerve sensitization and
264 visceral hypersensitivity in the absence of active inflammation, after intracolonic
265 administration of chloroquine and BAM(8-22), the respective ligands for MrgprA3 and
266 MrgprC11 (Brizuela et al., 2021; Castro et al., 2019; Van Remoortel et al., 2019). BAM(8-22), a
267 ligand specifically acting on both MrgprC11 and MRGPRX1, has been reported as a potential
268 cleavage product of the opioid precursor proenkephalin A (PENK) (Lembo et al., 2002). Even
269 though the expression of BAM(8-22) itself has not been directly detected endogenously so far,
270 the expression of its precursor PENK and some PENK-derived opioid peptides have been
271 reported in the myenteric plexus and in colitogenic T-cells of the colonic mucosa (Boué et al.,
272 2014; Denning et al., 2008; Ferri et al., 1988; Porcher et al., 1999). As such, the presence of
273 these BAM(8-22) precursors indirectly reinforces the assumption that this
274 MrgprC11/MRGPRX1 ligand is present in the wall of the intestine as well. Furthermore,
275 MrgprC11 is known to be activated by two FMRF-amide peptides, neuropeptide AF (NPAF)
276 and neuropeptide FF (NPFF), which have been reported to act as mediators in MC-sensory
277 nerve communications (Lee et al., 2008; Lee et al., 2011; Tatemoto et al., 2006). We were able
278 to identify, by means of RT-PCR, possible RF-amide sources in intrinsic and extrinsic
279 components of the ENS, i.e. in DRGs and in enteric neurons and intestinal MCs (Abdellah et
280 al., 2018). Furthermore, immunofluorescent staining with a FMRFamide antibody clearly
281 revealed the presence of FMRFamide in nerve fibers in both the myenteric and submucous
282 plexuses as well as in neuroendocrine cells with typical neuropods. Somewhat surprisingly,
283 co-labeling experiments did not reveal co-staining with CGRP (used as a marker for sensory
284 fibers) despite the presence of FMRFamide-positive DRG neurons, but yielded co-expression
285 with tyrosine hydroxylase, indicative of extrinsic postganglionic sympathetic axons.
286 Interestingly, intracolonic administration of NPAF in healthy mice induces afferent nerve
287 sensitization and visceral hypersensitivity in an Mrgpr-dependent manner (Castro et al., 2019).
288 NPAF and NPFF are associated with pain signaling and inflammation, and thus might act, in

289 association with MrgprC11, as a possible neuro-immune axis on gut afferents (Abdellah et al.,
290 2018; Han et al., 2002; Yang et al., 2008) . In addition to being activated by these RFamide
291 peptides, Mrgprc11 has also been linked to protease-mediated signaling. Established
292 protease-activated receptor 2 (PAR-2) agonists, such as the PAR-2 tethered ligand peptide
293 SLIGRL, and distinct cysteine proteases, including cathepsin S, papain and the house dust mite
294 Der P1 protease, also act as MrgprC11 agonists, thereby inducing effects that were previously
295 considered to be solely PAR2-mediated (Liu et al., 2011; Reddy and Lerner, 2017; Reddy et al.,
296 2015). In the GI tract, several of these MrgprC11/PAR2 ligands have been linked to pain
297 sensation and visceral hypersensitivity. For example, Coelho and co-workers (Coelho et al.,
298 2002) showed that intracolonic administration of the PAR2 agonist SLIGRL induces an
299 inflammatory response and drives visceral hypersensitivity. Moreover, PAR2-mediated
300 visceral hypersensitivity was seen to be accompanied by increased cathepsin S activity and
301 release from macrophages in a mouse colitis model (Cattaruzza et al., 2011) and the house
302 dust mite protease Der P1, present throughout the human GI tract, has been proposed as a
303 potential contributor to intestinal barrier dysfunction in GI disorders such as IBS and IBD (Tulic
304 et al., 2016). At this point, these proteases are mainly thought to exert their effects in the gut
305 through PAR-2-mediated signaling in the visceral afferent pathways, but the emerging role of
306 Mrgprs in the gut afferent innervation adds a new dimension to the current concepts of
307 protease-mediated signaling in the bowel, implying that protease-mediated signaling, which
308 was previously considered to be mediated via PAR-2 only, might also be mediated - at least in
309 part - through Mrgprs.
310
311
312 ***4. Conclusion***
313 Mrgpr research has grown substantially in the past few years and a multitude of recent studies
314 have provided evidence that Mrgprs are important, if not key players in the underlying
315 mechanisms of itch and pain, as well as in initiating and modulating inflammatory/allergic
316 responses not only in the skin, but also in visceral organs such as the respiratory and GI tracts.
317 It has become clear that the GI tract and its innervation in close association with the immune
318 system represent a novel expression site for Mrgprs (Fig. 1). Our lab performed some
319 pioneering work in visualizing and documenting the expression of several murine Mrgpr family
320 members in the intestinal wall, more specifically in the ENS and spinal afferents of the GI tract,

321 and has demonstrated that several of these members undergo marked expressional changes
322 in intestinal inflammatory conditions. More recently, we and others have shown that multiple
323 Mrgprs are also expressed in the gut extrinsic splanchnic innervation, play a direct and/or
324 indirect role in visceral pain (hyper)sensitivity and are involved in interoceptive pathways
325 monitoring this internal organ's status to maintain physiological homeostasis and to generate
326 an internal perception. Considering that the family of Mrgprs still contains many other
327 uncharacterized members and that many of these receptors are still orphan, we assume that
328 only the tip of the iceberg has been exposed and that the largest part of this intriguing and
329 clinically highly relevant story in terms of potential treatment strategies for neuro-immune
330 disorders such as IBD and IBS is still beneath the water surface. Further studies are therefore
331 warranted, and these future efforts should be focused on discovering the upstream drivers of
332 aberrant Mrgpr-mediated signaling in gut sensory pathways in health and disease. Moreover,
333 our and others' efforts are currently aimed at specific Mrgprs in MCs and at their role in
334 hitherto less well understood or undiscovered MC activation pathways.
335
336
337
338 Figure 1:



339

Figure 1: Schematic overview of the expression and cellular location of Mrgprs in the gastrointestinal tract.

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