

Role of mast cells and basophils in pruritus

Martin Steinhoff^{1,2,3,4,5} | Jörg Buddenkotte^{1,2} | Ethan A. Lerner⁶ 

¹Department of Dermatology and Venereology, Hamad Medical Corporation, Doha, Qatar

²Translational Research Institute, Hamad Medical Corporation, Doha, Qatar

³Weill Cornell Medicine-Qatar, Doha, Qatar

⁴Medical School, Qatar University, Doha, Qatar

⁵Department Of Dermatology and UCD Charles Institute for Translational Dermatology, University College Dublin, Dublin, Ireland

⁶Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA

Correspondence

Martin Steinhoff, Department of Dermatology and Venereology, Hamad Medical Corporation, Doha, Qatar.

Email: msteinhoff@hamad.qa

and

Ethan A. Lerner, Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital/Harvard Medical School, Charlestown, MA, USA.

Email: elerner@mgh.harvard.edu

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Summary

To protect our body systems, there is a constant interactive conversation between the skin nervous and immune system. Important elements of this conversation in the skin include mast cells, basophils, and sensory nerve fibers. These cells employ a vast array of sensors that detect danger and react accordingly. This reaction, summarized as neurogenic inflammation, manifests at the conscious level as sensations including pain and itch. Here we provide a perspective on the blossoming knowledge that is illuminating connections between mast cells, basophils, and sensory nerve fibers in the mediation of itch. We discuss established mediators and receptors, in particular cytokine and neuropeptide pathways, upstream proteases, and proteinase-activated receptors, and the emerging role of mas-related G-protein-coupled receptors in itch.

KEYWORDS

atopic dermatitis, itch, mas-related G-protein-coupled receptor, neuron, protease-activated receptor

1 | INTRODUCTION

Mast cells are heterogeneous cells of the innate immune system that reside in connective tissues and concentrate in sites exposed to the external environment. They are found in close anatomical association to myelinated and unmyelinated neural structures, dendritic cells, lymphatic as well as blood vessels, seemingly forming an important

functional regulatory unit that provides the anatomical and physiological foundation to react to insults and to re-establish and maintain homeostasis.^{1–5}

The dominant cutaneous mast cell types are chymase-, tryptase-, and carboxypeptidase-expressing mast cells (MC_{CT}). Mast cells are in close proximity to CGRP⁺ and/or SP⁺ nerves in various mucosal tissues including the gastrointestinal tract, urinary bladder, and skin.^{1,6,7} The presence of anatomical mast cell-nerve units, however, is not limited to normal tissue but is often increased in pathological conditions such as atopic dermatitis (AD),⁸ psoriasis,⁹ irritable bowel syndrome,¹⁰

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allergic rhinitis,¹¹ and neuropathic pain.¹² Placed in this unique position, mast cells function as protective sentinel cells detecting microenvironmental changes important to immunological counter-regulation.

2 | CROSS TALK BETWEEN MAST CELLS AND NERVES

Mast cells interact with various cell types, and the mediators produced by such cells, to generate physiological and immunological response and their modulation to restore homeostasis.⁶ A critical part of this multicellular cross talk includes elucidating details of the now well-established close communication between mast cells and sensory nerves. To coordinate intercellular communication, mast cells exploit a variety of approaches, including differential granule release of preformed intracellular mediators (eg, histamine, tryptase), or de novo products such as neuropeptides, (eg, calcitonin gene-related peptide [CGRP], substance P [SP], endothelin-1 [ET-1], vasoactive intestinal peptide [VIP]) lipid mediators (eg, leukotrienes, prostaglandin D₂), cytokines (eg, tumor necrosis factor- α (TNF α), interleukins, including IL-4, IL-13, IL-31), or chemokines (eg, granulocyte-macrophage colony-stimulating factor [GM-CSF], and CC-chemokine ligand 5 [CCL5]; Table 1).

In contrast, sensory nerves express neuropeptides (eg, CGRP, SP, VIP, pituitary adenylate cyclase-activating polypeptide [PACAP]) and neurotransmitters that facilitate neural-immune communication (Table 1). Both cell types share the expression of cognate receptors to many of the preformed or newly expressed agents. For example, the neuropeptide CGRP released by sensory nerves can activate its mast cell-expressed target receptor CALCRL/RAMP1 (calcitonin receptor-like receptor/receptor activity-modifying protein 1), which in turn could lead to mast cell degranulation of products that could then activate sensory neurons via various receptors. However, mast cells also secrete CGRP that potentially could activate CALCRL/RAMP1 expressed on sensory nerve fibers. This loop of neuro-immune activation and re-activation could potentially present a vicious cycle in dysregulated pathological conditions that feature neurogenic inflammation. The communication between multiple agonist/receptor axes that connect mast cells to nerves indicates the presence of an intricate intercellular communication network. This network includes simple uni-directional information transmission, bidirectional cross talk and possibly a complex multi-loop dialogue.

Mrgprs provide a recently recognized additional link between mast cells and sensory nerves, with expression of these receptors largely limited to these 2 cell types. Mrgpr activation by pruritogens, including SP,¹³ and allergens, is proving to be a fertile area of investigation and is discussed below.

3 | ITCH MEDIATORS AND RECEPTORS WITH LINKS TO MAST CELLS AND BASOPHILS

3.1 | Histamine and serotonin

Itch is generally considered to be histamine-dependent or histamine-independent. While it is now acknowledged that most clinical itches

are predominately independent of histamine, this amine remains the best-known pruritogen and deserving of discussion. Mast cells constitute the main source for histamine although it is also released from basophils and possibly keratinocytes.¹⁴⁻¹⁶ Histamine release is mediated by various exogenous (itch-inducing) stimuli. Histamine accounts at least in part for the itch sensation associated with insect bites,¹⁷ although these require prior sensitization to the arthropod. UV irradiation is also linked to histamine release and maybe to UV triggered itch.¹⁸ Histamine release can also be associated with endogenous mediators (eg, SP, VIP, somatostatin, ET-1).^{19,20} On a molecular level, histamine targets 4 distinct G-protein coupled receptors, H₁R, H₂R, H₃R, and H₄R. The effects of HR activation are dependent upon the receptor subtype and cellular location of its expression (reviewed in 21). Skin innervating sensory neurons express H₁R, H₃R, and H₄R.²² Histamine release can therefore lead to HR-dependent activation of pruriceptive sensory nerves and the release of neuropeptides to the microenvironment where they exert neurogenic inflammation as well as itch-associated flare and wheal (see also under neuropeptides). In particular, H₁- and H₄-receptors play a major role in peripheral histamine-induced itch reactions.²³ H₁-receptor antagonists are highly effective at suppressing itch, wheal and axon-reflex flare in human skin although local erythema remains.^{24,25} H₄-blockage, in contrast, effectively ameliorates histamine-induced itch but does not affect wheal and flare in healthy subjects.²⁶ With respect to clinical effectiveness, H₁R-antagonists are effective in only a few pruritic diseases including some, but not all cases of urticaria, and have not been shown to be effective in many other diseases, especially AD.^{25,27,28} Amelioration of itching often results from the sedative effects of antihistamines.^{27,29} However, recent evidence supports H₄R as a promising pharmacological target for the treatment of inflammatory skin diseases associated with chronic pruritus.³⁰ Feasibility, efficacy, and therapeutic impact of H₄R antagonism remain to be investigated for a plethora of human pruritic diseases. H₂R and H₃R do not appear to have a role in histamine-dependent pruritus. H₃R appears to have a role in sleep and its expression in the central nervous system suggests a possible contribution to the mediation of itch via a central mechanism.³¹ Because histamine is expressed by mast cells and not in sensory nerves, communication of the histamine/HR axis most likely follows a uni-directional pattern from immune cell to the nervous system. The effect of histamine on further HR expressing cell types and tissues has been summarized in recent reviews (eg, Panula et al²¹).

Serotonin (5-Hydroxytryptamine [5-HT]) is a potent vasoconstrictor³² and inflammatory mediator³³ that elicits mild to moderate itch accompanied by wheal and flare in humans when applied intradermally.³⁴⁻³⁸ In mice, the itch response to serotonin is histamine-independent. Serotonin is produced and released by mast cells,³⁹ and also found in melanocytes,⁴⁰ platelets,⁴¹ and neurons of central origin.⁴² The majority of systemic mammal serotonin, however, is synthesized by enterochromaffin cells of the gastrointestinal tract.⁴³ Mast cells are the more important source of serotonin in rodents, whereas serotonin is mostly found absent or expressed in marginal concentrations in human mast cells. However, mast cell serotonin levels can be upregulated in distinct pathologies such as carcinoid tumors

TABLE 1 Mediators and receptors ultimately or potentially involved in pruritus associated with mast cell and/or neurogenic inflammation

Expression				
Mast cell		DRG neuron		
Agonist	Receptor	Agonist	Receptor	Comments
Calcitonin gene-related peptide (CGRP) ⁸⁵	Calcitonin gene-related peptide receptor (CGRPR = CALCRL/ RAMP1) (r) ^{11,87}	CGRP (m) (r) (h) ⁸¹⁻⁸⁴	CGRPR, CNS ⁺⁺ , TRG ⁺⁺ , ⁸⁷ DRG ⁺⁸⁸	Pain transmission → Prolongation of itch latency (preadministered to SP application) → itch inhibition; ⁹⁵ Sensitization of sensory nerves; Vasodilation in arterioles and venules; TNFα release from mast cells and minimal histamine release ¹⁹
Substance P (SP) ⁵⁷	Tachykinin (neurokinin) receptor-1, -2, -3 (NK ₁ R, NK ₂ R, NK ₃ R), ^{11,68} MRGPRX2, MrgprB2	SP (h) ⁸³	NK ₁ R ⁺ , NK ₂ R ⁺ , NK ₃ R ⁺ (?), ^{191,192} MrgprA1 (m), MRGPRX2 ? RNAseq ⁻ , regulation in inflammatory (micro)environment ?	Priming of mast cells; TNFα, histamine, leukotriene B4, and prostaglandin release from mast cells (pruritus-involved substances); Itch via MrgprA1 ⁷³
Endothelin-1 (ET-1), ²⁰ Dependent on IL-4 signaling during mast cell maturation ¹¹²	Endothelin Receptor A (ETAR), ²⁰ Endothelin Receptor B (ETBR) (e.n.r.)	Peripheral expression for ET-1 is not reported	ETAR (m) (r) (h) ^{102,193,194}	Burning itch, vastly histamine-independent Inflammation; Mediates Cathepsin G-induced itch; ¹⁹⁵ ET-1 degranulates mast cells (eg, histamine, leukotriene C ₄ , serotonin); ^{112,113} ETAR (in)dependent, release of mast cell-TNFα and IL-6; Maturation of big ET-1 by chymase; ¹⁹⁶ ET-1 degradation by chymase ¹⁹⁷ and ECE-1 ¹⁰²
Gastrin-releasing peptide (GRP) (e.n.r.)	Gastrin-releasing peptide receptor (GRPR) /Bombesin receptor BB ₂ (BB ₂) (m) ? (e) ¹⁹⁸ (n.e.) ¹⁹⁹	GRP (m) ? (n.e.) ²⁰⁰⁻²⁰² (e) ²⁰³⁻²⁰⁵	BB ₂ /GRPR (m) (r) (h) (n.e.) ²⁰⁰	Peripheral role in itch not fully elucidated, central itch mediator; Mast cell degranulation; ¹⁹⁸ AD phenotype dependent on IL-22 signaling; ²⁰⁵ Epidermal GRP source, enhanced in murine atopic-like dermatitis ²⁰⁵
Pituitary adenylate cyclase-activating polypeptide (PACAP): (e.n.d.) ²⁰⁶ Vasoactive intestinal peptide (VIP) (e.n.r)	VPAC1R +/- (h) ^{68,206} VPAC2R (h) ^{68,207} AD ↓ IgE ↑ PAC1R (e.n.r.)	PACAP (h) ^{206,208} VIP ⁺ (h) ²⁰⁸ VIP ⁻ (h) ^{191,209}	PAC1R Ciliary ganglion (CG) ²¹⁰ VPAC1R, VPAC2R, PAC1R ?	In vitro: PACAP can activate mast cells via direct G-protein activation; ²¹¹ Release of histamine and serotonin; Involved in vasodilation and flush; Not involved in pruritus (own observation)
Substance P, ⁵⁷ Mucunain, ¹⁸⁶ Drugs (eg, Vancomycin)	MRGPRX2, ^{61,186} MrgprB2	SP, VIP, cortistatin (GABAergic interneurons)	MRGPRX2, ^{187,212} MRGPRX1, ? MrgprA1	Mast cell degranulation; Itch, cowhedge-induced itch; ¹⁸⁶ Pseudo-allergic drug reactions, eg, red man syndrome and staphylococcal delta toxin associated with AD ^{187,188}
Tumor necrosis factor-α (TNFα)	TNFR1, TNFR2-(e.n.r.)	TNFα (r) (n.e.) ²¹³	TNFR1 (CD120a) (m) ^{171,214} (r), ²¹³ TNFR2 (m) ²¹⁵ (r) ²¹³	TNFα triggers tryptase release from MCs; ¹⁵⁶ Mast cell-derived TNFα primes sensory nerves; Degraded by chymase ²¹⁶
Interleukin-4 (IL-4) (m) ²¹⁷ (r) ²¹⁸ (h), ¹⁶⁰ Interleukin-13 (IL-13) (h) ^{161,219} (m) ^{220,221}	IL-4Rα (h), ¹⁶⁶ L-13α1 (h), ²²² IL-13α2, ²²³ Human mast cell line-1 (HMC-1)	IL-4 (e.n.r.)	IL-4Rα (h) (m) ¹⁷⁰ (and own observation), IL-13α1 (m) (h), ¹⁷⁰ IL-5Rα not expressed ¹⁷⁰	Sensitizer of histamine-dependent itch, AD concomitant itch in mice; Possibly involved in chronic (idiopathic) itch via JAK1 activation in humans; ¹⁷⁰ IL-13 is degraded by chymase ²¹⁶

(Continues)

TABLE 1 (Continued)

Expression				
Mast cell		DRG neuron		
Agonist	Receptor	Agonist	Receptor	Comments
Interleukin-31 (h), ^{162,224} Oncostatin M (h) ¹⁶³	IL-31R α , OSMR (e.n.r.) but anticipated	IL-31 (e.n.r.)	IL-31R α (m) ¹⁶⁸ (h), ²²⁵ OSMR (m) ¹⁶⁹	Delayed onset of itch in humans; ¹⁷⁵ Involved in chronic itch of AD patients; ²²⁶ OSM treatment initiates mast cell growth ²²⁷
Histamine ¹⁴	Histamine receptor H1 (H ₁ R), H ₂ R, H ₄ R ²²⁸	Histamine (n.e.)	H ₁ R, H ₄ R ¹⁰⁹	Histamine induces itch in humans (dependent on administration route); H ₁ R and H ₂ R antagonism alleviate itch; In mice, H ₃ R antagonists induce itch; Induction of vasodilation and plasma extravasation
Serotonin (m) (r) ↑↑↑ (h) -/+, ^{12,39,45,46,229} Regulated expression in pathophysiologic conditions; Prerequisite for serotonin production: Tryptophan hydroxylase 1 (m) (h) ⁴⁶	5-HT _{1A} , ^{51,230} MRGPRX2 putative serotonin receptor	Serotonin is expressed in the central rather than peripheral nervous system ⁴²	5-HT _{1B} , 5-HT _{1D} , 5-HT _{1F} , 5-HT _{2A} , 5-HT ₃ , 5-HT ₄ , 5-HT _{5B} and 5-HT ₇ receptor subtypes, ^{37,52} MRGPRX2 ? RNAseq ⁻ , regulation in inflammatory (micro)environment ?, MRGPRX1 ?	SP, CGRP, VIP, somatostatin trigger serotonin release from MCs; ⁴⁷ Serotonin evokes mild to moderate histamine-independent itch in humans; Serotonin-mediated wheal reaction is sensitive to H ₁ R antagonism in human skin
Tryptase (Granular Content), ¹¹ Synthetic PAR-activating peptides (eg, SLIGRL)	PAR ₁ , PAR ₂ , PAR ₄ ^{136,231} MrgprC11	Tryptase (e.n.r.)	PAR ₂ ^{1,137}	Tryptase induces neurogenic inflammation and itch via PAR ₂ ; PDE ₄ inhibition ameliorates PAR ₂ -agonistic itch; ²³² Attenuates vasodilatory function of CGRP; Tryptase secretion from mast cells is eg, triggered by NGF; Tryptase matures NGF from pro-NGF; ²³³ Tryptase degrades VIP and CGRP, but not SP; ¹⁴⁹ TRPV1 and TRPA1 cross-activation ^{234,235}
Chymase (Granular Content) ¹¹	Degrades numerous extracellular substances, eg, ET-1	Chymase (e.n.r.)	Degrades numerous extracellular substances	Chymase degrades inflammatory, pruritic and anti-pruritic peptides, thereby counterbalances their impact; Degrades neuropeptides ET-1 ¹¹⁹ SP and VIP ¹⁴⁹ and cytokines IL-5, IL-6, TNF α ; ²¹⁶ Chymase-dependent conversion/activation of big ET-1 ²³⁶
Opioids, proopiomelanocortin (POMC) peptides ²³⁷	Opioid receptors, ^{121,122} Opioid receptor-independent MC activation via MRGPRX2, Direct G-protein activation, Others ?, μ , κ , δ -opioid receptors are absent in mononuclear cells ¹⁰⁹		μ -opioid receptors (h) ¹²⁰ μ -, κ -, δ -opioid receptor (r), ¹²³ MRGPRX2 ?	μ -opioid antagonism has central anti-pruritic effect Downregulation of κ -opioid in atopic dermatitis; ¹²⁶ Intradermal morphine evokes itch Enkephalins and β -endorphins intensify histamine-induced itch Methionine-enkephalin triggers histamine-sensitive flare reaction ¹⁰⁹ Morphine suppresses TNF α exocytosis ¹²¹

(Continues)

TABLE 1 (Continued)

Expression				
Mast cell		DRG neuron		Comments
Agonist	Receptor	Agonist	Receptor	
Endovanilloids (heat, acidosis, eicosanoids, histamine, bradykinin, extracellular ATP, Prostaglandins, neutrophins), Drugs (?) ²³⁸	TRPV1 (m) (r) (h) ^{239,240} TRPV2 (m) ²³⁹ TRPV4 (m) ²³⁹	Physical stimuli, Drugs (?) ²³⁸	TRPV1 (h), TRPV2 (h), ²⁴¹ TRPV4 (m) ²⁴²	TRPV1 is involved in itch induction; Agonist capsaicin depletes neuropeptides from sensory nerves, thereby long-term anti-pruritic effects, interrupts sensory nerve-MC communication; TRPV2 is not described in itch-mediating mechanisms; TRPV4 regulates serotonin-evoked itch
Prostaglandins (eg, PGD ₂)	Prostanoid receptors, eg, cysteinyl leukotriene receptor 1 (CysLT1R) and E-prostanoid receptor (EP) ₃ ²⁴³	Prostaglandin ⁺ (eg, PGE ₂) ²⁴⁴	Prostanoid receptors (eg, E-type prostanoid receptor) ²⁴⁵	PGE ₂ induces cutaneous vasodilation, pain, less itch in humans; ²⁴⁶ PGD ₂ inhibits IgE-mediated scratching ²⁴⁷

e, expressed; e.n.d., expression not detected; e.n.r., expression not reported; h, human; m, mouse; n.e., not expressed; r, rat; ?, contradictory reports; ↓-↑↑↑, grading of expression.

or mastocytosis.^{39,44-46} In the intestine-associated mesentery, the release of serotonin from mast cells is at least in parts under control of pruritogenic SP/CGRP/VIP-positive nerve fibers closely associated to mast cells.⁴⁷ This microanatomical association is also probable in human skin tissue but not established. Serotonin is upregulated in lesional skin of pruritic dermatoses including AD,⁴⁸ psoriasis,⁴⁹ and contact dermatitis⁵⁰ consistent with an as yet undefined role of serotonin in the pathogenesis of these conditions. Numerous 5-HT-receptors exist, of which mast cells express the 5-HT_{1A} receptor subtype and appears to be upregulated in the dermis of lesional AD skin.⁵¹ Sensory nerves in rodents express 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2A}, 5-HT₃, 5-HT₄, 5-HT_{5B}, and 5-HT₇ receptor subtypes.^{37,52} To date, only a few studies have been directed toward the identification of the precise 5-HT receptor subtype(s) acting as pruriceptor(s). Based on murine models 5-HT_{2A} has been suggested to convey the pruritic serotonin effect.^{53,54} A more recent study, however, suggests that 5-HT₇ is the key mediator of serotonergic chronic and acute itch and is dependent on the ion channel TRPA1⁵⁵ and probably TRPV4.³⁷ Because serotonin-mediated itch is not completely abrogated in *Htr7*^{-/-} mice, it is unlikely that the receptor represents the sole serotonergic pruriceptor in vivo.⁵⁵ Consistently, a 5-HT₂ agonist induces itch in mice independent of TRPA1 and 5-HT₇ receptor function.^{37,55} The identity of the receptors which convey serotonin-mediated itch in humans is not clear. Neither 5-HT₂ nor 5-HT₇ receptor antagonists have been evaluated for anti-pruritic capacity in humans. Also, although serotonin is a cationic neurotransmitter, a link to pruritogen receptors of the Mrgpr family has not been described.

The cutaneous serotonin reactions of wheal and flare present hallmarks of a partly histamine-dependent mechanisms, consistent with mast cell activation. H₁R-antagonism abolishes serotonin-induced wheals in healthy subjects without impacting serotonin-evoked itch.

These findings support a distinct pruritic mode of action for serotonin, while mast cell function facilitates the generation of serotonin-initiated wheals.³⁴ It is not known whether mast cells serve as the cellular source for serotonin in selected pruritic diseases. Further research is needed to determine the molecular links between the serotonergic system, mast cell, and pruritic skin disease.

3.2 | Neuropeptides

Neuropeptides represent a heterogeneous class of small peptides associated with and produced predominantly but not exclusively by neural tissues. They act as signaling molecules within the microenvironment of release and can regulate functions of the central nervous system and in the peripheral, their effects extend beyond nerve. Neuropeptides can also be expressed and secreted by immune and epithelial cells. The range of their function is diverse, including analgesia, itching, inflammation, learning, memory, and behavior. Here we address neuropeptides involved in mast cell/sensory nerve communication including substance P, CGRP, and ET-1 and their impact on neurogenic inflammation and itch (see subchapters and Table 1).

Substance P (SP), the first member of the tachykinin family of peptides to be identified, and its conventional receptor NK₁R (neurokinin 1 receptor) are localized with multiple additional neuropeptides in dorsal root ganglia sensory neurons (30%-50%), basophils,⁵⁶ and mast cells.⁵⁷ SP-positive nerve fibers are found in the dermis and epidermis and innervate the dermal vasculature, keratinocytes, dendritic cells, basophils, and mast cells.⁵⁸ Many exogenous and endogenous stimuli can initiate the release of SP from mast cells, basophils, and cutaneous sensory nerves. These stimuli include heat, scratching, and inflammatory mediators such as bradykinin, prostaglandin, proteases, and cytokines.⁵⁹ Other mediators, for example, pituitary adenylate

cyclase-activating polypeptide-38, inhibit tachykinin release from cutaneous sensory nerves, thereby promoting anti-inflammatory effects.⁶⁰ When SP is released from peripheral nerve endings, this tachykinin can generate neurogenic inflammation by initiating mast cell degranulation, arteriolar dilatation, plasma extravasation and immune cell infiltration from postcapillary venules.⁵⁹

Although the (patho)physiological SP-function was thought to be mediated by its cognate NK receptors, an alternative route via MRGPRX2 has been established very recently in mast cells.⁶¹ SP-positive cutaneous sensory nerves are closely associated with mast cells enabling bidirectional communication between the immune cells and primary sensory neurons by sensory neuropeptide stimulation of the immune cells, and immune cell products controlling neuropeptide release from sensory nerves. Neuronal SP can rapidly activate mast cells to secrete small and predominantly spherical granule structures^{62,63} that can contain histamine,⁶⁴ TNF- α ,⁶⁵ leukotriene B₄,⁶⁶ or vascular endothelial growth factor (VEGF),⁶⁷ mediators that contribute to inflammation, immunity, and angiogenesis. SP neither affects expression of the inflammatory cytokines IL-4, IL-5, IL-6, and IL-13 in skin mast cells nor their release.⁶⁴ However, substance P can initiate the release of IL-3 and IL-8, and various chemokines (eg, monocyte chemoattractant protein-1 [MCP-1], CCL-5).^{68,69} The significance of SP in inflammatory processes is further reflected by its increased expression in lesional skin of patients with AD,^{70,71} which concomitantly present elevated numbers of SP/NK₁R⁺ mast cells and basophils.^{57,72} Additionally, the chronic itching routinely observed in AD patients has been associated with SP, but not with mast cell function. The acute pruritic effects of the SP, at least in rodents, is now attributed to neuronal MrgprA1 activation rather than NK₁R,⁷³ whereas in humans the peripheral receptor(s) that mediate substance P in itch is uncertain. Of note, the route of administration of molecules associated with nociception is critical. Thus, itch is not observed when SP is administered via microdialysis,^{74,75} perhaps because it is being delivered to the dermis. However, when administered via inactivated cowhage spicules to the epidermis or superficial dermis, SP evokes itch (EAL, personal communication), a finding analogous to capsaicin evoking itch rather than pain when administered via spicules.⁷⁶

Neurokinin 1 receptor antagonism with aprepitant can lead to a reduction of itch intensity in patients suffering from chronic pruritus.⁷⁷ Whether the effect of the drug is central, peripheral or both, remains to be determined as the effect of topical aprepitant was matched by the vehicle.⁷⁸ Additional NK₁R antagonists are now being evaluated and early clinical reports from industry, although not peer-reviewed, are promising. Recent findings further implicate an important role for SP and its cognate receptor in the pathophysiology of allergic contact dermatitis where mast cell function is involved. During perennial allergic rhinitis, NK₁/NK₂R⁺ mast cells are increased leading to an enhanced association of mast cells with neural projections.¹¹ Finally, the classical "mast cell disorder" chronic spontaneous urticaria is associated with increased accumulation of SP/NK₁R⁺ basophils.⁵⁶

Convincing evidence for neuropeptide release from peripheral nerve terminals, a prerequisite for physiological function, is available for only a limited number of neuropeptides, notably SP and calcitonin

gene-related peptide (CGRP). The CGRP peptides include CGRP α and CGRP β .^{59,79-84} CGRP α is found in mast cells,⁸⁵ but not in basophils, while keratinocytes produce the β form.⁸⁶ CGRP targets and activates the CGRP receptor (CGRP_R), a heterogeneous receptor dimer consisting of calcitonin receptor-like receptor (CALCRL or CRLR) and receptor activity-modifying protein class 1 (RAMP1). Functional CGRP_R expression has been demonstrated for mast cells^{11,87} and trigeminal root ganglia neurons and in low levels for sensory DRG neurons.^{87,88} CGRP is not a pruritogen but it is a potent vasodilator. CGRP regulates nociception⁸⁴ and orchestrates multiple functions in the vicinity of its release. Multiple studies have described, both, anti- as well as pro- inflammatory immune responses in the aftermath of neuronal CGRP release. CGRP contributes to neurogenic inflammation by promoting vessel dilatation and erythema⁸⁹ but suppresses edema formation in a serotonin-mediated mouse model when applied intradermally.⁹⁰ Anti-inflammatory effects of cutaneous CGRP include inhibition of Langerhans cells and absent response to T_H1 haptens, while responses to T_H2 haptens are augmented.⁹¹ With regard to its function on mast cells, multiple divergent reports have been published. An anti-inflammatory effect for CGRP on mast cells by preventing degranulation in inflammatory milieu has been reported,⁹² while others do not find a mast cell-activating effect for CGRP.⁶⁸ There is also a report of CGRP-induced mast cell degranulation and subsequent DRG neuron activation which would indicate an important bidirectional cross talk between mast cells and afferent neurons.⁹³ Another group hints that CGRP induces epithelial cell migration and proliferation via transforming growth factor release from mast cells.⁹⁴ The precise relationship of CGRP and mast cells might not be uniformly formulated and is probably dependent on multiple factors such as tissue-dependent mast cell identity and the microenvironmental milieu. However, when applied intradermally to healthy subjects, CGRP evokes a flare reaction without wheal formation.⁷⁰ When followed by intradermal histamine injection, CGRP prevents the histamine-induced wheal without altering the flare reaction supporting functional interplay of the CGRP system and mast cell products *in vivo*.^{90,95}

In AD patients, CGRP-expressing sensory neurons are increased in lesional skin.⁹⁶ Enhanced plasma CGRP levels and keratinocyte-derived CGRP_R component RAMP1 have been found.⁹⁷ Despite providing proliferative input for keratinocytes,⁹⁸ the detailed roles of CGRP/CGRP_R in the epidermal compartment as well as in AD pathophysiology are not clear. While CGRP does not evoke itch, genetic ablation of CGRP α -expressing sensory neurons reduces sensitivity to histamine-mediated scratching in mice.⁹⁹ Additionally, itch transmission via TRPV1-positive neurons demonstrates CGRP dependence.¹⁰⁰

We have recently delineated central parts of the intracellular mechanism that engage in the pruritic function of the vasoconstrictor endothelin-1 (ET-1),¹⁰¹ the parent compound of the endothelin family of peptides.¹⁰² It has been firmly established previously that regulatory ET-1 peptide released by cutaneous sensory neurons, mast cells, or skin cells represents an important mediator involved in pruritus as well as in inflammation and pain^{20,103-107} (and reviewed in 108, 109). ET-1 is synthesized in a 3-step process from pre-pro-ET-1 toward the mature biologically active ET-1 mainly by enzymatic shaping through endothelin-converting enzyme-1 (ECE-1). Mature ET-1 can finally

activate 2 distinct GPCRs, endothelin receptor A (ETAR) and endothelin receptor B (ETBR) (for detailed information see Davenport et al¹¹⁰). The functional components of the ET-1 signaling complex are fully expressed in murine and human sensory DRG neurons and are complete in mast cells, suggesting autocrine-paracrine interactions, and are up-regulated in pathological skin conditions such as prurigo nodularis and AD.^{20,102,111} The capability of mast cells to produce and release ET-1, however, might be critically dependent on proper IL-4 signaling during mast cell maturation.¹¹² ET-1 induces a rapid release of mast cell mediators in pattern that is comparable to that associated with SP-activation. Like SP, ET-1 leads to secretion of small predominately spherical granule structures⁶² that could contain histamine, serotonin or pro-inflammatory agents such as IL-6, leukotriene C₄, TNF α , VEGF, or transforming growth factor- β (TGF β) that can drive cutaneous inflammation.^{113–117} The precise characteristics of mast cell-mediated cutaneous inflammation are dependent on the nature of the primary mast cell activation stimulus.⁶²

In human subjects, intradermal application of ET-1 induces local erythema that is comparable to that induced by histamine but without significant wheal or axon-reflex flare.¹⁰² Also, ET-1-mediated pruritus is only minimally driven by ET-1 triggered histamine release in mice^{107,118} indicating a direct role of neuronally expressed ETAR in itch. This has been confirmed in a study of ours in which we find that ET-1 induces only a very short pain response but a marked itch induction in human volunteers, indicating a direct role of ET-1 in human itch at nanomolar levels.¹⁰² The role of ET-1-dependent serotonin release from mast cells in ET-1-mediated itch remains to be investigated. In contrast, ET-1-initiated secretion of IL-6, TNF α , TGF β , and VEGF is involved in inflammatory angiogenesis.¹¹⁷ In fact, the biological impact of uncontrolled ET-1 expression can be deleterious. Mast cell-deficient mice develop hypothermia, diarrhea, and present a markedly elevated death rate when ET-1 is systemically administered. In contrast, wildtype mice demonstrate a mast cell-dependent protection from ET-1 toxicity.^{20,119} Maurer et al identified ETAR-dependent mast cell degranulation of chymase as the main step in resistance to pathophysiological ET-1 function. The released chymase effectively counterbalances malevolent ET-1 abundance by proteolytic degradation of the neuropeptide.¹¹⁹ Our understanding of the need of tight regulation of ET-1 function in order to prevent harmful ET-1/ETAR function in humans has grown over the years and mast cells are central in this task by providing ET-1-degrading components (ECE-1/2, chymase) to restore tissue homeostasis. Given that inhibition of the ET-1/ETAR system achieves beneficial effects in vivo,^{102,119} it will be critical to further study the impact of ETAR or ET-1 blockers on the treatment of therapy-resistant itch and in distinct mast cell-dependent pathologies. ECE-1 inhibition, however, that would partly block maturation of the ET-1-precursor, could also result in enhanced itching¹⁰² and needs to be characterized with regard to its drug target properties dependent on the particular disease pathophysiology in detail.

3.3 | Endogenous opioids and their receptors

Endogenous opioids are peptidergic neurotransmitters known primarily for their analgesic effects although their effects are much broader.

More than 20 opioids have been identified and these are grouped into 3 classes, the endorphins, enkephalins, and dynorphins.¹⁰⁹ Pre-opioids are converted to their active forms following proteolytical processing. These then activate their respective opioid receptor subtypes including μ (MOR), δ , or κ (KOR). The expression and tissue distribution of opioid receptors includes the central and peripheral nervous system but expression in peripheral non-neural tissues has been reported.^{120–122} In cutaneous sensory nerves, opioid receptors mainly co-localize to preprotachykinin-containing fibers and, indeed, opioids, tightly regulate the release of SP, thus underlining their analgesic function in the periphery.¹²³ Less is known about the role of the opioid system in peripheral itch or mast cell-dependent pruritus. It has been reported that intradermal application of morphine induces pruritus and low doses of enkephalins and β -endorphins can intensify histamine-induced pruritus while single doses of the latter opioids are ineffective.^{124,125} Opioid receptors might play a role in the pathophysiology of pruritic skin disorders, based on the observation that the cutaneous κ opioid system, but not the μ opioid system, is downregulated in AD patients and restored under effective AD therapy.¹²⁶ To understand how the mast cells and the opioid-complex interconnect in histamine-mediated pruritus might help to predict the role of opioids in the pathogenesis of other pruritic diseases. However, the severe itch experienced by patients along with μ -opioid agonist treatment (eg, morphine, codeine) is very likely attributed to a response at the level of the spinal cord and is independent of peripheral contributions.^{127,128}

In contrast to analgesia, the impact of opioids on cutaneous neurogenic inflammation is gaining traction. For example, inflammation results in a marked upregulation of opioid peptides in the skin, probably as a direct counterbalance reaction to inflammation.¹²⁹ In turn, cutaneous application of methionine-enkephalin induces a flare reaction that is sensitive to antihistamines, indicating a histamine-dependent and -independent involvement in neurogenic inflammation.¹³⁰ Furthermore, opioid mediators are known for their capacity to cause mast cell degranulation that can lead to histamine-driven urticaria or anaphylactic reactions.^{122,131–133} Recent findings indicate that MRGPRX2 (and a mouse ortholog MrgprB2) might be the essential receptor for the transmission of opioid function in human skin mast cells^{134,135} and is discussed further, below. An involvement for direct G-protein activation by opioids has also been reported. Thus, codeine activates human mast cell chemokine (CCL2) and cytokine (IL-8) production and secretion by activating protein kinase A and PI3 kinase.¹³³ The particular involvement of mast cell MRGPRX2 and the “classical” mast cell-expressed opioid receptor system, respectively, in opioid-induced itch and neurogenic inflammation is under active investigation. At least in human skin, opioid-induced mast cell activation and vascular responses appear not to be mediated by μ -opioid receptors.¹³¹

3.4 | Proteases and PARs

Tryptase-containing mast cells are closely associated with proteinase-activated receptor-2 (PAR₂)-expressing C-fibers during itch and inflammation.^{113,136,137} The serine protease tryptase, like cathepsin G and

trypsin, is capable of proteolytically activate neuronal PAR₂, thereby inducing widespread neurogenic inflammation that is characterized by vasodilation (erythema), plasma extravasation (edema), influx of immune cells, and activation of a network of cytokines.^{1,109,138,139} At the same time, these proteases can inactivate PARs.

Mast cells themselves express the PAR family members PAR₁ and PAR₂ not only on the plasma membrane but also on the membrane of intracellular tryptase-positive granules, indicating a potential autocrine regulation of tryptase-mediated cutaneous inflammation and itch via PAR₂ activation.^{136,140} This observation may be consistent with the increasing appreciation of endosomal signaling by GPCRs.¹⁴¹

Proteinase-activated receptor-2-positive sensory neurons overlap with peptidergic CGRP and SP neurons and these neuropeptides,¹ which can be released upon PAR₂ activation via mast cell or basophil-derived tryptase, as well as keratinocyte-derived kallikreins or trypsins, or cathepsin S. The PAR₂-mediated release of neuronal CGRP and SP subsequently generates neurogenic inflammation or itch, and might be involved in additional mast cell activation. Tryptase from degranulating mast cells can degrade CGRP and terminate its neurogenic (also see sub-chapter CGRP and SP) inflammatory effects.

Activation of neuronal PAR₂ indeed leads to nociceptive responses, including pruritus in human skin.¹³⁷ How much is pure itch vs pricking, stinging, burning, or pain may depend upon the relative dermal and epidermal location of the PAR activation. The pruritic capability of tryptase has been known for a long time and might be mediated—at least in part—by activation of PAR-expressing sensory neurons.¹⁴² Thus, mast cells and PAR₂ are involved in pruritus of AD patients, which is independent of histamine.^{137,143,144} However, AD patients demonstrate an upregulation of tryptase, underlining a potential role for mast cell-driven PAR₂-signaling in AD.¹³⁷ These results in humans have been confirmed in multiple pruritic mouse models.^{142,144–146} Interestingly, nerve growth factor (NGF) efficiently releases preformed histamine as well as tryptase from mast cells, and is upregulated in AD.^{147,148} Therefore, NGF-mediated release of PAR₂-activating tryptase could present an early step in a PAR-complex-driven induction of AD. Mast cell-derived chymase, however, predominantly appears to counteract inflammatory and pruritic signals. It terminates the effects of the neurogenic skin inflammation mediator SP¹⁴⁹ and counterregulates pruritic signals such as ET-1 by degradation.¹¹⁹ Chymase underlies regulation in AD and was found decreased in activity in lesional skin mast cells.¹⁵⁰

Proteases such as tryptase released by mast cells or trypsins secreted by keratinocytes have thus been identified as key regulators of neuro-immune communication and play an essential role in skin inflammation^{1,136,151,152} and pruritus.¹³⁷ Serine proteases activate PAR₂ and PAR₄. In 2003, PAR₂ has been identified as the first histamine-independent itch pathway receptor in humans.¹ Notably, while histamine concentration in the skin did not differ between AD patients and healthy controls after stimulation of mast cells, the concentration of tryptase was significantly higher in AD patients consistent with the tryptase/PAR₂ pathway contributing to the pathophysiology of AD. Subsequently, the PAR₂ activating serine proteases squamous cell tryptic enzyme (SCTE), matriptase, prostatic, trypsin-4, and kallikreins

have been validated as players in innate and adaptive immunity, skin barrier dysfunction and pruritus, all of which is dysregulated in AD.^{139,153–155} Thus, akin to Toll-like receptors, PAR₂ may be an important upstream contributor to inflammation, skin barrier dysfunction, and itch in AD patients by regulating mast cells, keratinocytes, macrophages, T cells, B cells, and nerves. It follows that topical or systemic protease inhibitors or PAR₂ antagonists may be of benefit in the treatment of the pathophysiological pathways responsible for AD.

3.5 | Cytokines and cytokine receptors

Cytokines, including TNF α ,¹⁵⁶ IL-33,¹⁵⁷ can activate mast cells while IL-10 and TGF β ¹⁵⁸ can antagonize mast cell activation.¹⁵⁹ Mast cells are also a source of cytokines. They express T_H2-type cytokines IL-4, IL-13, IL-31, as well as—among others—oncostatin M (OSM) and TNF α , depending on the tissue, environment, and activation status.^{160–164} Concentration and binding affinity of allergens to the Fc epsilon receptor I (Fc ϵ RI), for example, skew mast cells toward a general secretion pattern of predominantly chemokines or cytokines, respectively. Furthermore, human CD300 receptor family member activation can lead to secretion of cytokines (CD300c) or inhibition of cytokine release (CD300a).¹⁵⁹ Also classical pro-inflammatory mediators such as lipopolysaccharide directly release cytokines from mast cells, in this case via Toll-like receptor (TLR)-mediated release of TNF α , IL-4 to -6, and IL-13.¹⁶⁵ Because human mast cells express cytokine family receptors IL-4R α ¹⁶⁶ and IL-13 α 1¹⁶⁷ themselves, an autocrine regulation of cutaneous inflammation might exist. Sensory neurons have been found to be positive for the heterodimer IL-31 target receptor IL-31R α /OSMR,^{168,169} the heterodimer IL-4, and the IL-13 target receptor IL-4R α /IL-13 α 1,¹⁷⁰ and TNF α target receptor TNFR1 as well as TNFR2. Together, these observations indicate a complex neuronal regulation by mast cell cytokine products. In fact, mast cell-derived TNF α primes sensory neurons in an inflammatory vascular hyperpermeability response in mice.¹⁷¹

During neuronal induced cutaneous inflammation, the neuropeptides CGRP, VIP, and SP capably initiate Fc ϵ RI-independent mast cell degranulation of histamine,¹⁹ eicosanoids,¹⁷² chemokines such as MCP-1 and CCL5, as well as cytokines such as TNF α , IL-3, and eotaxin.⁶⁸ These mediators can subsequently recruit and activate multiple target cells, such as T-lymphocytes, neutrophils, and eosinophils. However, the cytokines released by activated mast cells can be released selectively dependent on the primary activating agonist and, by means of interactive neuro-immune communication, activate neuronal cytokine receptors, and lead to sustained neurogenic inflammation. As an example, activation of Toll-like receptors on rodent mast cells leads to selective release of cytokines including TNF α , IL-4, IL-6, and IL-13.¹⁶⁵ While overexpression of either mast cell-expressed cytokines IL-4, IL-13, or IL-31 appears to be sufficient for the development of atopic dermatitis in genetically modified mice,¹⁷³ it is acknowledged that the relevance of such overexpression to a specific cutaneous inflammatory condition in humans is speculative. Recent prominent findings indicate that IL-4/IL-13 signaling via IL-4R α is closely associated with AD and development of chronic itch.^{170,174}

Our understanding of the role and mechanism of the IL-31/IL31R α axis in AD pathophysiology, and itch onset in humans is increasing. Careful observation reveals a potent but delayed pruritic response to intradermally injected IL-31 in healthy and AD-affected subjects.¹⁷⁵ Overexpression of IL-31 in transgenic mice results in a severe pruritic phenotype and skin lesions¹⁷⁶ similar to those observed in human AD patients. The contribution of mast cells to IL-4-, IL-13-, and IL-31-mediated itch and their distinct or overlapping roles in AD onset and perpetuation will benefit from further investigation. Furthermore, thymic stromal lymphopoietin (TSLP), a cytokine considered to be important in the atopic march from AD to asthma, interacts with mast cells and sensory neurons express receptors for TSLP although a direct role for TSLP in human itch has not been established.¹⁷⁷

3.6 | Mrgprs on mast cells and sensory nerves

We now turn to a discussion that has informed our approach to the role of Mrgprs in itch. Mrgprs are involved in itch that is independent of histamine. Mrgprs may thus be involved in most clinical itches. The range of mediators and selected aspects of receptors and channels associated with the links between pruritus, mast cells, and sensory neurons has been discussed above. While numerous mediators capable of evoking itch have been identified, the specific mediators and relevant receptors of chronic itch have yet to be defined. Most studies in mice and humans are in the context of acute itch and all studies published to date that implicate Mrgprs are in acute itch models.

This family of orphan GPCRs was identified early in this century by an academic group led by Xinzhong Dong now at Johns Hopkins¹⁷⁸ and a pharmaceutical group led by Paola Lembo and Sultan Ahmad, both at Astra Zeneca at that time.¹⁷⁹ The groups referred to the receptors as Mrgprs and sensory neuron-specific GPCRs, respectively. A number of subclasses of these receptors exist. In mice, these are referred to primarily as A, B, C, and D, while in humans they are X and D. Specific receptors are called, for example, MrgprA1 in mice and MRGPRX1 in humans. The tissue distribution of Mrgprs tends to be limited to mast cells and neurons, primarily sensory neurons, consistent with a role in sensing and nociception. A role for Mrgprs in pruritus was first suggested in 2009.¹⁸⁰

Two of the authors (EAL and MS) have had long-standing overlapping interests in neuropeptides and their receptors.^{181,182} While studies by Steinhoff were directed at serine proteases, including tryptase and trypsin and their cognate PARs¹³⁸ with respect to itch, those by Lerner were directed at cysteine proteases, including mucunain from cowhage and cathepsin S, and initially PARs with respect to this sensation.¹⁸³ These parallel interests resulted in a collaboration in which the capacity for cathepsin S to evoke scratching behavior was evaluated in PAR₂ mutant mice. Surprisingly, scratching behavior remained intact in these animals (M. Steinhoff & E. A. Lerner, unpublished). To explain this result, the capacity of cysteine proteases to cleave and activate Mrgprs was explored. Indeed itch from cathepsin S occurs via activation of Mrgprs rather than PARs.¹⁸⁴ One aspect of this finding leads to the following important questions that remain to be addressed

definitively: What is the relative nociceptive contribution of PAR vs Mrgpr activation in humans in the context of itch and the related sensations of burning, pricking and stinging? What about within the context of pain?

The surprising finding that a cysteine protease could evoke itch via activation of a Mrgpr served to inform further studies that would explore a long-standing discrepancy, this one involving SP. SP is linked to both itch and neurogenic inflammation. The conventional receptor for SP is the neurokinin 1 receptor or NK1. Potent NK1 antagonists have been developed and have been effective in mouse models of itch and inflammation but have failed in the treatment of inflammation in humans. The effectiveness of NK1 antagonists for the treatment of chronic itch is under intense investigation. Promising data have been generated but the failure of topical aprepitant relative to vehicle⁷⁸ suggests that therapeutic benefit may arise from targeting central NK1. Moreover, NK1 mutant mice still scratched when injected with SP. As SP had been shown by others to activate selected Mrgprs (see below), that scratching behavior was blocked by the tripeptide Mrgpr antagonist termed QWF, and SP did not cause scratching in mice in which a cluster of Mrgprs had been removed genetically, it was deduced that itch from SP resulted from activation of Mrgprs.¹⁸⁵

Mice express more than twenty Mrgprs while humans express a much smaller number. As there are many more Mrgprs in mice relative to humans, it is reasonable to suggest that homology mapping with respect to ligand specificity is not 1:1. In addition, while most GPCRs are highly selective with respect to ligand specificity, Mrgprs are distinct. Mrgprs are broad innate sensors that may be set to trigger upon exposure to many ligands. Two receptors in humans, MRGPRX1 and MRGPRX2, may be considered to fulfill the roles of the entire A, B, and C subclasses of Mrgprs in mice. (In contrast, MRGPRD in humans and MrgprD in mice may overlap more closely.) Expression of individual Mrgprs has been considered by some investigators to be limited to either mast cells or nerves. MRGPRX1 is expressed only on sensory nerves. MRGPRX2 is expressed on mast cells but has also been identified on dorsal root ganglion neurons.¹⁸⁶ While MRGPRX2 is not readily identified in neurons on the basis of RNAseq of resting neurons, expression has not been considered during the process of neurogenic inflammation, a critical knowledge deficit in the context of intercellular and mediator cross talk. MRGPRX2 is activated by basic secretagogues, of which SP is one, and by small molecule drugs associated with pseudo-allergic reactions, including vancomycin which causes red man syndrome and staphylococcal delta toxin associated with AD.^{187,188}

Let us now delve further into the function of Mrgprs on mast cells vs nerves. Expression of MrgprB2 is limited to mast cells in mice and some investigators consider MRGPRX2 to be the human homolog. In contrast, MrgprA1 is expressed only on nerves in mice with high expression during development and low expression in adults. However, a lower level of expression has not been demonstrated to correlate with a lack of function. Rather the affinity for ligand receptor interaction is critical. Thus, SP activates MrgprB2, MrgprA1, and MRGPRX2. MrgprB2 is still present in the Mrgpr cluster knockout mice but SP does not cause scratching in these mice. Thus, in mice, SP causes itch by

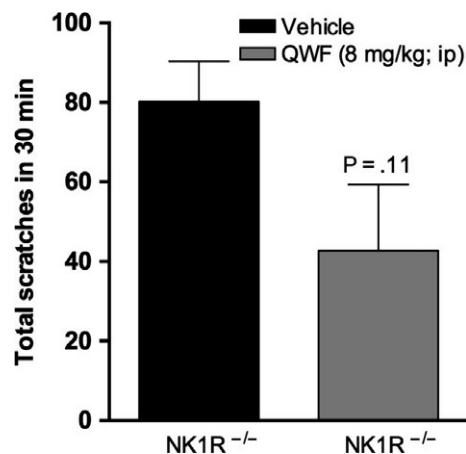


FIGURE 1 MrgprA1 contributes to scratching behavior in a model of neurogenic inflammation. Two groups of mice, each including 4 females and 1 male, were sensitized with a single epicutaneous application of 10 μ L of 2% oxazolone (OXZ) in acetone to the shaved abdomen. Mice were then challenged with 0.5% OXZ applied to the ears beginning 7 days later, and every other day, for 5 additional applications of OXZ. Mice were treated with QWF or vehicle as follows. About 8 mg/kg of QWF was delivered intraperitoneally and 2 mmol/L topically to the ears at the same time as each challenge with OXZ. While a p value of 0.05 was not achieved, limitations of this study include the relatively small numbers of mice and that while most behavioral studies use only male mice, female mice were included so as to better consider sexual differences

activating MrgprA1 on sensory nerves rather than activating MrgprB2. In humans, if MRGPRX2 is only on mast cells, then SP evokes itch by stimulating mast cells. In this scenario, the mouse receptors MrgprA1 and MrgprB2 share functional homologies with human MRGPRX2. This concept is consistent with single human receptors sharing function with multiple mouse Mrgprs. This concept is supported further by the following observations. SLIGRL, the hexapeptide derived from PAR₂, but which stimulates MrgprC11 on sensory nerves in mice to evoke itch, also activates MRGPRX2 while the potent itch inducer, compound 48/80, activates a plethora of Mrgprs expressed on mast cells and neurons. Returning to cysteine protease activation of Mrgprs, while cathepsin S activates MrgprC11 and MRGPRX2, the dust mite cysteine protease Der p1 associated with allergy and inflammation activates MrgprC11 and MRGPRX1 while cowhage activates human MRGPRX1 and MRGPRX2. The overlap of ligand-associated receptor activation, whether on mast cells or neurons, reveals that the sensation of itch occurs within the context of SP and neurogenic inflammation.

Consistent with the contributions of the interaction between SP and Mrgprs in neurogenic inflammation, we have found that the Mrgpr antagonist QWF modulates scratching behavior in a chronic model in mice sensitized to oxazolone (OXZ; Figure 1). The oxazolone (OXZ) model of delayed type hypersensitivity and AD has been studied in detail and involves SP¹⁸⁹ but an appreciation of the interaction between SP and Mrgprs in this model of chronic itch has not been considered previously. In this model, OXZ-primed T cells drive an IFN- γ -mediated Th1 effector response. Upon repeated exposure to the hapten OXZ, the effector response is shifted from Th1 to Th2 resulting

in hapten-specific IgE production, eosinophilic inflammation, and other features of AD-like pathology. Since SP activates NK1 and MrgprA1, and QWF is an antagonist of NK1 and MrgprA1, we sought to determine the contribution of MrgprA1 alone. We thus evaluated the effect of QWF on scratching behavior in NK1^{-/-} mice sensitized to OXZ and found that it was diminished. We interpret these findings as consistent with chronic itch from SP being dependent upon activation of MrgprA1 on sensory nerves. If these preliminary data can be extended, it would suggest that targeting Mrgprs indeed could be effective in the treatment of chronic itch and neurogenic inflammation. For completeness, we note that chloroquine can induce acute itch and activates MrgprA3 and MRGPRX1, while beta-alanine activates MrgprD, all on sensory neurons. The physiologic relevance of these observations is not clear.

Mrgprs are considered to be critical for histamine-independent itch. This concept is simple to understand with respect to activation of Mrgprs on sensory nerves. The situation is different with respect to mast cells. It is summarized here but discussed in detail elsewhere in this volume. Selective or differential granule release is critical to the contribution of mast cells to histamine-dependent and histamine-independent itch. Granule release can be IgE-dependent or IgE-independent. Activation of mast cell Mrgprs results in selective granule release and is independent of IgE. This situation contrasts with that of histamine.⁶² Human basophils also express MRGPRX2 ([www.jacionline.org/article/S0091-6749\(16\)32068-1/pdf](http://www.jacionline.org/article/S0091-6749(16)32068-1/pdf)) and the role of basophils in itch remains to be clarified.

Taken together, Mrgprs on mast cells and neurons provide powerful links between itch, allergy, and neurogenic inflammation. The recent identification of a Mrgpr antagonist¹⁸⁵ and residues critical for activity in which a combination of in silico modeling and in vitro activity have been employed by our group and others^{135,190} suggest that drugs that target this class of receptors will be identified and evaluated for therapeutic benefit.

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CONFLICT OF INTEREST STATEMENT

MS, JB, and EAL declare no financial or commercial conflicts of interest.

ORCID

Ethan A. Lerner  <http://orcid.org/0000-0001-5480-4133>

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