Peripheral Mechanisms of Itch
Changxiong J. Guo1, Nathaniel S. Grabinski1 and Qin Liu1

Itch is a universally experienced sensation, and chronic itch can be as diabolically debilitating as pain. Recent advances have not only identified the neuronal itch sensing circuitry, but also have uncovered the intricate interactions between skin and immune cells that work together with neurons to identify itch-inducing irritants. In this review, we will summarize the fundamental mechanisms of acute itch detection in the skin, as well as highlight the recent discoveries relating to this topic.

INTRODUCTION

Itch, also known as pruritus, is generally defined as an unpleasant sensation that evokes an instinctive urge to scratch. Similar to pain, itch evolved as a protective mechanism against harmful external elements. Whereas pain triggers immediate withdrawal from hazard, itch-induced scratching removes irritants from the skin. Despite the distinctiveness of itch sensation and its associated behavioral reflexes, itch was not fully appreciated as a separate sensory process until relatively recently. Itch and pain-sensing neural circuits were once theorized to be overlapping. Itch had been thought to be simply a low-intensity activation of pain-sensing circuits, largely due to the broad expression of noxious chemical receptors in itch-sensing neurons, such as the capsaicin receptor TRPV1 or the mustard oil receptor TRPA1 (Ward et al., 1996). Nevertheless, starting with the discovery of itch-selective MRGPRs in primary afferents of the peripheral nervous system (Han et al., 2013; Liu et al., 2012a, 2011, 2009) and gastrin-releasing peptide (GRP) signaling circuits in the CNS (Sun and Chen, 2007), itch is now recognized as a distinct biological process.

Research progress has also transformed itch research into a multidisciplinary field of study. Despite its conceptual simplicity, itch detection is an extraordinary task. The skin is continually assaulted by a barrage of diverse environmental irritants—ranging from UV, chemicals, and allergens to a host of bioactive compounds, peptides, and proteases from bugs, microbes, and pathogens—that all must be promptly detected and deciphered. Although itch-sensing neurons possess a repertoire of receptors that allows for a degree of direct exogenous irritants detection, itch is often initiated by epidermal and immune cells, and passed to sensory afferents through endogenous itch mediators. Findings over the past decade have clearly shown that itch detection is not purely a neuronal phenomenon but rather a complex orchestra of skin, immune, and neural processes that work in concert to complete this enormous task (Wang and Kim, 2020).

In this review, we will summarize the recent advances of this field and our current understanding of the main mechanisms of itch detection. We will first briefly summarize the neuronal receptors and primary afferent populations that mediate itch sensation. Then, we will discuss the cellular and molecular mechanisms of itch detection in the skin (Figure 1).

NEURONAL AND NONNEURONAL ITCH RECEPTORS

Although itch biology is an extraordinarily complex process, itch detection and signaling are largely accomplished through only six receptor families. Many of these receptors were first characterized in the skin and immune cells, where they play important roles in irritant detection and immune response. Their functions in these contexts are far too diverse and complex for the purposes of this review. Nevertheless, their involvement in itch initiation or mediation will be briefly discussed in the following section.

Histaminergic signaling was the earliest described itch process and remains the gold standard of the field (Bickford, 1938; Han et al., 2006; Inagaki et al., 1999). There are four known histamine receptors, HRH1–4, with distributed expression across a variety of tissues (Hill et al., 1997). These receptors play enormously important roles in both innate and adaptive immunity, mediating chemotaxis, cellular polarization, and cytokine release in a highly compartmentalized and dose-dependent manner (Dunford et al., 2006; Gützmer et al., 2005; Hofstra et al., 2003; Jutel et al., 2001; Strakhova et al., 2009). In the context of itch, histamine is liberally released by mast cells upon degranulation and produces itch sensation directly through neuronal HRH activation (Dimitriadou et al., 1994; Rossbach et al., 2011; Shim et al., 2007). In mice, HRH1 and HRH4 are expressed by dorsal root ganglia (DRG) neurons, where their expression is localized to two populations of itch-sensing nonpeptidergic (NP) nociceptors (Chiu et al., 2014; Usoskin et al., 2015; Zeisel et al., 2018). In humans, histaminergic itch signaling is largely similar, although only HRH1 has been conclusively shown to be expressed by primary sensory afferents (Timmerman et al., 2016).

The MRGPR family of receptors was discovered and characterized relatively recently, but has quickly become a quintessential family of neuronal itch sensors (Dong et al.,...
These receptors are broadly required for the detection of both exogenous pruritogens, as well as endogenous itch mediators from keratinocytes (KCs) and immune cells—especially in histamine-independent itch processes. In mice, MRGPRs comprise a family of ~50 receptors, half of which are pseudogenes. Many MRGPRs show highly restricted expression to NP nociceptors (Usoskin et al., 2015). Genetic deletion of a cluster of 12 MRGPR receptors in mice produced overarching itch defects, including insensitivity to many acute pruritogens as well as significantly attenuated itch in chronic models (Han et al., 2013; Huang et al., 2016; Liu et al., 2012a, 2011, 2009; Meixiong et al., 2019b). In humans, this family is condensed into just eight known members. Nevertheless, the functional scope of human MRGPRs is remarkably well-conserved, with some receptors showing condensed functions of two or more murine counterparts (Dong and Dong, 2018).

Serotoninergic (5-hydroxytryptamine [5-HT]) signaling comprises the third major neuronal itch pathway. In the skin, serotonin receptors (HTRs) are expressed by both immune cells and sensory neurons, and modulate both immune response and sensory perception in a highly complex, context-dependent manner (Mössner and Lesch, 1998). In the context of itch, HTRs are expressed by both murine and human DRG neurons (Flegel et al., 2015; Usoskin et al., 2015), and serotonin application is acutely pruritogenic in both species (Morita et al., 2015; Weisshaar et al., 2004). In mice specifically, 5-HT is released by mast cells and acts as a pruritogen at lower doses than histamine (Morita et al., 2015). Genetic deletion of Htr7 in mice has been shown to completely abolish serotonergic itch as well as to attenuate many types of chronic itch (Morita et al., 2015).

Toll-like receptors (TLRs) are pattern-recognizing receptors that detect pathogen-associated molecular patterns (Akira et al., 2006; El-Zayat et al., 2019). There are 14 known TLRs in mice, with expression across KCs, immune cells, and neurons alike (Diogenes et al., 2011; Duan et al., 2018; El-Zayat et al., 2019; Liu et al., 2012b, 2010; Qi et al., 2011). Four of these receptors, TLR3, TLR4, TLR5, and TLR7, have been reported to mediate itch in mice (Duan et al., 2018; Liu et al., 2016b, 2012b, 2010). In addition, TLR3 and TLR7 activation may also lead to prolonged enhancement of itch neuron excitability, likely through a transcriptional mechanism because genetic deletion of these TLRs leads to broad attenuation of acute itch sensitivity (Liu et al., 2012b, 2010). However, the exact mechanism behind TLR-mediated itch is unclear. Direct neuronal activation has been proposed, but influence from epidermal and immune cell TLR is difficult to isolate in experimental settings.

Protease-activated receptors (PARs) detect a range of exogenous and endogenous proteases, including mast cell–derived tryptases, chymases, and cathepsins (Kempkes et al., 2014; Reddy et al., 2008; Reddy and Lerner, 2010). These receptors are expressed by KCs, immune cells, and neurons (Böhm et al., 1996), and of the four known receptors, PAR2 and PAR4 have been implicated in itch initiation (Akiyama et al., 2009; Steinhoff et al., 2003). PAR activation is a two-step process involving proteolytic cleavage of its N-terminal

Figure 1. Schematic of acute itch detection in the mouse skin. Allergens and pruritogens induce itch through three main mechanisms: (i) certain external pruritogens, such as chloroquine or proteases, activate itch-sensing primary afferent neurons directly through their MRGPR receptors. (ii) Allergens and other pruritogenic compounds activate mast cells through their IgE-bound FceRI and MRGPRB2 receptors, respectively. Activated mast cells activate itch-sensing neurons in turn through the release of histamine, serotonin, proteases, and MRGPR agonists. (iii) Pruritogenic compounds may also, directly or indirectly, activate keratinocytes or induce cutaneous inflammation. Activated keratinocytes induce itch through the release of neuronal agonists or through mast cell activation. 5-HT, 5-hydroxytryptamine; HTR, 5-hydroxytryptamine receptor; miR, microRNA; NP, nonpeptidergic; Th, T helper.
tethered ligand, followed by autoactivation by the released ligand. In the case of PAR2, the tethered ligand SLIGRL-NH2 or SLIGKV-NH2 from mouse and human PAR2, respectively, is also a potent agonist of murine MRGPRC11 and human MRGPRX2 receptors (Akiyama et al., 2015; Liu et al., 2011). However, the mechanism of PAR-induced itch is somewhat contentious. In mice, deletion of the MRGPR cluster leads to complete abolishment of PAR2 ligand–induced itch, whereas deletion of PAR2 receptors did not result in appreciable attenuation of itch response (Liu et al., 2011; Reddy et al., 2015). Moreover, activation of PAR2 using a truncated, PAR2-specific peptide ligand was found to elicit pain instead of itch (Liu et al., 2011), strongly suggesting that PAR2 ligands induce itch indirectly through MRGPRS.

Finally, cytokines related to type 2 immune response and their receptors are becoming increasingly recognized for their role in itch modulation. TSLP is a major instigator of T helper (Th) 2 response (Kim et al., 2013), and its receptor complex, IL7Rα/TSLPR, was reported to be expressed by a small subset of itch-sensing DRG neurons (Wilson et al., 2013). High TSLP skin expression is a hallmark feature of atopic dermatitis (AD) (Jariwala et al., 2011), and TSLP is released by KCs in response to a broad range of stimuli, including in allergy and proteolytic PAR2 activation (Kouzaki et al., 2009; Moniaga et al., 2013). In mice, intradermal TSLP injection was reported to induce acute itch, and direct neuronal TSLPR activation has been purported to be a major pruritogenic mechanism (Wilson et al., 2013).

IL-33 is another important KC-derived initiator of Th2 response (Brandt and Sivaprasad, 2011), and its receptor complex, ST2/IL1RAP, is likewise expressed by a subset of histamine-sensitive DRG neurons. However, the exact involvement of IL-33 in itch is less clear. Although this cytokine is important for the development of chronic itch conditions such as allergic contact dermatitis (ACD) and xerosis, IL-33 is a weak neuronal agonist and does not induce acute itch in naive mice (Liu et al., 2016a). Alternatively, IL-33 has been reported to stimulate enkephalin production in group 2 innate lymphoid cells (Brestoff et al., 2015). The derivative of proenkephalin A—bovine adrenal medulla 8–22—is a potent MRGPR agonist (Dong et al., 2001). However, a direct functional connection has not yet been demonstrated.

Finally, type 2 immune cell–derived ILs—IL-4, IL-13, and IL-31—and their receptors play a major role in AD-associated itch (Oetjen et al., 2017). The involvement of these cytokines in AD skin disease had already been known, but these cytokines have recently also been implicated in the direct modulation of itch neurons. In mice, the IL-4 and IL-13 receptor complex IL-4Rα/IL-13Rα1 is broadly expressed by itch-sensing DRG neurons, and the IL-31 receptor complex IL-31Rα/OSMRβ is further expressed by some 5-HT–sensitive fractions of these neurons (Chiu et al., 2014; Usoskin et al., 2015; Zeisel et al., 2018). The expression of these receptors has also been detected in human DRGs (Oetjen et al., 2017). Intradermal injection of IL-31 produces mild itch in mice, but perhaps more importantly, these cytokines broadly enhance itch neuronal excitability, thereby potentiating both histaminergic and nonhistaminergic itch pathways (Oetjen et al., 2017). Anti–IL-4 therapies have already shown great efficacy in treating moderate to severe cases of AD (Thaçi et al., 2016). Now, Jak inhibitors, which target the shared downstream signaling pathways of these IL receptors, are beginning to show promising anti-itch potential in clinical trials (Guttman-Yassky et al., 2019; Kim et al., 2020; Nakagawa et al., 2018).

**ORGANIZATION OF MURINE ITCH-SENSING PRIMARY AFFERENT NEURONS**

The current model clusters itch-sensing primary afferents into several distinct populations on the basis of their gene expression profile, physiological properties, and function. Most of these neuronal populations are unmyelinated, small-diameter C-fiber DRG or trigeminal ganglia (TG) neurons, whose peripheral axons innervate the superficial layers of the skin, usually terminating as free nerve endings, and the central axons synapse with CNS neurons in the upper laminae of either the dorsal horn of the spinal cord or the spinal trigeminal nucleus (Han et al., 2013; Morita et al., 2015; Patel and Dong, 2011). Unbiased single-cell RNA-sequencing studies of murine DRGs clustered itch-sensing neurons into three widely referenced NP afferent populations: MRGPRD-expressing NP1 neurons, MRGPRRA3-expressing NP2 neurons, and somatostatin/brain natriuretic peptide (NPPB)-expressing NP3 neurons (Li et al., 2018; Usoskin et al., 2015; Zeisel et al., 2018) (Figure 1 and Table 1). It should be noted that the NP nomenclature/classification stems from their lack of substance P (SP) or calcitonin gene-related peptide (CGRP) expression during late embryonic development (Woolf and Ma, 2007). NP neurons express neuropeptides, many of which such as NPPB or GRP are largely itch specific in the spinal cord (Dong and Dong, 2018).

NP1 neurons were first identified as mechanoreceptors that detect punctate mechanical pain (Cavanaugh et al., 2009; Dussor et al., 2008). These neurons form the largest of the three clusters, making up approximately 20% of murine primary afferents in the DRG and TG (Cavanaugh et al., 2009; Wang and Zylka, 2009; Zylka et al., 2005, 2003). Since then, this population of neurons has been implicated in the acute itch induced by the bodybuilding supplement β-alanine, which directly activates MRGPRD receptors to induce nonhistaminergic itch (Liu et al., 2012a; Shinohara et al., 2004). More recently, these neurons were implicated in ACD-associated pruritus, although the mechanism remains undefined (Meixiong et al., 2019a; Qu et al., 2014). Moreover, NP1 neurons express IL-4Rα/IL-13Rα1 receptors and the lysophosphatidic acid receptors LPAR3 and LPAR5 (Usoskin et al., 2015), suggesting potential involvement in other chronic itch conditions.

NP2 neurons are perhaps the best studied itch-sensing population and are broadly implicated in both histaminergic and nonhistaminergic acute itch and chronic pruritus (Han et al., 2013; Nattkemper et al., 2018; Zhu et al., 2017). These neurons are principally responsible for detecting histamine through their HRH1 receptors (Han et al., 2013). In addition, NP1 neurons detect the antimalarial drug chloroquine through direct activation of their MRGPRRA3 receptors (Liu et al., 2009), and are broadly sensitive to both exogenous and endogenous itch mediators through their highly
promiscuous MRGPRC11 receptor (Usoskin et al., 2015). Moreover, this population is frequently implicated in chronic itch conditions, including xerosis and AD (Han et al., 2013; Qu et al., 2014; Zhu et al., 2017), and was recently implicated in cholestatic itch through its MRGPR1 receptors (Meixiong et al., 2019b). Similar to NP1 neurons, NP2 neurons express IL-4Rα/IL-13Rα1 receptors and are sensitive to type 2 immune cell–derived ILs, but may also be sensitive to KC-derived IL-33 through ST2 receptors (Liu et al., 2016a).

NP3 neurons also show broad involvement in itch detection (Emery and Emfors, 2020; Huang et al., 2018b) and share some functional overlap with NP2 neurons through their HRH4 and IL-4Rα/IL-13Rα1 receptors (Usoskin et al., 2015). Nevertheless, NP3 neurons possess considerable unique functions. These neurons are sensitive to T4 lymphocyte–derived IL-31 through IL-31Rα/OSMRβ receptor and are largely responsible for the detection of serotonergic itch through its HTR2 and HTR7 receptors (Morita et al., 2015). Recently, NP3 neurons were further implicated in two unique chronic itch processes. This population was reported to detect KC-derived periostin through its Av-β3 receptors during allergy and in a murine AD model (Mishra et al., 2020). These neurons are also sensitive to basophil–derived cysteinyl leukotriene (LTC4) through its CYSLTR2 receptors and mediate allergic itch flares in AD (Wang et al., 2021).

Moreover, this neuronal cluster has not been determined.

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**Table 1. Organization of Murine Itch Sensing DRG Neuron**

<table>
<thead>
<tr>
<th>Neuronal Cluster</th>
<th>NP1</th>
<th>NP2</th>
<th>NP3</th>
<th>Unclassified</th>
<th>NF1/NF2 LTMR (subset)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuron type</td>
<td>C-fiber</td>
<td>C-fiber</td>
<td>C-fiber</td>
<td>C-fiber</td>
<td>Aβ</td>
</tr>
<tr>
<td>Identifying molecular marker</td>
<td>MRGPRD</td>
<td>MRGPRC3</td>
<td>SST/BNP</td>
<td>TSLPR</td>
<td>TLR5</td>
</tr>
<tr>
<td>Histamine receptor status</td>
<td>(–)</td>
<td>HRH1</td>
<td>HRH4</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>MRGPR status</td>
<td>(–)</td>
<td>Multiple</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>Serotonin receptor status</td>
<td>(–)</td>
<td>(–)</td>
<td>HTR2, HTR7</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>TLR status</td>
<td>(–)</td>
<td>TLR3?, TLR7?</td>
<td>(–)</td>
<td>(–)</td>
<td>TLR5</td>
</tr>
<tr>
<td>PAR status</td>
<td>(–)</td>
<td>(+)</td>
<td>(–)</td>
<td>(–)</td>
<td>(–)</td>
</tr>
<tr>
<td>IL receptor status</td>
<td>IL4R/IL13R</td>
<td>IL4R/IL13R, ST2</td>
<td>IL4R/IL13R, IL31R</td>
<td>TSLPR</td>
<td>(–)</td>
</tr>
<tr>
<td>TRP status</td>
<td>TRPA1, TRPC3, TRPM3, TRPA1, TRPV1</td>
<td>TRPA1, TRPV1</td>
<td>TRPA1</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Exogenous pruritogen sensitivity</td>
<td>β-alanine, Chloroquine, Defensins, proteases</td>
<td>?</td>
<td>?</td>
<td>Flagellin, mechanical stimuli</td>
<td></td>
</tr>
<tr>
<td>Chronic itch participation</td>
<td>ACD</td>
<td>Xerosis, AD</td>
<td>AD</td>
<td>AD</td>
<td>Xerosis, ACD, AD (mechanical alloknesis)</td>
</tr>
</tbody>
</table>

Abbreviations: ACD, allergic contact dermatitis; AD, atopic dermatitis; HTR, 5-hydroxytryptamine receptor; LTMR, low threshold mechanoreceptor; NF, neurofilament; NP, nonpeptidergic; TLR, toll-like receptor.

*Classification for this neuronal cluster has not been determined.

**EVOLUTIONARY CONSERVATION OF ITCH NEURON ANATOMY**

It should be noted that colocalization of known pruritogen-sensing receptors is variable between species, even among rodents (Han and Dong, 2014; Zylka et al., 2003). Owing to limitations in and the technical difficulties associated with studies involving human or nonhuman primate sensory neurons, genetic and functional characterization of human itch afferents remain largely incomplete. Because of this, it is not certain whether the organization of human and murine itch-sensing primary afferent populations shares a high degree of anatomical or genetic conservation. Nevertheless, the limited available evidence has thus far supported the notion that this process is well-conserved between mice and humans. Although there are some notable differences, the expression pattern of itch-related receptors in human sensory neurons is largely similar to what is observed in mice. MRGPRs, HRH, HTRs, and IL4/13/31 receptors are expressed by human DRGs, even though the exact distribution of these receptors across human DRG populations remains undefined (Oetjen et al., 2017; Ray et al., 2018). Moreover, MRGPRD and MRGPX1 expression can be used to define two distinct populations of C-fiber neurons in human DRGs, where the latter group is also sensitive to chloroquine, MRGPRC11 agonists, and histamine (Valtcheva et al., 2016). These two human primary afferent populations could represent the functional homologous of murine NP1 and NP2 neurons, respectively. Additional studies are needed to confirm whether this is the case.

Despite the unknowns, human and murine pruritogen receptors often form clear homologous pairs and show remarkable functional conservation in their ability to detect exogenous pruritogens (Meixiong and Dong, 2017). Moreover, clinical features of murine and human itch pathologies are highly similar; even the antihistamine-refractory nature of many chronic itch skin pathologies is conserved between the species. Because of these features and the broad availability of genetic mutants, murine models have been ideal for the study of both acute itch and translational studies of chronic pathologies involving pruritus.

**NEURONAL ITCH INITIATION**

Although itch is often initiated by immune or epidermal cells, all the peripheral itch signals, including both endogenous...
and exogenous, are detected and transmitted centrally by primary sensory neurons. As mentioned in the previous section, murine NP1 and NP2 neurons directly detect pruritogens \( \beta \)-alanine and chloroquine through their hallmark MRGPR receptors, MRGPRD and MRGPRA3, respectively (Liu et al., 2012a, 2009). More recent studies have shown that tick defensin peptides IPDef1 and IRDef2 and the dust mite cysteine protease Der p1 directly activate NP2 neurons through MRGPRC11 receptors (Li et al., 2021; Serhan et al., 2019). These functions are conserved in humans through the MRGPRD and MRGPRX1 receptors (Huang et al., 2003; Li et al., 2021; Liu et al., 2012a, 2009).

Two recent studies independently implicated MRGPRX4 receptors in neurogenic cholestatic itch (Meixiong et al., 2019c; Yu et al., 2019). Enhanced serotoningergic and MRGPR signaling as well as neuronal TGR5 receptor activation had already been associated with cholestatic itch (Alemi et al., 2013; Sanjel et al., 2019; Schwörer et al., 1995). These new studies further show that the human MRGPRX4 receptor, which is expressed by histamine-sensitive itch-sensing DRG neurons, is directly activated by bilirubin and bile acid components, especially deoxycholate. Bilirubin was further demonstrated to activate mouse MRGPRA1 receptors and NP2 neurons; however, the mouse neuronal receptor for deoxycholate remains unidentified (Meixiong et al., 2019b, 2019c; Yu et al., 2019). A third study further showed epithelial involvement in cholestatic itch, which will be discussed later in this review (Chen et al., 2021).

**MECHANICAL ITCH DETECTION**

Mechanically evoked itch, such as that from a bug walking across the skin or light pricking of a von Frey hair on a laboratory mouse, was recently reported to be mediated by a subpopulation of TLR5-expressing A\( \beta \)-LTMRs (Figure 2a)—this same population may also directly detect flagellin-induced acute itch. Silencing of these neurons blocked both acutely induced mechanical itch within their receptive field as well as eliminated histamine-induced mechanical alloknesis. Interestingly, silencing of these neurons or their postsynaptic spinal circuits in mice also attenuated mechanical alloknesis and spontaneous itch across a range of pruritic skin conditions, including acetone:ether water (AEW).
model of xerosis, ACD, and AD (Duan et al., 2018; Pan et al., 2019). However, the precise mechanism of neural gating of mechanical itch in the CNS appears quite complex. Loss or silencing of \( \beta \)LTMRs in other contexts has also, paradoxically, been reported to enhance mechanical allolokinesis (Sakai and Akiyama, 2019). Furthermore, in aging mice, the loss of touch-sensitive Merkel cells, one of the many touch-sensitive skin structures that complexes with \( \beta \)LTMRs, was correlated with increased mechanical allolokinesis and hyperkinesis (Feng et al., 2018). Additional studies are required to further delineate the mechanical itch mechanism as well as the overlap of mechanical and chemical itch-processing circuits in the CNS.

**NEUROIMMUNE AND NEURAL–EPIDERMAL MECHANISMS OF PRURITOGEN DETECTION**

**Mast cells**

Whereas most types of innate immune cells and T cells are involved in itch in some capacity, mast cells are the standouts in terms of itch functions and have been implicated in many pruritic skin diseases (Meixiong et al., 2020; Siiskonen and Harvima, 2019; Wang and Kim, 2020).

The mast cell–neuron complex is a highly conserved itch-sensing unit across mammalian species (Figure 1). Mast cells are closely associated with itch-sensing afferents in the skin (Huang et al., 2018a) and are perhaps best known for their canonical role as the principal mediator of allergic urticaria and anaphylaxis (Hennino et al., 2006). During this process, allergens cross a damaged skin barrier and bind to mast cells through FceRI receptor–bound IgE antibodies. Subsequent crosslinking of IgE triggers rapid degranulation of the mast cells and the release of an inflammatory soup containing, among other things, endogenous itch mediators, including histamine and 5-HT, as well as newly discovered endogenous itch mediators such as neuropeptide FF, LTC4, and type 2 cytokines. In mice, these endogenous pruritogens converge on NP2 and NP3 neurons to induce itch (Meixiong et al., 2020; Solinski et al., 2019; Wang and Kim, 2020).

Conversely, murine mast cells respond to a range of neuropeptides, including SP and CGRP, released from the peripherally terminals of nociceptive and pruritoceptive DRG neurons (Forsythe, 2019; Meixiong et al., 2020). During steady states, these neuropeptides promote the close association of mast cells with nerve terminals; and during allergy, these peptides can trigger mast cell chemotaxis to the affected skin and directly modulate mast cell responses (Kleij and Bienenstock, 2005; Rogoz et al., 2016; Xu et al., 2020). In an extreme example, one study reported that mGlur7 deficiency in NP2 itch neurons, which disrupts glutamate-dependent autocrine-negative feedback at the central terminals of these neurons, led to exaggerated mast cell–dependent itch and anaphylaxis responses during allergy (Rogoz et al., 2016).

The contribution of mast cells is not solely restricted to allergic histaminergic itch. In mice, the activation of mast cells alone through chemogenetics or its surface receptors (without concurrent allergy or inflammation) is sufficient to induce degranulation and subsequent itch (Solinski et al., 2019). Importantly, mast cells express the murine MRGPRB2 receptor and its human homolog MRGPRX2 (McNeil et al., 2015; Tatemoto et al., 2006). As mentioned previously, many MRGPRs are principally neuronal itch sensors, and mast cells are among the few non-neuronal tissues with MRGPR expression. Activation of MRGPRB2/X2 receptors can directly trigger mast cell degranulation, leading to nonhistaminergic itch, inflammation, and innate immune response (McNeil et al., 2015; Yuan et al., 2021). Even though these receptors were only deorphaned within the past decade, MRGPRB2/X2 receptors have been shown to demonstrate broad sensitivity toward both endogenous and exogenous chemicals, including many neuropeptides and neuroendocrine secretory proteins (Grimes et al., 2019; McNeil et al., 2015). These receptors also directly mediate drug-induced pseudoallergic reactions, including those caused by C48/80, atracurium, vancomycin, and a number of quinolone antibiotics (Babina, 2020; McNeil et al., 2015; Meixiong et al., 2019c; Yuan et al., 2021).

Recently, mast cells have also been implicated as a major effector of ACD-associated itch. ACD had been generally described as a Langerhans cell– and T-cell–driven process. However, Meixiong et al. (2019a) further reported that ACD-associated itch is primarily a mast cell–dependent process. Although T-cell–derived ILs undoubtedly rearrange the itch-sensing environment in the affected skin, MRGPRB2 deficiency significantly attenuated itch across multiple murine models of ACD. Moreover, the authors’ findings strongly suggest that ACD-associated itch is generated through a KC-mast cell mechanism. Under inflammatory skin conditions, KCs secrete PAM9–20, which activate mast cell MRGPRB2 receptors in a histamine- and IgE-independent manner. Activated mast cells, in turn, activate NP1 and, to a lesser extent, NP2, and NP3 neurons to elicit itch (Meixiong et al., 2019a).

**T cells**

In addition to mast cells, cutaneous CD4+ T cells, especially Th2 cells, are increasingly recognized for their involvement in the pruritogenic process. However, in contrast to mast cells, examples of direct T-cell involvement in acute itch detection or initiation are sparse because these cells are recruited by innate immune cells and arrive comparatively late in the allergen or pathogen response process. Instead, T-cell function in itch is much better understood in terms of their role in the chronic or dysfunctional remodeling of the itch-sensing microenvironment. T-cell–derived cytokines are integrally involved in the initiation and continuance of cutaneous inflammation as well as the enhancement of itch neuron excitability. These effects are most prominently observed in chronically itchy inflammatory skin disorders such as psoriasis and AD, which will be discussed in greater detail in a later section.

Nonetheless, T cells possess the ability to produce itch. The best example of T-cell–driven itch is perhaps cutaneous T-cell lymphoma (CTCL) (Figure 2b). CTCL is a class of non–Hodkin lymphoma characterized by the accumulation of neoplastic T cells in the skin. Severe itching of the affected skin is commonly associated with advanced-stage disease, which typically cannot be satisfactorily alleviated by steroids or antihistamines (Ahern et al., 2012; Meyer et al., 2010; Misery, 2014; Vij and Duvic, 2012). The pathophysiology of
CTCL-associated itch is complex and is not completely understood. Previous studies of CTCL tissues have typically reported Th2-like secretory profiles in the malignant cells and elevated Th2-derived cytokine levels in the affected epidermis and sera of patients with CTCL with pruritus. Moreover, this Th2 polarization was more prominent with disease progression (Nattkemper et al., 2016; Singer et al., 2013). As mentioned previously, receptors for Th2-derived cytokines are broadly expressed across all the three NP itch-sensing neuron populations. Intradermal IL-31 injection acutely induces itch in naïve mice through NP3 neurons, whereas IL-4 injection acutely potentiates histamine-induced itch (Oetjen et al., 2017). Elevated IL-4 and IL-31 are both pruritogenic. Although it remains to be determined whether IL-33 can directly induce itch in xerotic skin, this finding strongly suggests that IL-33 released by damaged KCs augments NP2 neuron excitability. Indeed, this same study noted that IL-33 may acutely promote chloroquine response in cultured mouse DRG neurons (Trier et al., 2021). Our own research is currently focused on identifying KC-derived MRGPR agonists, with promising progress.

MECHANISM OF PSORIASIS AND AD ITCH

Because of the prevalence of psoriasis and AD, intense psychophysical impacts on patients, and often treatment-refractory nature of these disorders, intense research efforts have been invested to decipher their mechanisms and to identify new therapeutic targets. The clinical presentations of psoriasis and AD are similar, that is, dry, inflamed skin with intense pruritus. These disorders are also both antihistamine refractory, with complex involvement and convergence of the dermal, immune, and neuronal components mentioned earlier. In addition, sensitization of CNS itch circuits is also associated with both psoriasis and AD. Despite the similarities, these two disorders are driven by different immunological and itch mechanisms, which are described in Figure 2c and d (Kapur et al., 2018; Rendon and Schäkel, 2019).

The mechanism behind psoriasis-associated itch is still not well-understood, largely owing to the complexity of the disorder and the fact that psoriasis is unique to humans. No single mouse model has fully recapitulated human psoriasis disease (Schön et al., 2021). Nevertheless, some contributing factors are known. Although psoriasis is a Th1/Th17-driven
process, TSLP and the Th2 cytokine IL-31 are reported to be elevated in psoriatic skin (Komiya et al., 2020; Nattkemper et al., 2018; Volpe et al., 2014) and induce itch through direct activation of neuronal itch receptors. Histamine and mast cells are also abundantly present in psoriatic skin. However, existing studies have not consistently correlated histamine or HRH1 levels with itch (Jaworecka et al., 2021), and psoriasis itch is rarely treatable using antihistamines. The neuropeptide SP and its receptor NK1R (which is expressed by KCs, immune cells, and neurons) levels are also frequently reported to be elevated in psoriatic skin (Komiya et al., 2020; Saraceno et al., 2006). Promisingly, the NK1R antagonist serlopitant recently completed phase 2 trials and reported mild attenuation of psoriasis itch (Pariser et al., 2020). However, the interpretation of this finding is difficult because SP signaling is involved in both ascending and descending itch circuits in the CNS (Ständer and Yosipovitch, 2020). However, the interpretation of this finding is difficult because SP signaling is involved in both ascending and descending itch circuits in the CNS (Ständer and Yosipovitch, 2019), and this trial did not exclude CNS effects. In addition, nerve GF (NGF), prostaglandin E2 (PGE2), endothelin-1 (ET-1), and κ-opioid receptor (KOR)—all of which, except for KOR—were specifically increased in lesional skin, which was decreased. PGE2 and ET-1 are both putative itch mediators, NGF has been suggested to promote itch by inducing hyperinnervation of skin nerve fibers, and KOR and its ligand dynorphin A have been reported to suppress itch but only in the context of the CNS (Jaworecka et al., 2021; Komiya et al., 2020).

Similar to psoriasis, AD itch is also a complex, multifaceted process with no shortage of pruritogens. There is some overlap with psoriasis because TSLP, IL-31, SP, NGF, opioid receptors, among others have also been implicated in AD pruritus. In addition, proteases such as cathepsin and tryptase have also been previously shown to contribute to AD itch (Mollanazar et al., 2016). More recently, epidermally derived periostin and basophil-derived LTC4 have been reported to promote AD itch by direct activation of murine NP3 neurons through Aβ3 and CYSLTR2 receptors, respectively (Mishra et al., 2020; Wang et al., 2021). The basophil axis is especially relevant during allergy-triggered acute itch flares. Our own study recently implicated the KC-derived serine protease kallikrein 7 as another important contributor of itch in mouse AD-like disease through an immune-independent mechanism (Guo et al., 2020).

Arguably, the most important discovery in relation to AD-associated itch has been the identification of Th2 receptor-mediated potentiation of neuronal excitability (Meng et al., 2021; Oetjen et al., 2017). IL-4R/IL-13R is expressed by all the three NP itch-sensing afferents groups, and IL-31R is expressed in serotonin-sensitive NP3 neurons, which detect periostin and LTC4 itch signals. Even though Th2 cytokines are weak acute pruritogens, neuron-specific deletion of IL-4Rα receptor subunits significantly attenuated AD itch in mice (Oetjen et al., 2017). Moreover, these same mice showed reduced skin disease severity, strongly suggesting that reciprocal neuronal modulation of immune populations is another major driver of this disorder. Consistent with these results, anti–IL-4 therapy has shown great efficacy in alleviating AD symptoms, including itch, in patients with moderate to severe AD (Beck et al., 2014; Silverberg et al., 2020). Jak1 inhibitors, which target the common intracellular signaling of IL4R, IL-31R, and TSLPR, are likewise showing great promise in clinical trials (Kim et al., 2020; Scuron et al., 2021).

CONCLUDING REMARKS

The past two decades of intense research have transformed the itch field. Itch biology grew from a subtext of the pain field to an interdisciplinary and highly collaborative area of study. Entirely unknown itch-sensing receptors and neural circuits were discovered and, more importantly, reconciled with existing inflammatory skin processes. These advances, for the first time, not only yielded an explanation for the psychophysiological manifestations of intensely pruritic skin disorders but also actionable therapeutic targets to manage what is perhaps the most debilitating symptom of these disorders. However, there are still many unknowns left to reveal. New itch mediators and processes are discovered continually, and the processes behind neural–immune–neuro–epidermal cross-modulation are finally beginning to decode. Itch is a quickly advancing field with ample openings for paradigm-shifting breakthroughs. The most exciting discoveries are still yet to come.

ORCIDs

Changxiong J. Guo: http://orcid.org/0000-0002-2886-8536
Nathaniel S. Grabinski: http://orcid.org/0000-0003-0291-5717
Qin Liu: http://orcid.org/0000-0003-4333-4951

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank Xingzhong Dong, Lian Han, Brian Kim, and Michael Panneton for their thoughtful critiques of this paper. Their invaluable feedback greatly improved the quality of this manuscript.

AUTHOR CONTRIBUTIONS

Visualization: CJG, NSG; Supervision: QL; Writing - Original Draft Preparation: CJG, NSG; Writing - Review and Editing: CJG, NSG, QL

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