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Mast Cells Get on Your Nerves in Itch

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Mast-cell-nerve interactions play an integral role in itch and inflammation. Meixiong et al. (2019) show that the receptors MRGPRB2 and FcεRI mediate distinct types of mast cell activation and nerve interactions and that mast cell activation through MRGPRB2 drives itch in allergic contact dermatitis.

Mast cells are often found to be closely associated with nerve fibers in peripheral tissues and in the central nervous system (Forsythe and Bienenstock, 2012). It is increasingly clear that bidirectional communication between mast cells and nerves contributes to itch, pain, and inflammation. Rita Levi-Montalcini, who won the Nobel Prize in Physiology in 1986 for her discovery of nerve growth factor (NGF), highlighted her interest in mast-cell-neuron interactions in her Nobel lecture. Her laboratory found that NGF injection induced mast cell accumulation next to nerve fibers in neonatal rats and that mast cells were a source of NGF (Leon et al., 1994). Because mast cells and neurons are in close contact with each other, it has been proposed that these two cell types serve as a functional unit in physiology (Forsythe and Bienenstock, 2012). However, only recently have studies begun to define the molecular mechanisms that mediate mast-cell-nerve communication. In this issue of *Immunity*, Meixiong et al (2019) make important findings that contribute to this emerging area of neuroimmunology. They demonstrate that Fc epsilon RI (FcεRI)-mediated mast cell activation and Mas-related G-protein coupled receptor member B2 (MRGPRB2)-mediated mast cell activation have distinct functional consequences on mast cell mediator release, sensory neuron activation, and itch (Figure 1).

A major advance in mast cell biology was the discovery of MRGPRX2, a human G-protein coupled receptor (GPCR), and its mouse orthologue, MRGPRB2, as mediators of mast cell activation (McNeil et al., 2015; Subramanian et al., 2011). In canonical allergic reactions, antigen leads to the crosslinking of immunoglobulin E (IgE) bound to the high-affinity IgE receptor FcεRI on the surface of mast cells to induce mast cell degranulation. By contrast, MRGPRX2 and MRGPRB2 are activated by basic secretagogues, which include 48/80, the neuropeptide substance P (SP), pro-adrenomedullin peptide 9-20 (PAMP9-20), and small molecules associated with pseudo-allergic reactions (Kamohara et al., 2005; McNeil et al., 2015; Subramanian et al., 2011). A recent study showed that the quality of mast cell degranulation is influenced by the type of mast cell stimuli (Gaudenzio et al., 2016). Mast cell degranulation through MRGPRB2 and other GCPRs (e.g., C3aR, C5aR) was distinct from degranulation induced by anti-IgE-mediated cross-linking of FcεRI (Gaudenzio et al., 2016). GPCR-induced mast cell degranulation occurred faster, with smaller and more uniform granules being released, whereas anti-IgE-induced mast cell degranulation was characterized by a sustained elevation of intracellular calcium and with a fusion of granules before exocytosis (Gaudenzio et al., 2016).

In this study, Meixiong et al. (2019) find that distinct types of mast cell stimuli induce a differential release of mast cell mediators, leading to the activation of distinct sets of sensory neurons and itch modalities. Mast cell activation through the MRGPRB2 ligand PAMP9-20 led to the secretion of Tryptase B2 and low levels of serotonin by mast cells. By contrast, mast cell activation through anti-IgE crosslinking led to the secretion of high levels of histamine and serotonin but not Tryptase B2. The authors next compared the functional outcome of the two types of mast cell degranulation in the context of itch. They show that intradermal injections of PAMP9-20 induced significant scratching behaviors in mice, and this itch was decreased in *Mrgprb2*$^{-/-}$ mice. By contrast, both anti-gen and anti-IgE-injection-induced itch were intact in *Mrgprb2*$^{-/-}$ mice. Itch induced by anti-IgE was inhibited using antagonists for the histamine receptors H1R and H4R or by serotonin receptor antagonists, whereas itch induced by PAMP9-20 was largely unaffected by these antagonists. These results indicate that the two types of mast cell degranulation (FcεRI versus MRGPRB2) lead to histamine-dependent and histamine-independent types of itch, respectively.

Itch is an unpleasant sensation mediated by pruriceptive sensory neurons whose cell bodies reside in the dorsal root ganglia (DRG). Meixiong et al. (2019) elegantly used intravital calcium imaging to characterize the types of DRG neurons activated by mast cell stimulation in live anesthetized mice. The authors imaged lumbar DRG neurons that genetically expressed a fluorescent calcium indicator and recorded neuronal responses after injection of mast cell stimuli and pruritogens into the skin of the footpad. Although both anti-IgE and PAMP9-20 injection induced calcium influx in DRG neurons, the types of responsive neurons and kinetics of activation differed. The majority of neurons that responded to anti-IgE cross-linking also responded to histamine, whereas only ~30% of the neurons responding to PAMP9-20 also responded to histamine. By contrast, neurons that responded to PAMP9-20 were more likely to respond to the pruritogenic ligands chloroquine (MRGPRA3 agonist), serotonin (5-HT1F agonist), and β-alanine (MRGPRD agonist). Histamine receptor antagonists for H1R and H4R strongly decreased neuronal activation induced by anti-IgE but affected PAMP9-20-induced activation to a much lesser degree. Expression of histamine receptors in DRG neurons differs from expression of MRGPRA3, 5-HT1F, and MRGPRD

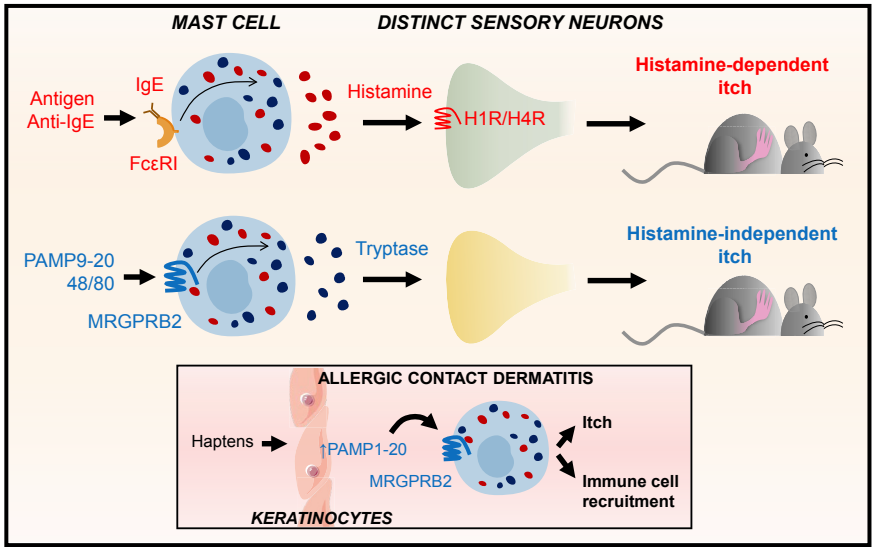

Figure 1. Mast Cells Communicate with Sensory Neurons to Drive Distinct Types of Itch
Upon activation by either FcεRI or MRGPRB2, mast cells interact with different sensory neurons to drive distinct types of itch. Mast cell activation through crosslinking of IgE bound to FcεRI induces degranulation and release of histamine, which activates sensory neurons through H1R and H4R to produce histaminergic itch. Mast cell activation through MRGPRB2 by its ligands 48/80 or PAMP9-20 induces mast cell release of Tryptase B2 and activation of a distinct set of sensory neurons to produce non-histaminergic itch. In allergic contact dermatitis (ACD), haptens induce itch and immune-cell recruitment dependent on MRGPRB2. Human ACD skin shows upregulation PAMP1-20, a ligand for MRGPRX2 (inset).

(Usoskin et al., 2015), suggesting that anti-IgE and PAMP9-20 activate distinct neuronal subtypes. DRG neuronal calcium influx was delayed until 5 min after anti-IgE injection, whereas neuronal calcium influx was induced within 1 to 2 min by PAMP9-20. PAMP9-20 induced greater numbers of neuronal activation peaks in smaller areas of analysis compared to anti-IgE, which may relate to the larger numbers of smaller granules released by mast cells when activated by PAMP9-20 (Gaudenzio et al., 2016).

Meixiong et al. (2019) next investigated the functional role of MRGPRB2 in pathological conditions of itch. Allergic contact dermatitis (ACD) is caused by repeated exposure of the skin to a contact allergen, which is a hapten that penetrates the skin barrier and induces a delayed-type hypersensitivity reaction. An example of ACD is poison-ivy-induced dermatitis, which is characterized by severe itch and inflammation. Although pruritis is a major part of the phenotype of ACD, the underlying mechanisms of itch are not well understood. Meixiong et al. (2019) set up three different mouse models of ACD using the haptens SADBE, oxalozone, and DNCB to investigate itch mechanisms after skin sensitization and challenge. They showed that itch behavior was significantly reduced in *Mrgprb2*$^{-/-}$ mice compared to wild-type (WT) mice for all three models of ACD. Total CD45^{+} immune-cell numbers were reduced in *Mrgprb2*$^{-/-}$ mice compared to WT mice in DNCB-treated mice, indicating that MRGPRB2 may also mediate immune-cell recruitment in ACD. By contrast, there were no differences in itch between *Mrgprb2*$^{-/-}$ mice and WT mice in mouse models of atopic dermatitis (MC903) or dry-skin-induced itch (AEW). With potential relevance in human pathology, Meixiong et al. (2019) found that skin from patients suffering from ACD displayed increased levels of PAMP1-20, an MRGPRX2 agonist, as well as increased numbers of mast cells compared to healthy controls. These data demonstrate that MRGPRB2 (mice) and MRGPRX2 (human) may drive itch in ACD.

This study highlights the growing importance of neural-immune interactions in itch and inflammation. Meixiong et al. (2019) found that distinct types of mast cell stimuli drive distinct activation of sensory neurons and itch. Although anti-IgE-induced cross-linking of FcεRI led to mast-cell-induced histaminergic itch, MRGPRB2 activation led to mast-cell-induced non-histaminergic itch that plays a role in ACD. Some outstanding questions remain. Mast cell degranulation releases a broad array of molecules that could act on sensory neurons. The mediators that activate pruriceptive neurons downstream of MRGPRB2 activation remain unclear. One candidate identified by Meixiong et al. (2019) is Tryptase B2. Tryptases are known to activate protease-activated receptors (PARs), including PAR1, PAR2, and PAR4, on sensory neurons that can sensitize itch (Akiyama et al., 2015). Tryptases could also act through PARs on keratinocytes to release mediators that act on neurons to induce itch. It would also be interesting to determine whether mast cells and sensory neurons form a bidirectional positive feedback loop in itch and inflammation. After activation, sensory neurons release neuropeptides, including SP, which is a potent activator of MRGPRX2 and MRGPRB2 (McNeil et al., 2015). A recent study found that SP-mediated mast cell activation through MRGPRB2 drives neurogenic inflammation and the recruitment of immune cells in a mouse model of incisional wound (Green et al., 2019). It would be interesting to determine whether itch fibers express SP and whether pruriceptive neurons activate mast cells to drive inflammation and immune-cell recruitment in ACD. The types of connective-tissue-type mast cells (CTMCs) and mucosal mast cells (MMCs) that express MRGPRB2 or MRGPRX2 also remain to be fully determined. Future work may focus on imaging mast-cell-nerve interactions in different tissues and determining how molecular crosstalk occurs in acute and chronic inflammatory diseases. Targeting mast cells and their signaling to neurons could lead to novel approaches to treat itch and inflammation.

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Exploiting Allelic Variation in CD8$^+$ T Cells

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In this issue of *Immunity*, van der Veeken et al. (2019) leverage genetic variation between mouse strains to assess epigenetic and transcriptional regulation dynamics in CD8$^+$ T cells responding to acute infection.

CD8$^+$ T cells can adopt a multitude of cellular states in the context of acute infections. Infection causes naive pathogen-specific CD8$^+$ T cells to differentiate into effector (also referred to as activated) cells. When the infection is cleared, most effector CD8$^+$ T cells die, but some survive long-term in a resting state termed memory. These distinct cellular states are largely determined by epigenetic and transcriptional patterns (Henning et al., 2018; Kakaradov et al., 2017; Youngblood et al., 2017; Yu et al., 2017). Some of the infection-induced epigenetic and transcriptional changes are transient and therefore only apparent in the effector state. Other changes are, once induced, maintained throughout the life of the cell, i.e., stable, and thus apparent in both the effector and memory populations. In this issue of *Immunity*, van der Veeken et al. (2019) investigated the mechanisms underlying transient versus stable gene regulation in CD8$^+$ T cells during the course of an acute infection.

Gene expression can be controlled in *cis* or *trans* (Emerson and Li, 2010). *Trans*-regulatory elements include transcription factors, non-coding RNAs, and DNA elements that control distant genes—i.e., where there is a spatial disconnect between the loci encoding the regulatory factor and its target. *Cis*-regulatory elements, on the other hand, regulate proximal genes located on the same chromosome and include promoters, enhancers, repressors, and non-coding RNA that act on the same DNA chain in which they are encoded. Of note, the gene-regulatory action of *cis*-elements (e.g., promoter) might additionally require the binding of *trans*-acting factors (e.g., transcription factor).

Previous studies have implicated numerous transcription factors in CD8$^+$ T cell state-specific gene expression (Kaech and Cui, 2012; Yu et al., 2017). However, the role of transcription factors has primarily been assessed in genetic deletion settings. Such data are difficult to interpret, because multiple transcription factors might have redundant functions and might normally act in competition. Furthermore, how exactly these transcription factors interact with *cis*-regulatory regions to induce chromatin remodeling and gene expression is not fully understood.

To explore the role of transcription factors without the drawbacks associated with genetic deletion approaches, van der Veeken et al. (2019) make use of naturally occurring sequence variation, i.e., polymorphisms between mouse strains. Specifically, they crossed mice from either the Cast/Eij or Spret/Eij strain to C57BL/6. The resulting F1 mice harbor 20 and 40 million allelic variants, respectively. If such genetic variation is located in *cis*-regulatory regions that play a role in epigenetic or transcriptional regulation in a particular cell state, this might result in differential chromatin accessibility or transcription from the two alleles (Figure 1). Thus, allelic imbalances in