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Mast cell-neural interactions contribute to pain and itch

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Summary

Mast cells are best recognized for their role in allergy and anaphylaxis, but increasing evidence supports their role in neurogenic inflammation leading to pain and itch. Mast cells act as a "*power house*" by releasing algogenic and pruritogenic mediators, which initiate a reciprocal communication with specific nociceptors on sensory nerve fibers. Consequently, nerve fibers release inflammatory and vasoactive neuropeptides, which in turn activate mast cells in a feedback mechanism, thus promoting a vicious cycle of mast cell and nociceptor activation leading to neurogenic inflammation and pain/pruritus. Mechanisms underlying mast cell differentiation, activation, and inter-cellular interactions with inflammatory, vascular and neural systems are deeply influenced by their microenvironment, imparting enormous heterogeneity and complexity in understanding their contribution to pain and pruritus. Neurogenic inflammation is central to both pain and pruritus, but specific mediators released by mast cells to promote this process may vary depending upon their location, stimuli, underlying pathology, gender and species. Therefore, in this review we present the contribution of mast cells in pathological conditions, including distressing pruritus exacerbated by psychologic stress and experienced by the majority of patients with psoriasis and atopic dermatitis and in different pain syndromes due to mastocytosis, sickle cell disease and cancer.

Keywords

Mast Cell; pain; itch; pruritus; sickle cell disease; mastocytosis; cancer; atopic dermatitis; psoriasis; neurogenic inflammation

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Contribution

KG wrote the segment on mast cell heterogeneity, activation and pain; designed the cartoons and supervised the analysis of mast cell activation in the skin of sickle mice; edited and prepared the manuscript for communication. IH wrote the segments on itch, reviewed and edited the entire manuscript.

Introduction

Mast cells are the “first responders” of the immune system. They defend against invading foreign organisms and act as central regulators of the immune system by instantly releasing chemical mediators that orchestrate immune defense and by attracting other cellular entities involved in immune defense. As a result, mast cells are a part of the innate and adaptive immune systems.(1, 2) Mast cells are omnipresent in the body and found near externally exposed surfaces such as the epithelial lining of the skin, mucosa of the gastrointestinal tract, meningeal membrane of the CNS, and airways of the lung. This close proximity to the external environment allows them to induce a rapid immune response to environmental stimuli, such as allergens and pathogens. Mast cells that become extremely sensitive can lead to well-known pathologic condition, allergy and anaphylaxis. In addition to defense against environmental stimuli, mast cells are also sensitive to the endogenous microenvironment and therefore contribute to many biological and pathological processes including vascular physiology, pain, itch and cancer.(1, 3–10) Increasing evidence suggests that mast cells contribute to various pathologies via their interactions with the vasculature and CNS. This review presents the advances made recently in understanding the contribution of mast cells to itch and pain via neuro-immune interactions. We also provide a general overview of mast cell origin and biology, which supports their diversity of function in different pathological conditions.

Origin and heterogeneity

Mast cells are derived from the myeloid lineage of hematopoetic stem cells (HSC) from the bone marrow (Figure 1).(1) Unlike most cells of myeloid lineage, mast cells do not exist in the blood, but reside in tissues similar to macrophages. HSCs in the bone marrow differentiate into mast cell precursors (MCP), which transmigrate to the tissues via blood. Once in the tissues MCPs differentiate into mast cells and propagate further. Their long life and ability to proliferate in a conducive microenvironment endows them the ability to have a long-lasting, sustained effect. These processes are highly regulated, yet mast cells display tremendous heterogeneity depending upon the microenvironment, anatomical location, and species.(11, 12) They accomplish both positive and negative immunoregulatory roles.(13) Mast cell heterogeneity dates back to 1895, when Hardy and West documented morphological differences between mast cells from different anatomical regions of rat.(14) Subsequently, increasing evidence shows tremendous differences in mast cell size, staining with chromogenic dyes, structure, histochemical properties, secretagogue, localization, sensitivity to stimuli/drugs, species and function.(12) For example, it is worth noting that in rodents, mast cells from the peritoneal cavity are responsive to inhibition with sodium cromoglycate, whereas those derived from intestinal mucosa are not.(12) Therefore, it is not surprising that sodium cromoglycate has not been significantly effective in mice or in clinical studies for sickle cell disease (SCD), which is characterized by significantly high mast cell degranulation.(7, 15) It is therefore critical to bear mast cell heterogeneity in mind while performing and/or comparing studies.

Mast cells as a “power house”

Mast cells act as “*power houses*” by releasing substances from their preformed granules within minutes of stimulation, which include proteoglycans, proteases, leukotrienes, biogenic amines, and cytokines (Figure 2).(12, 16) In addition to these immediate actions, mast cells also release cytokines, neuropeptides, and chemokines as a late response to stimuli following *de novo* synthesis. Therefore, mast cells exert instant effects that can be sustained or opposed through a late response. Mast cells shed extracellular vesicles by exocytosis including exosomes formed in the endosomes and/or by budding off of their plasma membrane.(16, 17) These extracellular vesicles transport mast cell contents, including micro RNA and major histocompatibility complex-II, to distant cells.(18) A distinguishing feature of mast cell-derived exosomes is sphingomyelin rich membrane compared to cholesterol in exosomes derived from other cells.(19) Exosomes are also found in biological fluids including cerebrospinal fluid and are involved in neuro-immune regulation and psoriasis.(16, 20–22) Several other processes have been recognized including tunneling nanotobes (TNT), physical extension via pseudopodia-like structures and extracellular trap formation, which may allow for increased interaction of mast cells with vasculature, nervous system and immune cells, described in detail below. Therefore, mast cells exhibit extraordinary complexity and contribute to diverse functions (1, 3–5, 8–10, 12, 16, 23–25). This review will examine the interactions of mast cells with the central and peripheral neural systems, pathophysiological mechanisms and therapeutic targets to treat pain and pruritus.

Mast cells interact with the nervous system

Mast cells localize in close proximity with afferents innervating the periphery, visceral organs, and the meninges.(26–28) Traditionally, mast cells co-exist with nerve terminals in the epidermis and in the meninges, but can be rarely found in the epidermis of normal skin. In psoriasis, epidermal hyperplasia and chronic inflammation, epidermal mast cells have been detected. We have observed mast cells in deep dermis in proximity to myelinated nerve bundles in transgenic mice with SCD (Figure 3), and others have reported mast cells in the brain.(26) Therefore, through the release of algogenic substances, mast cells can interact directly with the sensory terminals in the CNS and also through convergent pathways receiving inputs from the chemosensitive primary afferents in the skin. In the periphery mast cells are localized in the vicinity of primary afferent nerve terminals expressing pruriceptors and nociceptors involved in transmission of itch and pain, respectively.(29) Of the nerve fibers, unmyelinated small diameter C-fibers and myelinated A-delta fibers transmit nerve impulses activated by pruriceptors and nociceptors to the trigeminal and dorsal root ganglion, spinal cord and brain for the processing of itch and pain sensation. Histamine and substance P (SP) released from mast cells have been known for their algogenic- and itch-producing ability.(30) As described in Figure 2, many substances released through rapid degranulation, late response *de novo* synthesis, and vesiculation may contribute directly and/or indirectly to different processes underlying the generation of pain including neurogenic inflammation and neuroinflammation.

Mast cell influence on neurogenic inflammation and peripheral nociceptor sensitization

Neurogenic inflammation involves a multicellular system involving neurovascular interactions in the skin and visceral organs.(31, 32) It involves the release of vasoactive and proinflammatory neuropeptides SP and calcitonin gene-related peptide (CGRP) from the sensitized peripheral nerve endings; this leads to vascular dilatation, plasma extravasation, leukocyte infiltration, and mast cell activation.(33, 34) Upon activation, mast cells release neuropeptides, histamine, and other algogenic mediators that stimulate nerve endings to release more neuropeptides, leading to a vicious cycle of mast cell activation and peripheral nerve sensitization further amplifying vascular leakage and neurogenic inflammation.(33, 35) This process leads to painful wheals and flares (redness and heat), pruritus, and edema, and further sensitizes sensory nerve endings.(36–38) Additionally, substances such as tryptase, SP, and histamine released from mast cells in response to noxious stimuli have direct vasoactive effects on permaeability and leukocyte migration, adhesion, and trafficking. On the other hand, neuropeptides can be released from sensitized nerve endings antidromically and following axonal reflex. These feedback mechanisms result in a vicious cycle of mast cell activation and peripheral nerve sensitization and may be involved in the transition from acute to chronic pain and may lead to challenges in treating pain.(39)

Mast cell's role in central sensitization and neuroinflammation

Action potentials generated in sensitized nerve terminals travel orthodromically to the dorsal horn of the spinal cord and excite second order nociceptive neurons. However, continuous excitation of spinal dorsal horn neurons leads to central sensitization and antidromic release of neuromodulators from the spinal cord to the periphery and release of neuropeptides from peripheral nerve endings. This process occurs in chronic pain conditions such as sickle cell disease.(40–42) Brain neuroimaging also demonstrated the existence of central sensitization in patients with SCD.(41, 43) Mast cells are also present in the meninges and different regions of the brain including thalamus and are known to influence blood-brain barrier permeability.(26, 44–46) Their number and distribution in the brain is affected by the environment, age, gender, pathological condition, and stimuli such as stress.(26, 27, 47–50) In the brain mast cells may directly interact with the nociceptive neurons depending upon their location and proximity to nociceptive pathways and/or via their bidirectional interactions with microglial/astroglial cells.(51, 52) These interactions lead to increased release of cytokines and chemokines as well as histamine by glial and/or mast cells, thus leading to amplified neuroinflammation. Therefore, in spite of a relatively small number of mast cells in the brain, their effect on neuroinflammation is significant. Almost 50% of histamine and 25% of TNF-α is attributed to mast cells in the rat brain.(26, 53) Mast cell activation may therefore contribute to nociception via direct central interactions in the brain as demonstrated recently in a spinal nerve ligation model in mice.(44) This study also demonstrated that nerve growth factor (NGF), formalin, and dynorphin-induced hyperalgesia involved degranulation of mast cells in the brain of mice. It is likely that mast cells in the CNS are a critical player in the multicellular repertoire orchestrating central sensitization.

Molecular mechanisms of mast cell activation and interaction with nervous system

The neuroimmune interface is increasingly recognized as a central player in chronic and acute pain of inflammatory, neuropathic, and nociceptive origin. As discussed above, it may even underlie the transition from acute to chronic pain. Tremendous heterogeneity in mast cell presence, activation, species, gender, and secretagogue are further compounded by different pathological conditions, each with a unique molecular and cellular microenvironment. For example, in neuropathic pain in diabetes, high glucose is a major factor, while in SCD, sickle red blood cells (RBCs) occluding blood vessels is the underlying pathologic feature. This diversity is challenging in developing a unified mechanism underlying the contribution of mast cells in pain across different pathologies, mouse models, and *in vitro* studies. Molecular mechanisms are critical for developing targeted therapies, but, in the case of mast cell targeting, these have to be understood on a case-by-case basis using disease-specific validated mouse models and clinical studies. Molecular targets on mast cells are presented in an in-depth review by Harvima et al. 2014. (4) Here, we describe recent developments in the basic mechanisms of mast cell activation.

Mast cell degranulation

Within minutes of mast cell stimulation, degranulation releases secretory granules pre-loaded with lysosomal enzymes, proteoglycans, proteases, leukotrienes, biogenic amines, and cytokines (Figure 2).(16, 54, 55) It involves activation of FcεR1, followed by cytoskeletal rearrangement with disassembly of F-actin and microtubule formation.(56) This calcium-independent process mediated by FcεR1 activation of Fyn/Gab2/RhoA leads to microtubule polymerization permitting the translocation of secretory granules to the plasma membrane(57). The exocytosis of granules from the plasma membrane involves activation of Lyn and Syk downstream of activated FcεR1, leading to calcium mobilization, granule-membrane fusion, and degranulation. Several degranulation products play a key role in pain hypersensitivity at the peripheral and CNS level as discussed below.(8, 26, 58) Signaling pathways leading to degranulation offer targets to inhibit degranulation using recently developed pathway inhibitors. (4)

“Piecemeal degranulation (PMD)” refers to the selective release of a part of the granular content and occurs in many pathological conditions including chronic inflammation.(59) PMD may lead to the heterogeneous release of substances, which may depend upon the stimuli leading to degranulation, although this process is not fully understood. Another process called “*pseudopod translocation*” or “*transgranulation*” refers to the direct contact of mast cells with adjacent cells and transfer of granular content to the other cell.(16, 60) It involves direct transfer of granules from mast cells to cells in the vicinity by exocytosis followed by their intake by the recipient cell or release of mediators into the microenvironment. This is also accomplished by extensions of mast cells much like pseudopodia to reach distantly, followed by detachment from the cell body (Figure 3). This mode of mast cell communication has been demonstrated with vascular and neural system and others.(60–62) Because of the close proximity of mast cells with the vasculature and nerve fibers, it is likely that transgranulation may physically disrupt physiological structures,

similar to axonal injury, and thereby contribute to neuropathic pain and alterations in the vessel wall. Indeed, functional synapses between mast cells and nerve endings were demonstrated to underlie pain in irritable bowel syndrome.(63) These processes are demonstrated in Figures 3 and 4 and described under the section, "*Mast cells contribute to pain in SCD*".

Release of substances synthesized de novo

As a late response to various stimuli, mast cells release cytokines and chemokines synthesized de novo via several different mechanisms involving multiple receptors including an array of cytokine receptors. These mechanisms are complex and involve cross-talk between several different receptors depending upon the microenvironmental cues particularly from adjacent inflammatory cells such as T-cells, neutrophils, macrophages, and glial cells. IgE receptors also mediate this response in cooperation with pattern recognition receptors including Toll-like receptors (TLRs). TLRs require recruitment of CD14 or functional CD48 for the effect of TLR ligands.(64) Murine mast cells express CD14, whereas human mast cells show relatively weak expression of CD14. TLRs have attracted significant attention in the progression and maintenance of neuropathic pain involving the peripheral and central nervous systems at a multicellular level.(65) IgE-independent receptors also regulate mast cell *de novo* synthesis. This involves *c-kit*, a receptor tyrosine kinase (RTK) for the stem cell factor (SCF), which is expressed on murine as well as human mast cells.(66) In addition to the release of products from mast cells, SCF also promotes the development, survival, and proliferation of mast cells.(67) Several cytokines including IL-3 and IL-4 have been demonstrated to influence mediator release from mast cells. IL-4 synergizes with SCF to facilitate the release of mediators via the MAPK/ERK signaling pathway and transcriptional regulation by AP-1 (activator protein-1).(68) The mechanisms of different cytokine-induced mediator release are not clear, but depending upon their cross-talk with SCF/*c-kit*, their activity can be inhibited with RTK inhibitors such as Imatinib.(4, 7, 8) Considering that a large number of inflammatory mediators including cytokines, chemokines, and neuropeptides are released from mast cells and their close proximity to axonal processes and potential to recruit/influence other inflammatory cells, mast cells may significantly contribute to pain hypersensitivity and activation of nociceptive mechanisms in inflammatory and neuropathic pain.

Exosomes/extracellular vesicles (EVs)

Exosome release is a process common to many cell types whereby they are formed in the endosomes and released by exocytosis, mostly delivering RNAs including microRNA.(16, 69, 70) Several substances affecting multiple cellular and pathological functions have been suggested to be released from EVs and exosomes (reviewed in (16)). Importantly, mast cell exosomes contain MHC-II molecules that can activate T-cells, which have been shown to contribute to neuropathic pain.(71) Mast cell exosomes contain precursors of TNF-α and proteases, both of which are critical players in inflammatory and neuropathic pain in several pain-associated conditions. Mast cell exosomes have been demonstrated to interact with sensory nerve fibers, leading to the release of SP and activation of both mast cells and nociceptors involved in pain hypersensitivity.(72) Because of their small size (30–150 nm), exosomes can migrate and elicit an effect at longer distances. Mast cell exosomes have been

observed in the cerebrospinal fluid (CSF) and influence synaptic interaction in the CNS.(21) On the other hand, larger EVs (100–5,000 nm) are challenging to distinguish from degranulation because of the common markers surrounding their membranes (Figure 3). Nonetheless, both release substances with the potential to influence neuroimmune interactions.

Tunneling nanotubes (TNTs) facilitate transport of mast cell products

According to Weng et al., TNTs offer rapid and direct communication to "*alarm*" other cells.(73) TNTs are F-actin-based structures that form cytoplasmic as well as non-cytoplasmic connections with distant cells in a process called "*reticulation*".(74) TNTs are much like filopodia/pseudopodia described above, except that TNTs are non-adherent and span distances longer than 100 microns. Murine mast cell TNTs were observed first with co-stimulation of FcεR1 with CCR1 and MIP-1α.(75) Distinct differences between EVs and nanotubes and their function are still emerging but they have been implicated in tumor progression and resistance to therapy, which can be important in cancer pain.(76) In the present context, TNTs offer another mechanism for mast cells to interact directly with neural, vascular, and immune systems as all of these systems engage in TNT formation.

Mast cell extracellular trap (MCET) formation

Extracellular trap formation is a feature of cells of innate immune system in which they ensnare invading organisms in self-defense.(77) This process involves citrullination of histones in the chromatin leading to the decondensation and unfolding of chromatin, disassembly of DNA, and eruption of web-like nuclear contents ejected extracellularly.(78) Citrullination of histones is catalyzed by protein arginine deaminase 4 (PAD 4).(79) Similar to neutrophil extracellular traps (NETs), mast cells also form extracellular traps.(80) MCET formation is dependent upon the formation of reactive oxygen species (ROS) and engages TLR4. In addition to DNA and histones, MCETs contain fibers with mast cell-tryptase. Increasing evidence suggests the presence of NETs and MCETs in pathological conditions including psoriasis and SCD.(81) MCET is not stimulated by pathogen invasion, rather it is stimulated with IL-23 and IL-1β, resulting in the release of IL-17. It is believed that MCETs may contribute to immunopathology in chronic inflammatory conditions.(82) Secukinumab (Cosentyx), an inhibitor of IL-17A, is approved for the treatment of plaque psoriasis and psoriatic arthritis in many countries including the USA and Europe.(83) IL-17 is also implicated in pain, and it is likely that MCET formation-induced release of tryptase and IL-17 activate the nociceptors on nerve endings via protease-activated receptor-2 (PAR2) and contribute to inflammation, itch and pain.

Mast cell products that contribute to pain

Many substances are released from mast cells that either directly or indirectly contribute to pain via their effect on nervous system or immune and/or other cells. We describe a few substances that support the role of mast cells in nociception. Several substances with a potential for drug targeting have been described in a review by Harvima et al.(4)

Histamine

Histamine via its several receptors (namely H1R, H2R, H3R, and H4R) plays a critical role in neurogenic inflammation and pain transmission in the periphery and CNS; its specific effects in pain and itch have been reviewed in.(33) Histamine released from mast cells acts in an autocrine and paracrine manner by stimulating mast cells and nerve endings, respectively.(4) Both C- and A-delta fibers are responsive to histamine in the periphery and CNS. Histamine stimulates SP and glutamate release from nerve terminals in inflammatory conditions.(84) Interestingly, histamine interacts with neuropeptides in a bi-directional manner. CGRP stimulates mast cell release of histamine, whereas histamine administration peripherally induces CGRP release into the CSF and trigeminal ganglion, suggesting a synergism between neuronal mast cell interactions involving histamine and CGRP in animal models.(85–87) Antagonists of H1–H4 receptors have been demonstrated to inhibit neurogenic inflammation, pain, mechanical and/or thermal hyperalgesia and itch in animal models in a case-specific manner.(33) In clinical trials, H1R hydroxyzine hydrochloride improved the symptoms in about 30% patients with interstitial cystitis.(88) The H2R antagonist cimetidine given orally demonstrated a significant improvement in pain symptoms in painful bladder syndrome in a randomized, double-blind, placebo-controlled trial.(89) However, evidence of the effectiveness of histamine receptor targeted therapies in pain is still needed in randomized controlled phase 3 trial versus standard of care or placebo for different pain conditions.

Tryptase

Serine proteinases, tryptase, chymase, and cathepsin-G are stored in secretory granules of mast cells and released upon degranulation associated with matrix remodeling. Of these, tryptase is a predominant protein of mast cell granules and interacts with PAR-2 on nerve endings.(90, 91) PAR-2, in turn, co-activates TRPV1 channels stimulating the release of neuropeptides SP and CGRP from nerve terminals.(92) These neuropeptides activate neurogenic inflammation discussed above, and also initiate nociceptive transmission by activating nociceptors on nerve terminals as well as co-activating mast cells in an autocrine/paracrine manner. However, no satisfactory inhibitors are available to target tryptase or PAR-2 clinically, but these mechanisms remain a potentially attractive target to develop pharmacotherapeutics to treat pain. Serum tryptase can also serve as a biomarker for mast cell load in the body because the majority of circulating tryptase is contributed by mast cells.(93)

Nerve growth factor

Mast cell NGF has autocrine functions that include differentiation, proliferation, and survival.(94) Mast cells synthesize NGF by the cleavage of pro-NGF by tryptase, and upon stimulation stored NGF is released.(95, 96) In an autocrine manner, NGF stimulates mast cells to release pro-nociceptive mediators including histamine as well as NGF, supporting a positive feedback loop. NGF and its receptor TrkA evoke sustained pain hypersensitivity via TRPV1 channels on the neurons in the periphery and CNS.(97) NGF is present in remarkably high levels in inflamed tissue and persistent pain states.(98) Once activated, mast cells and NGF may therefore amplify each other’s effects, resulting in a highly noxious

microenvironment leading to uncontrolled pain. In clinical trials NGF targeted therapies using NGF-sequestering antibodies were highly successful in controlling pain, but were put on hold by the FDA because of adverse events involving the sympathetic nervous system and bones.(94, 99)

Sphingosine-1-phosphate

Sphingosine-1-phosphate (S1P), a sphingolipid metabolite, acts as a second messenger in a variety of cellular processes.(100) S1P is produced from its precursor sphingosine by the catalytic action of sphingosine kinase (SphK) 1 and 2.(101) Mast cells release S1P, which acts in an autocrine manner by binding to its receptors $S1P_1$ and $S1P_2$ on mast cells. SphK1 mediates antigen-induced degranulation and migration of mature and developing mast cells via S1P receptors after antigen-induced FcεR1 aggregation.(101, 102) S1P activity is thought to support mast cell recruitment to sites of inflammation and to augment chemokine release to attract other inflammatory cells.(101) Thus, S1P is important in the early phase of inflammation, and because of its autocrine activity, it may contribute to sustained and amplified mast cell degranulation. Several antagonists of S1PRs have been developed, including FTY720 (fingolimod), which has been approved for clinical use in multiple sclerosis.(103) FTY720 is phosphorylated by SphK and binds to 4 of the S1P receptors and modulates receptor activities.(104) S1PR antagonists including FTY720 have been demonstrated to inhibit bone cancer pain and chemotherapy-induced painful neuropathy in murine models.(105, 106) It is likely that mast cells contribute to these S1P-dependent painful conditions; however, this remains to be proven.

Mast cell's contribution to the pathobiology of pain and pruritus

Mast cells have roles in a variety of pathological conditions including chemotherapy-induced neuropathic pain, osteoarthritis and pelvic pain.(7, 107–110) We will review recent research suggesting that mast cells are important to the pathobiology of pain and pruritus, in mast cell activation disease (MCAD), SCD, and cancer, and in atopic dermatitis and psoriasis, respectively.

Mast cell activation disease

MCAD is an umbrella diagnosis for mast cell-associated conditions including mastocytosis and mast cell activation syndrome (MCAS).(8, 111) Mastocytosis is a rare disease characterized by increased mast cell proliferation. In contrast, MCAS is a more common non-proliferative condition with increased activation of mast cells. Symptoms range from hypotension to pain. However, pruritus and pain are among the most common symptoms with variable location and intensity across MCAS and mastocytosis. Common features of pain include headache, abdominal pain, musculoskeletal pain, and wheals and flares such as those may occur in neurogenic inflammation. Mast cell release of the many different substances discussed above underlies the symptoms of MCAD. Additionally, tissue-specific localization of mast cells may be important in eluding a diagnosis of MCAD. Therefore, presentation of MCAD symptoms is markedly heterogenous and difficult to diagnosis clinically. For this reason, cutaneous mastocytosis with mast cell activity limited to the skin is relatively easy to diagnose and treat, but diagnosis and treatment of systemic mastocytosis

with mast cells infiltrating visceral organs remains a major challenge.(112, 113) Circulating tryptase is a common feature of mastocytosis and MCAS, but the proposed diagnostic criteria include elevated tryptase during symptomatic episodes.(114) In some chronic conditions with inflammation and pain, circulating levels of tryptase may be insightful in determining the activation of mast cells during episodes of symptoms similar to MCAS. In chronic conditions, endogenous alterations in the tissue microenvironment with disease progression and organ pathology as well as use of drugs may stimulate mast cell differentiation, proliferation, and activation as described below for SCD and cancer. Targeting of c-*kit* with RTK inhibitors including imatinib mesylate and similar inhibitors is a promising strategy for the control of mast cell activity.(111)

Mast cell activity in pain in sickle cell disease

Globally SCD is the most common inherited monogenic disorder. It is caused by a mutation in the b-globin gene of the hemoglobin molecule, which leads to sickling of red blood cells (RBC). Clinical sequelae of this single mutation are extremely complex including hemolytic anemia, multi-organ dysfunction, often life-long pain, and reduced survival. Pain in SCD can occur as chronic pain and unpredictable and recurrent episodes of acute pain due to vasoocclusive crises (VOC); these conditions lead to hospitalization and opioid analgesia (reviewed in (32, 42, 115–118)). Mast cell involvement in SCD appears to underlie many features of sickle pain. Most evidence is based on studies using a transgenic mouse model of SCD, HbSS-BERK expressing human sickle hemoglobin exclusively and presenting characteristics of pain similar to those observed clinically.(42, 119, 120) These sickle mice show extensive mast cell degranulation directly affecting nerve bundles and blood vessels in the skin (Figures 3 and 4). In the 3-D rendition of the confocal image of sickle mouse skin, degranulating mast cells are seen as continuous projections surrounding nerve fibers. Tryptase-loaded granules containing mast cells nest near the nerve plexus, with pseudopodia-like extensions and vesicular structures scattered all along nerve bundles and their vicinity (Figure 3). The spread of mast cell extracellular extensions also suggests the presence of extracellular traps. We have demonstrated the presence of MCETs in sickle mouse skin.(121) Similarly, degranulating tryptase-releasing mast cells are seen hugging and invading blood vessels, suggestive of a direct effect on sickle cell vasculopathy and contribution to VOC (Figure 4). Increased transmigration of myeloid cell progenitors from bone marrow to the periphery was demonstrated in sickle mice.(122) Increased cytokines in the SCD microenvironment—including RANTES, GM-CSF, and MCP-1—may contribute to the transmigration of myeloid progenitors and their differentiation and proliferation in tissues. Therefore, mast cells may contribute to different pain conditions in SCD described below.

[i] Dactylitis

Dactilytis is painful swelling of hands and feet, increased local temperature, and erythema, which occurs in a significant percent of children with SCD within one year of age.(123, 124) It is thought to involve bone marrow necrosis, medullary infarction, and leukocytosis. We observed that an intravenous injection of Evans blue dye revealed increased vascular leakage and swelling in the skin, hands, and feet in HbSS-BERK sickle mice as compared to control

HbAA-BERK mice expressing normal human hemoglobin A; these findings are suggestive of plasma extravastion and neurogenic inflammation.(7) Skin biopsies of these mice revealed increased release of neuropeptides SP and CGRP and several inflammatory cytokines including TNF-α, GM-CSF, IL-6, and MP-1α. Treatment with the mast cell stabilizer sodium cromoglycate and the c-kit inhibitor imatinib significantly reduced Evans blue leakage as well as neuropeptide and cytokine release from skin biopsies; this indicates a role for mast cells in this neurogenic process. It is therefore likely that dactylitis may involve neurogenic inflammation and mast cell-mediated erythema, swelling, and pain.

[ii] Chronic Pain and Requirement of High Dose of Morphine

Chronic pain is challenging to treat in SCD and requires relatively larger doses of morphine than analogous pain in other conditions.(125) Similarly, sickle mice require a significantly high dose of morphine (20 mg/Kg) to treat tonic hyperalgesia.(119) Electrophysiological recordings show peripheral and central sensitization in these sickle mice.(40, 126) The mechanisms of sickle pain are still emerging, but mast cell activation may contribute to this process based on clinical observations and sickle mice. The skin of sickle mice constitutively has a dense population of degranulating mast cells expressing *c-kit*, FcεR1, and tryptase with significantly higher transcripts of TLR4.(7) Treatment of sickle mice with cromolyn for five days showed a trend in reduction of hyperalgesia, but it was not statistically significant. Treatment with imatinib for 5 days significantly reduced hyperalgesia in these mice. Co-treatment with a sub-analgesic dose (10 mg/Kg) of morphine significantly reduced hyperalgesia in both cromolyn and imatinib-treated mice. Furthermore, genetic deletion of mast cells in sickle mice completely attenuated hyperalgesia. These data demonstrate the role of mast cells in hyperalgesia in sickle mice and suggest that inhibition of mast cell activity may improve opioid analgesia. It is therefore likely that morphine, a known activator of mast cell degranulation, stimulates mast cell degranulation concomitant to its analgesic effect on the CNS. These co-treatment strategies deserve consideration in treating pain in SCD and conditions associated with mast cell activation. In a well-controlled clinical study, sickle patients with chronic pain showed significantly elevated plasma tryptase levels, almost twice that of ethnicity-matched control subjects ($p=0.0053$).(127) Comparisons between sickle patients with no pain, sickle patients with pain, and controls were statistically significant ($p=0.006$). Opioid use was significantly higher in sickle patients with chronic pain than in patients with no pain; this could contribute to the elevated plasma tryptase levels in patients with chronic pain. We observed increased release of neuropeptides from the dorsal root ganglion (DRG) and skin in response to morphine treatment, but a decrease with cromolyn or imatinib in sickle mice, suggesting that mast cells contribute to neuropeptide release in sickle mice in the periphery, which may contribute to pain and have direct effects on vascular permeability and vascular physiology. Together, data from sickle mice and clinical observations demonstrate the contribution of mast cells to chronic sickle pain, and further investigation is merited. In summary, mast cells release tryptase and SP, which sensitize nociceptors on nerve endings by activating PAR-2 and TRPV1 channels, leading to the release of neuropeptides SP and CGRP (Figure 5). Neuropeptides stimulate arteriolar dilatation and vascular leakage resulting in neurogenic inflammation. Additionally, neuropeptides co-activate mast cells and nociceptors in an autocrine and paracrine manner,

thus promoting a vicious cycle of mast cell activation, neurogenic inflammation, and pain. (7)

[iii] Acute pain

Acute pain in SCD is evoked during vasoocclusive crises (VOC). Vascular occlusion is caused by sickle RBCs obstructing blood flow leading to impaired oxygen supply and nutrients to the organs, vascular dysfunction, ischemia/reperfusion injury, inflammation, and end organ damage.(42, 115, 128) Ischemia/reperfusion injury stimulates mast cell degranulation.(129) VOC pain is associated with ischemia/reperfusion injury in SCD, and we simulated VOC-like pain following hypxia/reoxygenation in sickle mice.(120) Incitement of hypoxia/reoxygenation in sickle mice led to an appreciable increase in cutaneous mast cells and a significant increase in their degranulation as compared to sickle mice in normoxic conditions.(130) Increased mast cell degranulation in the skin was accompanied by a significant elevation of circulating tryptase, β-hexosaminidase, serum amyloid protein (marker of inflammation), and SP; this finding is indicative of mast cell degranulation and neurogenic inflammation during VOC. These molecular markers of mast cell degranulation were accompanied by significantly elevated Evans blue leakage following hypoxia/reoxygenation as compared to normoxia. Together, these alterations under hypoxia/reoxygenation suggest an increase in neurogenic inflammation associated with mast cell degranulation in sickle mice. Consistent with our findings of increased SP after hypoxia/reoxygenation and neurogenic inflammation, an increase in circulating SP in acute pain during VOC has been reported in patients with SCD.(131–133) Together, these pre-clinical and clinical observations support the contribution of mast cells in acute pain during VOC.

[iv] Headache and regional pain syndromes

Acute pain may manifest in different regions of the body in SCD, including headaches and painful penile erections in priapism.(124) The pathophysiology of these specific regional pain syndromes is not understood. However, mast cells may have a role in migraine headaches.(134–137) The frequency of migraine headaches is higher in females than males, and it has been demonstrated that estrogen-related mast cell recruitment from the spleen and increased density of dural mast cells and their activation are higher in females than male rats.(138) This observation suggests that pain should fluctuate with the estrous cycle in females. The dural layer, which is rich in mast cells, is also highly innervated with nociceptive afferents and thus may facilitate mast cell-nociceptor interactions because of their close proximity and sensitivity to environmental cues.(139, 140) SCD female mice and female patients experience more pain than their male counterparts and is challenging to treat. (141)

Novel mast cell-targeted therapies for SCD pain

The mast cell inhibitors cromolyn and imatinib reduce hyperalgesia in sickle mice.(7) Targeting mast cells may improve opioid analgesia in patients with SCD or other pain conditions in which mast cells are activated. We have also observed that mast cell inhibition with nociceptin receptor (NOP/R) agonists and cannabinoids have an analgesic effect.(32, 142, 143) The combined NOP/R and mu opioid receptor agonist AT-200 and cannabinoid CP 55940 were able to attenuate both chronic and acute hyperalgesia in sickle mice.(130,

144) The anti-nociceptive effects of AT-200 and cannabinoids also inhibited mast cell degranulation and circulating neuropeptides and tryptase under normoxia and hypoxia/reperfusion evoked hyperalgesia as well as neurogenic inflammation in sickle mice. Chronic treatment with AT-200 or cannabinoids did not lead to tolerance, whereas morphine tolerance and morphine-induced hyperalgesia are thought to occur in several pain conditions. Morphine promotes mast cell degranulation, and it is likely that in chronic pain conditions morphine may cause hyperalgesia via the activation of mast cells. Therefore, analgesic strategies, such as those described herein to target mast cells, may be potentially beneficial in pain treatment without causing tolerance and/or hyperalgesia. Tryptase and mast cell markers appear to have a prognostic value in strategizing mast cell-based treatment strategies. Sickle patients frequently use morphine, and pruritus is a common feature in sickle patients.(145) Other drugs also lead to pruritus in SCD, including crizanlizumab therapy, a recently developed and promising new therapy.(146) It is conceivable that co-treatment with mast cell stabilizing agents along with morphine or other drugs may prevent and/or reduce the discomforting symptoms of pruritus.

Although the evidence is limited, clinical observations support a role for mast cells in chronic and acute pain in SCD. In a case report, a patient with SCD having multiple VOCs per year stopped having VOCs with imatinib treatment.(147) Upon discontinuation of imatinib, VOCs recurred. No effect was observed on hematologic analysis or HbF levels. We believe that imatinib's effect could have occurred via inhibition of mast cells. Therefore, clinical trials are warranted to test mast cell inhibitory strategies in pain refractory to opioid therapy. Several naturally occurring and pharmacotherapeutics for mast cell stabilization have been reviewed, offering the advantage of carefully selecting the agents for preventive and therapeutic purposes (Table 1).(148)

Mast cells contribute to pain in pancreatic cancer

One of the mechanisms of pain in pancreatic cancer is direct damage to the nerves in pancreatic tissue, also called pancreatic neuropathy, neurogenic inflammation, and neuritis.(5) Increased hypertrophy and nerve density, immune cell infiltration in the parenchyma, and increased release of neurotrophic growth factors have been observed in specimens from patients with pain in pancreatic cancer, but not in the normal pancreas.(149) TRPV1 channel expression is activated on pancreatic nerve endings, which are involved in the release of SP and CGRP.(150, 151) In addition to their contribution in pain conductance directly, these neuropeptides cause neuroinflammation by activating neutrophils, mast cells, and macrophages and chemotaxis.(149) Immune cells observed around the pancreatic nerves are predominantly infiltrated with mast cells in patients with pain, whereas with T-lymphocytes in patients without pain.(152) NGF, a key contributor to pain/hyperalgesia, and its receptor tropomyocin kinase receptor (TrkA) mRNA were increased several-fold in the pancreatic tissue of patients with high pain scores.(149) NGF is involved in neural repair and released by multiple cell types including pancreatic and immune cells in response to nerve injury and from mast cells as discussed above.(151) NGF promotes chemotaxis and augments SP and CGRP release. Thus, it is likely that mast cells contribute to pain in pancreatic cancer and perhaps other cancers. We found that chronic morphine treatment led to increased mast cell degranulation and SP in tumors of transgenic mice with breast cancer.(6) SP

immunoreactivity co-localized with mast cells, indicative of a pro-nociceptive microenvironment produced by morphine-induced mast cell activation.

We have reviewed above the potential role of mast cells in many painful conditions and their role is increasingly being demonstrated in pain.(8) However, a recent report elegantly demonstrates that peripheral sensitization is not mast cell-dependent using a CFA model of pain in male mice.(153) This could be because the CFA model stimulated responses were confined to the paw, which contrast with testing in actual disease models with constitutive mast cell activation and testing paradigms using responses akin to the disease being examined. We have demonstrated that analgesic responses vary between transgenic mouse models of SCD or cancer showing the evolutionary spectrum of the human disease as compared to one time CFA-induced hyperalgesia in C57BL/6 mice.(154)

Skin innervation and itch

The free nerve endings in the basal, spinous, granular and sometimes corneal layers of the epidermis are essential for itch sensation possibly through a reciprocal synaptic-like interaction between keratinocytes and nerves.(155) In addition, keratinocytes positive for β-endorphin have been reported to be clustered around the terminal ends of unmyelinated fibers, and these nerves can be activated through the μ-opioid receptor.(156) However, it is not clear whether there are itch-specific peripheral sensory neurons in the skin. They are probably a subgroup of nociceptive neurons, e.g., similar to those expressing the MrgprA3 receptor in mice.(157) In contrast, nonpruriceptive nociceptive neurons have been identified, including nociceptors with C- or A-fibers that are activated by mechanical stimuli, heat or capsaicin. Subsets of mechano-insensitive nociceptive C-fibers respond to histamine and consequently can release neuropeptides, such as SP and CGRP. The mechano/heat-sensitive nociceptors do not react markedly to histamine, but react to a cowhage plant protease, producing stinging itch possibly through protease-activated receptor-2 or -4 (PAR-2 or -4). However, the nerves express a high variety of receptors and ion channels, and therefore it is not clear, which combinations of receptors contribute to each type of itch and pain sensation. Additional complexity is caused by inhibitory and excitatory interneurons, different spinothalamic tract neurons and the central nervous system in the brain.(155, 158, 159) Furthermore, inflammation *per se* produces marked changes to the cutaneous neural network.

Mast cell-neural interactions and itch in psoriasis

Psoriasis is a chronic inflammatory and scaly skin disease that is aggravated by psychosocial stress in 40–80% of patients.(160) Another common feature affecting 60–90% of psoriatic patients is pruritus that can have different forms, such as stinging, pinching, tickling, crawling, burning or even pain sensations.(161)

Emotional stress leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis and consequent release of stress hormones, including corticotropin-releasing hormone (CRH), adrenocorticotropic hormone and glucocorticoids. In addition, human skin has its own functional peripheral equivalent of the HPA axis.(162) Other systems activated during

stress include prolactin, α-MSH, neuropeptides, neurotrophins and the sympathetic nervous system.(162) This plethora of systems activated during stress means that it is difficult to pinpoint a specific mechanism that is responsible for pruriception in psoriasis. However, one major focus in this context has been the concept of neurogenic inflammation where cutaneous nerve fibers and mast cells interact.(161)

Psoriasis typically presents symmetric cutaneous lesions, e.g., on the knees and elbows, suggesting an involvement of peripheral nerves. Furthermore, there are clinical cases where nerve damage has produced clearance of chronic psoriasis, but the lesions reappeared with the recovery of cutaneous sensation.(163) Several studies have shown that sensory fibers and neuropeptides, such as SP, neurokinin A and vasoactive intestinal peptide (VIP), are increased in the psoriatic lesion compared to nonlesional skin.(164–166) In double-staining experiments, the apparent contacts between neurofilament$^+$ sensory nerves and tryptase$^+$ mast cells are more numerous in developing and mature psoriatic lesions than in nonlesional psoriatic, normal or lichen planus skin.(167, 168) In addition, the contacts between SP$^+$ and CGRP$^+$ fibers and tryptase$^+$ mast cells are increased in the psoriatic lesion, but not the contacts between VIP$^+$ fibers and tryptase$^+$ mast cells.(169) This suggests an interactive role for SP/CGRP$^+$ sensory nerves and mast cells in psoriasis.

The MC_{TC}-type (tryptase$^+$, chymase$^+$) of mast cell is increased in number in the upper dermis of psoriatic lesions. However, chymase undergoes partial inactivation during the lesion development possibly through the action of protease inhibitors, whereas tryptase retains its activity [reviewed in (170)]. This decrease in chymase activity is interesting, as chymase can cleave neuropeptides SP and VIP, whereas tryptase can cleave VIP and CGRP, but not SP.(171–173) The net outcome may be enhanced SP-mediated neurogenic inflammation. As tryptase can activate PAR-2, the serine proteinase released may further activate mast cells in a para- or autocrine fashion, which is supported by the finding that the proportion of tryptase$^+$ mast cells expressing PAR-2 is increased in psoriatic lesions.(174) Further, PAR-2 activation sensitizes TRPV1 resulting in enhancement in SP and CGRP release (175), and TRPV1 is present in SP$^+$ fibers and mast cells in human skin.(176) Furthermore, the traditional itch-mediator, histamine, can stimulate sensory nerves and neurogenic flare.(177) The increased interstitial histamine concentration in the psoriatic lesion (178) as well as some clinical efficacy of the H1-antihistamine cetirizine (179) suggest some role for histamine in the modulation of neuronal activity in psoriasis. The interaction between CRH and its receptor CRH-R1 on mast cells has been suggested to mediate some of the stress-related neuroendocrine actions in psoriasis.(25) This theory is supported by findings that the expression of CRH is reportedly increased in the epidermis, sweat glands and hair follicles in the psoriatic lesion (180), and the proportion of tryptase$^+$ mast cells containing CRH-R1 immunoreactivity is higher in the lesional (53±19%) than nonlesional (28±19%) psoriatic skin (unpublished results).

When compared with the non-pruritic-type of psoriasis, the pruritic one has been reported to associate with the following parameters: the psoriasis area and severity index (181, 182); increased levels of SP, its receptor NK1R or NK2R, and nerve fibers (166, 181, 183); decreased levels of SP-degrading neutral endopeptidase (183); increased levels of NGF and/or its receptor TrkA (181, 183) as well as decreased expression of semaphorin-3A (a

molecule that inhibits neurite outgrowth) (182, 184) suggesting an imbalance between nerve elongation and repulsion factors; increased numbers of total mast cells, degranulated mast cells, and free mast cell granules in close apposition to nerve fibres; increased numbers of IL-2$^+$ cells and strong vascular expression of E-selectin (183); decreased epidermal expression of dynorphin A and κ-opioid receptor, but no change in the μ-opioid receptor (184, 185); and increased numbers of inflammatory cells immunopositive to gamma-aminobutyric acid and its $GABA_A$ receptor.(186) Therefore, the psoriatic pruritus is associated with neural, mast cell and molecular changes in skin lesions.

Mast cell-neural interactions and itch in atopic dermatitis

Atopic dermatitis (AD) is characterized by chronic or chronically relapsing eczematous skin inflammation, IgE-mediated sensitization to environmental allergens, and epidermal barrier dysfunction. Itch is a hallmark of AD, and it can be aggravated by a range of pruritogens, inflammatory mediators, activation of receptors on sensory nerves, skin dryness, heat, sweat and psychosocial stress. Even the healthy-looking atopic dry skin can be itchy.(187, 188)

Mast cells are crucial in the IgE-mediated skin wheal reaction and its associated itch in AD. In the lesional AD skin, the number of tryptase$^+$ mast cells is increased to some extent, but the enzyme activity of chymase is decreased, when compared to nonlesional skin.(189, 190) Partial inactivation of chymase has been detected also in the IgE-mediated prick-test wheal reaction.(191) A possible explanation for the reduced chymase activity is the localization of chymase inhibitors in mast cells.(191) The inactivation of chymase may have consequences to the course of inflammation, as chymase can degrade to a varying extent IL-6, IL-13, TNF-α, IL-4, IL-5, SP and VIP.(172, 192, 193) For example: the proportion of mast cells containing the immunoreactivity of TNF-α, IL-4, IL-6 and CD30 ligand is higher in the lesional than nonlesional AD skin.(190, 194–196) IL-4$^+$ mast cells in the healthy-looking atopic skin correlate with the extent of prick-test wheal induced by cow allergen.(197) Mast cells generated from CD34$^+$ peripheral blood progenitor cells of AD patients exhibit enhanced IL-6 release in response to *Malassezia sympodialis* yeast than those of healthy controls.(198) Dermal tryptase$^+$ and IL-6$^+$ mast cells and the severity of itching correlate inversely with the (pro)filaggrin expression in the epidermis of AD patients.(199) Therefore, the partial inactivation of chymase may allow prolonged survival of proinflammatory cytokines and neuropeptides, such as SP, giving rise to enhanced inflammation and itching.

The lesional AD skin is characterized by increased sensory nerve density in the epidermis and dermis (200–204), which may explain, at least in part, the distressing pruritus in AD. (187) A plausible explanation for increased nerves is the increased epidermal expression of nerve elongation factors, NGF and amphiregulin, and decreased expression of nerve repulsion factors, semaphorin-3A and anosmin-1, in the lesional AD skin.(187, 200, 203) Mast cells can also be a source for soluble NGF in an IgE-dependent reaction, and NGF expression is increased in mast cells in AD lesions. Furthermore, mast cells also express the high-affinity receptor of NGF, i.e., TrkA.(205–207) Thus, the treatment of mast cells with NGF *in vitro* results in increased TrkA expression and tryptase and histamine contents.(207) Interestingly, tryptase has the capacity to hydrolyze pro-NGF to mature NGF (96), and thereby it may further enhance the feed-forward loop. The contacts between tryptase$^+$ mast

cells and neurofilament$^+$ fibers are significantly increased in the lesional AD skin, and probably also so in the nonlesional skin, when compared to healthy controls (204), a finding which provides the morphologic basis for enhanced mast cell-nerve interaction. Interestingly, the exposure of patients with AD to acute Trier Social Stress Test (TSST) resulted in decreased numbers of NGF$^+$ and PGP 9.5$^+$ fibers and decreased contacts between PGP 9.5$^+$ fibers and tryptase$^+$ mast cells in the lesional skin, whereas, these parameters increased, rather than decreased, in the nonlesional skin. In addition, a significant positive correlation between itch and nerve fiber-mast cell contacts in the nonlesional (after TSST) or lesional (before TSST) skin was noted.(208) In another study, stress induced by video games or frequently ringing mobile phone increased allergen-induced skin wheal reactions and serum levels of SP, VIP and NGF in AD patients, but not in patients with allergic rhinitis or in healthy controls.(209) Therefore, acute psychologic stress is apparently associated with changes in cutaneous nerve fibers, neuropeptides and mast cells.

The histaminergic itch is induced through H1-receptors on sensory nerves. However, H4-receptors on nerves can be involved in pruritus as well, and combined antagonism of both H1- and H4-receptors may be a useful strategy in controlling itch and inflammation in AD.(177) The nonhistaminergic itch may be associated with several distinct neural pathways. One of them is PAR-2 as PAR-2$^+$ fibers are increased in the lesional AD skin, like is increased the codeine-induced release of the PAR-2 agonist, tryptase, in the nonlesional AD skin detected by the microdialysis technique. As a result of the activation of PAR-2$^+$ fibers, SP and CGRP are released leading to potentiation of neurogenic inflammation.(210, 211) Another pathway to the nonhistaminergic itch is IL-31, a cytokine that is derived from Th2 cells. The expression of the mRNA of IL-31 and its receptor IL-31RA is increased in the lesional AD skin, but not in psoriatic lesions, and the receptor has been localized in small-diameter neurons of human dorsal root ganglia.(212, 213) There are differences between histamine- and IL-31-induced itch in human skin: histamine induces immediate itch starting within 5 min after skin pricking, whereas IL-31-induced mild itch appears more slowly starting at 143 min after skin pricking accompanied by a long-lasting erythema.(214) In addition to pruritus, IL-31 may increase the elongation, branching and density of nerve fibers in AD, as demonstrated in mouse models.(215) The number of dermal cells containing IL-31 immunoreactivity is preferentially increased in the AD lesion compared to several other skin diseases.(216) However, the cell types producing IL-31 may include also other cells than Th2 cells: mast cells in psoriatic and AD lesions show increased expression of IL-31 immunoreactivity when compared to normal skin.(217)

Mast cell-neural interactions, stress and itch in animal models

The neuroendocrine signals may travel from the central nervous system to peripheral tissues and vice versa. The circuitry between mind and mast cells was demonstrated by MacQueen *et al.* in 80´s who showed that rats exposed to both audiovisual cue and antigen injection were conditioned to mast cell protease II release after reexposure to the audiovisual cue only.(218) Mast cells can be activated in the brain of mice and rats using an acute non-traumatic immobilization stress, an effect which is related to CRH.(48, 219) Furthermore, also cutaneous mast cells in rats can be activated by immobilization stress.(220) A prolonged sonic stress for 24 hours in mice has been shown to induce mast cell

degranulation, endothelial changes, increased SP^+ fibers and their contacts with mast cells, and increased NGF expression in skin mast cells.(221–223)

The interaction between mast cells and sensory nerves has been clarified in a mouse model of hapten-induced AD, where lesional skin mast cells express high levels of cell adhesion molecule-1 (CADM1), a molecule that enhanced sensory nerve–mast cell adhesion and communication *in vitro*.(224) In a mouse model of AD induced by IL-13, itch-evoked scratching behaviour showed marked association with increased dermal $PGP9.5^+$ and $CGRP^+$ nerve density, mast cell number, $TRPA1^+$ (transient receptor potential ankyrin 1) nerves, and especially with $TRPA1^+$ mast cells. In contrast, TRPV1 was not increased in the inflamed mouse skin. The intraperitoneal administration of TRPA1-specific antagonist attenuated the scratching behaviour, whereas the H1-antagonist cetirizine did not. Furthermore, $TRPA1^+$ mast cells and nerves and their apparent mutual contacts were increased in the lesional skin of patients with AD.(225) This study points to the role of TRPA1 in skin inflammation-associated itch, rather than TRPV1. However, pruritus induced in mouse skin by intradermal injections of β2-microglobulin is related, at least in part, to $TRPV1^+$ primary sensory neurons.(226) In addition, pruritus induced in mice by IL-31 injections is dependent on both TRPV1 and TRPA1 as verified in knockout mouse models.(213) In another study in mice, itch behaviour induced by PAR-4 activation using intradermal injections of the selective agonist AYPGKF-NH_2 was not dependent on mast cells, even though 32% of the skin mast cells expressed PAR-4 and the cells reacted to the ligand. Possibly the itch in this model was evoked directly through PAR-4 on gastrin-releasing peptide-positive pruriceptive fibers. Also, the itch behaviour was diminished by TRPV1 knockout or antagonism, but unexpectedly it was diminished only by TRPA1 antagonism and not by TRPA1 knockout.(227)

The cutaneous nerve fibers and their interactions with mast cells may be influenced by therapeutic molecules resulting in decreased itch and inflammation. A promising example from this is the NC/Nga mouse model of AD, where the spontaneously developed dorsal skin lesions were treated with intracutaneous injections of semaphorin-3A twice a day for 5 days and then were biopsied on day 11.(228) The treatment with this nerve repulsion factor led to the decrease 1) in the clinical skin score of the treated lesion on day 5, but not elsewhere in the skin, 2) in the scratching behaviour already on day 3, 3) in $PGP9.5^+$ fibers in the epidermis, and 4) in mast cells, $CD4^+$ T cells, IL-4 production and epidermal thickness.(228)

Summary and Future Perspectives

Mast cell inhibition poses a major therapeutic challenge. Mast cell heterogeneity is influenced by multiple chemical mediators and inflammatory cells in the tissue microenvironment, by gender, and by species. Mast cells can act immediately or have long-term, sustained responses to a whole host of microenvironment stimuli. Recent research has shown that mast cells have different roles in the pathologic mechanisms underlying different diseases. Thus, it is paramount to use highly specific disease models and clinical condition rather than applying findings from one model or clinical condition to another.

Despite the fact that mast cells have disease-specific functions, several major lacunae remain to be addressed in the pathologic roles of mast cells. First and foremost is the transmigration and homing of mast cell progenitors and their differentiation. Next is identifying the process that is influenced whether it is differentiation, proliferation, and/ or activation. Similarly, acute and chronic activation are important to consider. Strategies to prevent mast cell activation may be more effective than strategies to reverse activation once it has been initiated. Once mast cells are activated, inhibition can pose a major challenge because of positive feedback loops that continually signal mast cell activation.

Even though experiments in mice and rats provide growing evidence for the intimate communication between mast cells and sensory nerves in stressful conditions and models of pruritus and pain, thus far the evidence in human skin and other tissues is still sparse and indirect. Therefore, controlled and experimental models using standardized stress, pruritus and pain techniques should be translated in future human studies/clinical trials. Such studies can form the basis for developing new therapeutic strategies to resolve the unmet clinical problem, i.e., distressing itch/pain and reducing opioid requirement for pain.

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References

1. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol. 2005; 6:135–142. [PubMed: 15662442]
2. Kubo M. Innate and adaptive type 2 immunity in lung allergic inflammation. Immunol Rev. 2017; 278:162–172. [PubMed: 28658559]
3. Maurer M, Theoharides T, Granstein RD, et al. What is the physiological function of mast cells? Experimental Dermatology. 2003; 12:886–886. [PubMed: 14719507]
4. Harvima IT, Levi-Schaffer F, Draber P, et al. Molecular targets on mast cells and basophils for novel therapies. J Allergy Clin Immunol. 2014; 134:530–544. [PubMed: 24767877]
5. Koulouris AI, Banim P, Hart AR. Pain in Patients with Pancreatic Cancer: Prevalence, Mechanisms, Management and Future Developments. Dig Dis Sci. 2017; 62:861–870. [PubMed: 28229252]
6. Nguyen J, Luk K, Vang D, et al. Morphine stimulates cancer progression and mast cell activation and impairs survival in transgenic mice with breast cancer. Br J Anaesth. 2014; 113:i4–i13. [PubMed: 24861561]
7. Vincent L, Vang D, Nguyen J, et al. Mast cell activation contributes to sickle cell pathobiology and pain in mice. Blood. 2013; 122:1853–1862. [PubMed: 23775718]
8. Aich A, Afrin LB, Gupta K. Mast Cell-Mediated Mechanisms of Nociception. Int J Mol Sci. 2015; 16:29069–29092. [PubMed: 26690128]
9. Xanthos DN, Gaderer S, Drdla R, et al. Central nervous system mast cells in peripheral inflammatory nociception. Mol Pain. 2011; 7:42. [PubMed: 21639869]
10. Ammendola M, Sacco R, Sammarco G, et al. Mast Cell-Targeted Strategies in Cancer Therapy. TMH. 2016; 43:109–113.

11. Moon TC, St Laurent CD, Morris KE, et al. Advances in mast cell biology: new understanding of heterogeneity and function. Mucosal Immunol. 2010; 3:111–128. [PubMed: 20043008]
12. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997; 77:1033–1079. [PubMed: 9354811]
13. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat Rev Immunol. 2008; 8:478–486. [PubMed: 18483499]
14. Hardy WB, Wesbrook FF. The Wandering Cells of the Alimentary Canal. J Physiol (Lond). 1895; 18:490–i493.
15. Karimi M, Zekavat OR, Sharifzadeh S, et al. Clinical response of patients with sickle cell anemia to cromolyn sodium nasal spray. Am J Hematol. 2006; 81:809–816. [PubMed: 16941613]
16. Vukman KV, Försönits A, Oszvald Á, et al. Mast cell secretome: Soluble and vesicular components. Seminars in Cell & Developmental Biology. 2017; 67:65–73. [PubMed: 28189858]
17. D'Incà F, Pucillo CE. Exosomes: tiny clues for mast cell communication. Front Immunol. 2015; 6:73. [PubMed: 25741344]
18. Carroll-Portillo A, Surviladze Z, Cambi A, et al. Mast cell synapses and exosomes: membrane contacts for information exchange. Front Immunol. 2012; 3:46. [PubMed: 22566928]
19. Laulagnier K, Motta C, Hamdi S, et al. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J. 2004; 380:161–171. [PubMed: 14965343]
20. Cheung KL, Jarrett R, Subramaniam S, et al. Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a. J Exp Med. 2016; 213:2399–2412. [PubMed: 27670592]
21. Smalheiser NR. Exosomal transfer of proteins and RNAs at synapses in the nervous system. Biol Direct. 2007; 2:35. [PubMed: 18053135]
22. Bechter K, Schmitz B. Cerebrospinal fluid outflow along lumbar nerves and possible relevance for pain research: case report and review. Croat Med J. 2014; 55:399–404. [PubMed: 25165054]
23. Theoharides TC. Neuroendocrinology of mast cells: Challenges and controversies. Experimental Dermatology. 2017; 26:751–759. [PubMed: 28094875]
24. Harvima IT, Nilsson G. Mast cells as regulators of skin inflammation and immunity. Acta Derm Venereol. 2011; 91:644–650. [PubMed: 21879235]
25. Harvima IT, Nilsson G. Stress, the neuroendocrine system and mast cells: current understanding of their role in psoriasis. Expert Rev Clin Immunol. 2012; 8:235–241. [PubMed: 22390488]
26. Hendriksen E, van Bergeijk D, Oosting RS, et al. Mast cells in neuroinflammation and brain disorders. Neurosci Biobehav Rev. 2017; 79:119–133. [PubMed: 28499503]
27. Silver R, Curley JP. Mast cells on the mind: new insights and opportunities. Trends Neurosci. 2013; 36:513–521. [PubMed: 23845731]
28. Skaper SD, Giusti P, Facci L. Microglia and mast cells: two tracks on the road to neuroinflammation. FASEB J. 2012; 26:3103–3117. [PubMed: 22516295]
29. Azimi E, Xia J, Lerner EA. Peripheral Mechanisms of Itch. Curr Probl Dermatol. 2016; 50:18–23. [PubMed: 27578066]
30. Azimi E, Reddy VB, Pereira PJS, et al. Substance P activates Mas-related G protein-coupled receptors to induce itch. J Allergy Clin Immunol. 2017; 140:447–453.e443. [PubMed: 28219706]
31. Gouin O, L'Herondelle K, Lebonvallet N, et al. TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization. Protein Cell. 2017
32. Aich, A., Beitz, AJ., Gupta, K. Mechanisms of Pain in Sickle Cell Disease. In: Inusa, B., editor. Sickle Cell Disease. Croatia, EU: InTech Publishers; 2016.
33. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. Br J Pharmacol. 2013; 170:38–45. [PubMed: 23734637]
34. Schmelz M, Petersen LJ. Neurogenic inflammation in human and rodent skin. News Physiol Sci. 2001; 16:33–37. [PubMed: 11390944]
35. Foreman JC, Jordan CC. Neurogenic inflammation. Trends in Pharmacological Sciences. 1984; 5:116–119.

36. Foreman JC, Jordan CC, Oehme P, et al. Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. J Physiol (Lond). 1983; 335:449–465. [PubMed: 6192237]
37. Roosterman D, Goerge T, Schneider SW, et al. Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. Physiol Rev. 2006; 86:1309–1379. [PubMed: 17015491]
38. Teresiak-Mikołajczak E, Czarnecka-Operacz M, Jenerowicz D, et al. Neurogenic markers of the inflammatory process in atopic dermatitis: relation to the severity and pruritus. Postepy Dermatol Alergol. 2013; 30:286–292. [PubMed: 24353488]
39. Austin PJ, Moalem-Taylor G. The neuro-immune balance in neuropathic pain: involvement of inflammatory immune cells, immune-like glial cells and cytokines. J Neuroimmunol. 2010; 229:26–50. [PubMed: 20870295]
40. Cataldo G, Rajput S, Gupta K, et al. Sensitization of nociceptive spinal neurons contributes to pain in a transgenic model of sickle cell disease. Pain. 2015; 156:722–730. [PubMed: 25630029]
41. Darbari DS, Hampson JP, Ichesco E, et al. Frequency of Hospitalizations for Pain and Association With Altered Brain Network Connectivity in Sickle Cell Disease. J Pain. 2015; 16:1077–1086. [PubMed: 26291276]
42. Tran H, Gupta M, Gupta K. Targeting Novel Mechanisms of Pain in Sickle Cell Disease. Blood. 2017 **In Press**.
43. Case M, Zhang H, Mundahl J, et al. Characterization of functional brain activity and connectivity using EEG and fMRI in patients with sickle cell disease. Neuroimage Clin. 2017; 14:1–17. [PubMed: 28116239]
44. Kissel CL, Kovács KJ, Larson AA. Evidence for the modulation of nociception in mice by central mast cells. Eur J Pain. 2017
45. Esposito P, Gheorghe D, Kandere K, et al. Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. Brain Res. 2001; 888:117–127. [PubMed: 11146058]
46. Dropp JJ. Mast cells in the central nervous system of several rodents. Anat Rec. 1972; 174:227–237. [PubMed: 4627786]
47. Taiwo OB, Kovács KJ, Sun Y, et al. Unilateral spinal nerve ligation leads to an asymmetrical distribution of mast cells in the thalamus of female but not male mice. Pain. 2005; 114:131–140. [PubMed: 15733638]
48. Theoharides TC, Spanos C, Pang X, et al. Stress-induced intracranial mast cell degranulation: a corticotropin-releasing hormone-mediated effect. Endocrinology. 1995; 136:5745–5750. [PubMed: 7588332]
49. Silver R, Silverman A-J, Vitković L, et al. Mast cells in the brain: evidence and functional significance. Trends Neurosci. 1996; 19:25–31. [PubMed: 8787137]
50. Goldschmidt RC, Hough LB, Glick SD, et al. Mast cells in rat thalamus: nuclear localization, sex difference and left-right asymmetry. Brain Res. 1984; 323:209–217. [PubMed: 6084538]
51. Kim J, Kim J, Kim DW, et al. Wnt5a induces endothelial inflammation via beta-catenin-independent signaling. J Immunol. 2010; 185:1274–1282. [PubMed: 20554957]
52. Nautiyal KM, Liu C, Dong X, et al. Blood-borne donor mast cell precursors migrate to mast cell-rich brain regions in the adult mouse. J Neuroimmunol. 2011; 240–241:142–146.
53. Skaper SD, Facci L, Kee WJ, et al. Potentiation by histamine of synaptically mediated excitotoxicity in cultured hippocampal neurones: a possible role for mast cells. J Neurochem. 2001; 76:47–55. [PubMed: 11145977]
54. Lacy P. Mechanisms of degranulation in neutrophils. Allergy Asthma Clin Immunol. 2006; 2:98–108. [PubMed: 20525154]
55. Cromheecke JL, Nguyen KT, Huston DP. Emerging role of human basophil biology in health and disease. Curr Allergy Asthma Rep. 2014; 14:408. [PubMed: 24346805]
56. Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. Nat Rev Immunol. 2014; 14:478–494. [PubMed: 24903914]
57. Nishida K, Yamasaki S, Ito Y, et al. Fc{epsilon}RI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. J Cell Biol. 2005; 170:115–126. [PubMed: 15998803]

58. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. Nat Rev Immunol. 2007; 7:93–104. [PubMed: 17259966]
59. Dvorak AM, Kissell S. Granule changes of human skin mast cells characteristic of piecemeal degranulation and associated with recovery during wound healing in situ. J Leukoc Biol. 1991; 49:197–210. [PubMed: 1992000]
60. Greenberg G, Burnstock G. A novel cell-to-cell interaction between mast cells and other cell types. Exp Cell Res. 1983; 147:1–13. [PubMed: 6617756]
61. Dines KC, Powell HC. Mast cell interactions with the nervous system: relationship to mechanisms of disease. J Neuropathol Exp Neurol. 1997; 56:627–640. [PubMed: 9184654]
62. Wilhelm M, Silver R, Silverman AJ. Central nervous system neurons acquire mast cell products via transgranulation. Eur J Neurosci. 2005; 22:2238–2248. [PubMed: 16262662]
63. Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology. 2004; 126:693–702. [PubMed: 14988823]
64. Marshall JS. Mast-cell responses to pathogens. Nat Rev Immunol. 2004; 4:787–799. [PubMed: 15459670]
65. Thakur KK, Saini J, Mahajan K, et al. Therapeutic implications of toll-like receptors in peripheral neuropathic pain. Pharmacol Res. 2017; 115:224–232. [PubMed: 27894923]
66. Mitsui H, Furitsu T, Dvorak AM, et al. Development of human mast cells from umbilical cord blood cells by recombinant human and murine c-kit ligand. Proc Natl Acad Sci USA. 1993; 90:735–739. [PubMed: 7678463]
67. Bischoff SC, Sellge G, Schwengberg S, et al. Stem cell factor-dependent survival, proliferation and enhanced releasability of purified mature mast cells isolated from human intestinal tissue. Int Arch Allergy Immunol. 1999; 118:104–107. [PubMed: 10224352]
68. Lorentz A, Wilke M, Sellge G, et al. IL-4-induced priming of human intestinal mast cells for enhanced survival and Th2 cytokine generation is reversible and associated with increased activity of ERK1/2 and c-Fos. J Immunol. 2005; 174:6751–6756. [PubMed: 15905515]
69. Akers JC, Gonda D, Kim R, et al. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. J Neurooncol. 2013; 113:1–11. [PubMed: 23456661]
70. Chevillet JR, Kang Q, Ruf IK, et al. Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci USA. 2014; 111:14888–14893. [PubMed: 25267620]
71. Vicuña L, Strochlic DE, Latremoliere A, et al. The serine protease inhibitor SerpinA3N attenuates neuropathic pain by inhibiting T cell-derived leukocyte elastase. Nat Med. 2015; 21:518–523. [PubMed: 25915831]
72. Chen B, Li M-Y, Guo Y, et al. Mast cell-derived exosomes at the stimulated acupoints activating the neuro-immuno regulation. Chin J Integr Med. 2016
73. Weng Z, Zhang B, Tsilioni I, et al. Nanotube Formation: A Rapid Form of "Alarm Signaling"? Clin Ther. 2016; 38:1066–1072. [PubMed: 27085584]
74. Zaccard CR, Rinaldo CR, Mailliard RB. Linked in: immunologic membrane nanotube networks. J Leukoc Biol. 2016; 100:81–94. [PubMed: 26931578]
75. Fifadara NH, Beer F, Ono S, et al. Interaction between activated chemokine receptor 1 and FcepsilonRI at membrane rafts promotes communication and F-actin-rich cytoneme extensions between mast cells. Int Immunol. 2010; 22:113–128. [PubMed: 20173038]
76. Vignais M-L, Caicedo A, Brondello J-M, et al. Cell Connections by Tunneling Nanotubes: Effects of Mitochondrial Trafficking on Target Cell Metabolism, Homeostasis, and Response to Therapy. Stem Cells Int. 2017; 2017:6917941. [PubMed: 28659978]
77. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532–1535. [PubMed: 15001782]
78. Jorch SK, Kubes P. An emerging role for neutrophil extracellular traps in noninfectious disease. Nat Med. 2017; 23:279–287. [PubMed: 28267716]
79. Wang Y, Li M, Stadler S, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol. 2009; 184:205–213. [PubMed: 19153223]

80. von Köckritz-Blickwede M, Goldmann O, Thulin P, et al. Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. Blood. 2008; 111:3070–3080. [PubMed: 18182576]
81. Lin AM, Rubin CJ, Khandpur R, et al. Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. J Immunol. 2011; 187:490–500. [PubMed: 21606249]
82. Simon D, Simon HU, Yousefi S. Extracellular DNA traps in allergic, infectious, and autoimmune diseases. Allergy. 2013; 68:409–416. [PubMed: 23409745]
83. Baronaite Hansen R, Kavanaugh A. Secukinumab for the treatment of psoriatic arthritis. Expert Rev Clin Immunol. 2016; 12:1027–1036. [PubMed: 27550397]
84. Riedel W, Neeck G. Nociception, pain, and antinociception: current concepts. Z Rheumatol. 2001; 60:404–415. [PubMed: 11826734]
85. Mobarakeh JI, Takahashi K, Sakurada S, et al. Enhanced antinociceptive effects of morphine in histamine H2 receptor gene knockout mice. Neuropharmacology. 2006; 51:612–622. [PubMed: 16806305]
86. Bileviciute I, Lundeberg T, Ekblom A, et al. Substance P-, neurokinin A-, calcitonin gene-related peptide- and neuropeptide Y-like immunoreactivity (-LI) in rat knee joint synovial fluid during acute monoarthritis is not correlated with concentrations of neuropeptide-LI in cerebrospinal fluid and plasma. Neurosci Lett. 1994; 167:145–148. [PubMed: 7513839]
87. Tani E, Senba E, Kokumai S, et al. Histamine application to the nasal mucosa induces release of calcitonin gene-related peptide and substance P from peripheral terminals of trigeminal ganglion: a morphological study in the guinea pig. Neurosci Lett. 1990; 112:1–6. [PubMed: 1696704]
88. Theoharides TC, Sant GR. Hydroxyzine therapy for interstitial cystitis. Urology. 1997; 49:108–110.
89. Thilagarajah R, Witherow RO, Walker MM. Oral cimetidine gives effective symptom relief in painful bladder disease: a prospective, randomized, double-blind placebo-controlled trial. BJU Int. 2001; 87:207–212. [PubMed: 11167643]
90. Molino M, Barnathan ES, Numerof R, et al. Interactions of Mast Cell Tryptase with Thrombin Receptors and PAR-2. J Biol Chem. 1997; 272:4043–4049. [PubMed: 9020112]
91. Vergnolle N, Bunnett NW, Sharkey KA, et al. Proteinase-activated receptor-2 and hyperalgesia: A novel pain pathway. Nat Med. 2001; 7:821–826. [PubMed: 11433347]
92. Steinhoff M, Vergnolle N, Young SH, et al. Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med. 2000; 6:151–158. [PubMed: 10655102]
93. Schwartz LB, Metcalfe DD, Miller JS, et al. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med. 1987; 316:1622–1626. [PubMed: 3295549]
94. Skaper SD. Nerve growth factor: a neuroimmune crosstalk mediator for all seasons. Immunology. 2017; 151:1–15. [PubMed: 28112808]
95. Leon A, Buriani A, Toso RD, et al. Mast cells synthesize, store, and release nerve growth factor. PNAS. 1994; 91:3739–3743. [PubMed: 8170980]
96. Spinnler K, Fröhlich T, Arnold GJ, et al. Human tryptase cleaves pro-nerve growth factor (pro-NGF): hints of local, mast cell-dependent regulation of NGF/pro-NGF action. J Biol Chem. 2011; 286:31707–31713. [PubMed: 21768088]
97. Eskander MA, Ruparel S, Green DP, et al. Persistent Nociception Triggered by Nerve Growth Factor (NGF) Is Mediated by TRPV1 and Oxidative Mechanisms. J Neurosci. 2015; 35:8593–8603. [PubMed: 26041925]
98. Donnerer J, Schuligoi R, Stein C. Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: Evidence for a regulatory function of nerve growth factor in vivo. Neuroscience. 1992; 49:693–698. [PubMed: 1380138]
99. Kelleher JH, Tewari D, McMahon SB. Neurotrophic factors and their inhibitors in chronic pain treatment. Neurobiol Dis. 2017; 97:127–138. [PubMed: 27063668]
100. Olivera A, Spiegel S. Sphingosine kinase: a mediator of vital cellular functions. Prostaglandins Other Lipid Mediat. 2001; 64:123–134. [PubMed: 11324702]

101. Jolly PS, Bektas M, Olivera A, et al. Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. J Exp Med. 2004; 199:959–970. [PubMed: 15067032]
102. Oskeritzian CA, Alvarez SE, Hait NC, et al. Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions. Blood. 2008; 111:4193–4200. [PubMed: 18178871]
103. Brinkmann V, Billich A, Baumruker T, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov. 2010; 9:883–897. [PubMed: 21031003]
104. Brinkmann V, Davis MD, Heise CE, et al. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem. 2002; 277:21453–21457. [PubMed: 11967257]
105. Grenald SA, Doyle TM, Zhang H, et al. Targeting the S1P/S1PR1 axis mitigates cancer-induced bone pain and neuroinflammation. Pain. 2017; 158:1733–1742. [PubMed: 28570482]
106. Janes K, Little JW, Li C, et al. The development and maintenance of paclitaxel-induced neuropathic pain require activation of the sphingosine 1-phosphate receptor subtype 1. J Biol Chem. 2014; 289:21082–21097. [PubMed: 24876379]
107. Jaggi AS, Kaur G, Bali A, et al. Pharmacological investigations on mast cell stabilizer and histamine receptor antagonists in vincristine-induced neuropathic pain. Naunyn Schmiedebergs Arch Pharmacol. 2017
108. Sousa-Valente J, Calvo L, Vacca V, et al. Role of TrkA signalling and mast cells in the initiation of osteoarthritis pain in the monoiodoacetate model. Osteoarthr Cartil. 2017
109. Breser ML, Salazar FC, Rivero VE, et al. Immunological Mechanisms Underlying Chronic Pelvic Pain and Prostate Inflammation in Chronic Pelvic Pain Syndrome. Front Immunol. 2017; 8:898. [PubMed: 28824626]
110. Kaur G, Singh N, Jaggi AS. Mast cells in neuropathic pain: an increasing spectrum of their involvement in pathophysiology. Rev Neurosci. 2017
111. Komi DEA, Rambasek T, Wöhrl S. Mastocytosis: from a Molecular Point of View. Clin Rev Allergy Immunol. 2017
112. Hartmann K, Escribano L, Grattan C, et al. Cutaneous manifestations in patients with mastocytosis: Consensus report of the European Competence Network on Mastocytosis; the American Academy of Allergy, Asthma & Immunology; and the European Academy of Allergology and Clinical Immunology. J Allergy Clin Immunol. 2016; 137:35–45. [PubMed: 26476479]
113. Valent P. Mastocytosis: a paradigmatic example of a rare disease with complex biology and pathology. Am J Cancer Res. 2013; 3:159–172. [PubMed: 23593539]
114. Akin C. Mast cell activation syndromes presenting as anaphylaxis. Immunol Allergy Clin North Am. 2015; 35:277–285. [PubMed: 25841551]
115. Sins JWR, Mager DJ, Davis SCAT, et al. Pharmacotherapeutical strategies in the prevention of acute, vaso-occlusive pain in sickle cell disease: a systematic review. Blood Advances. 2017; 1:1598–1616. [PubMed: 29296801]
116. Ballas, SK. Sickle Cell Pain. 2. Washington, D.C: IASP Press; 2015.
117. Panepinto JA, Paul Scott J, Badaki-Makun O, et al. Determining the longitudinal validity and meaningful differences in HRQL of the PedsQL™ Sickle Cell Disease Module. Health Qual Life Outcomes. 2017; 15:124. [PubMed: 28606098]
118. Dampier C, Palermo TM, Darbari DS, et al. AAPT Diagnostic Criteria for Chronic Sickle Cell Disease Pain. The Journal of Pain. 2017; 18:490–498. [PubMed: 28065813]
119. Kohli DR, Li Y, Khasabov SG, et al. Pain-related behaviors and neurochemical alterations in mice expressing sickle hemoglobin: modulation by cannabinoids. Blood. 2010; 116:456–465. [PubMed: 20304807]
120. Cain DM, Vang D, Simone DA, et al. Mouse models for studying pain in sickle disease: effects of strain, age, and acuteness. Br J Haematol. 2012; 156:535–544. [PubMed: 22171826]
121. Tran H, Jha R, Nguyen J, et al. Hemin-Induced Mast Cell-Extracellular Traps Impart Resistance to Therapy in a Sickle Microenvironment. Blood. 2015; 126:3385–3385.

122. Lamming CED, Augustin L, Blackstad M, et al. Spontaneous circulation of myeloid-lymphoid–initiating cells and SCID-repopulating cells in sickle cell crisis. J Clin Invest. 2003; 111:811–819. [PubMed: 12639987]

123. Dampier C, Ely B, Brodecki D, et al. Pain characteristics and age-related pain trajectories in infants and young children with sickle cell disease. Pediatr Blood Cancer. 2014; 61:291–296. [PubMed: 24115743]

124. Ballas, SK. Acute Regional pain Syndrome. In: Ballas, SK., editor. Sickle Cell Pain. 2. IASP Press; 2015. p. 141-188.

125. Ballas SK, Gupta K, Adams-Graves P. Sickle cell pain: a critical reappraisal. Blood. 2012; 120:3647–3656. [PubMed: 22923496]

126. Uhelski ML, Gupta K, Simone DA. Sensitization of C-fiber nociceptors in mice with sickle cell disease is decreased by local inhibition of anandamide hydrolysis. Pain. 2017; 158:1711–1722. [PubMed: 28570479]

127. Kuei N, Patel N, Xu H, et al. Characteristics and Potential Biomarkers for Chronic Pain in Patients with Sickle Cell Disease. Blood. 2015; 126

128. Ballas, SK. The Acute painful episode. In: Ballas, SK., editor. Sickle Cell Pain. 2. IASP Press; 2015. p. 75-139.

129. Reichel CA, Lerchenberger M, Uhl B, et al. Plasmin inhibitors prevent leukocyte accumulation and remodeling events in the postischemic microvasculature. PLoS ONE. 2011; 6:e17229. [PubMed: 21364954]

130. Vincent L, Vang D, Nguyen J, et al. Cannabinoid receptor-specific mechanisms to alleviate pain in sickle cell anemia via inhibition of mast cell activation and neurogenic inflammation. Haematologica. 2016; 101:566–577. [PubMed: 26703965]

131. Brandow AM, Wandersee NJ, Dasgupta M, et al. Substance P is increased in patients with sickle cell disease and associated with haemolysis and hydroxycarbamide use. Br J Haematol. 2016; 175:237–245. [PubMed: 27539682]

132. Michaels LA, Ohene-Frempong K, Zhao H, et al. Serum levels of substance P are elevated in patients with sickle cell disease and increase further during vaso-occlusive crisis. Blood. 1998; 92:3148–3151. [PubMed: 9787150]

133. Douglas SD. Substance P and sickle cell disease-a marker for pain and novel therapeutic approaches. Br J Haematol. 2016; 175:187–188. [PubMed: 27539537]

134. Sicuteri F. Mast cells and their active substances: Their role in the pathogenesis of migrain. Headache. 1963; 3:86–92. [PubMed: 14094109]

135. Levy D. Migraine pain, meningeal inflammation, and mast cells. Curr Pain Headache Rep. 2009; 13:237–240. [PubMed: 19457286]

136. Smith JH, Butterfield JH, Cutrer FM. Primary headache syndromes in systemic mastocytosis. Cephalalgia. 2011; 31:1522–1531. [PubMed: 21960649]

137. Theoharides TC. Mast cells and migraines. Perspect Biol Med. 1983; 26:672–675. [PubMed: 6353354]

138. Boes T, Levy D. Influence of sex, estrous cycle, and estrogen on intracranial dural mast cells. Cephalalgia. 2012; 32:924–931. [PubMed: 22833613]

139. Rozniecki JJ, Dimitriadou V, Lambracht-Hall M, et al. Morphological and functional demonstration of rat dura mater mast cell-neuron interactions in vitro and in vivo. Brain Res. 1999; 849:1–15. [PubMed: 10592282]

140. Strassman AM, Weissner W, Williams M, et al. Axon diameters and intradural trajectories of the dural innervation in the rat. J Comp Neurol. 2004; 473:364–376. [PubMed: 15116396]

141. Brandow AM, Farley RA, Panepinto JA. Early insights into the neurobiology of pain in sickle cell disease: A systematic review of the literature. Pediatr Blood Cancer. 2015; 62:1501–1511. [PubMed: 25976161]

142. Small-Howard AL, Shimoda LMN, Adra CN, et al. Anti-inflammatory potential of CB1-mediated cAMP elevation in mast cells. Biochem J. 2005; 388:465–473. [PubMed: 15669919]

143. Khroyan TV, Polgar WE, Orduna J, et al. Differential effects of nociceptin/orphanin FQ (NOP) receptor agonists in acute versus chronic pain: studies with bifunctional NOP/μ receptor agonists

in the sciatic nerve ligation chronic pain model in mice. J Pharmacol Exp Ther. 2011; 339:687–693. [PubMed: 21859931]

144. Vang D, Paul JA, Nguyen J, et al. Small-molecule nociceptin receptor agonist ameliorates mast cell activation and pain in sickle mice. Haematologica. 2015; 100:1517–1525. [PubMed: 26294734]
145. Koch J, Manworren R, Clark L, et al. Pilot study of continuous co-infusion of morphine and naloxone in children with sickle cell pain crisis. Am J Hematol. 2008; 83:728–731. [PubMed: 18543345]
146. Ataga KI, Kutlar A, Kanter J, et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. N Engl J Med. 2017; 376:429–439. [PubMed: 27959701]
147. Stankovic Stojanovic K, Thiolière B, Garandeau E, et al. Chronic myeloid leukaemia and sickle cell disease: could imatinib prevent vaso-occlusive crisis? Br J Haematol. 2011; 155:271–272. [PubMed: 21488859]
148. Finn DF, Walsh JJ. Twenty-first century mast cell stabilizers. Br J Pharmacol. 2013; 170:23–37. [PubMed: 23441583]
149. Ceyhan GO, Bergmann F, Kadihasanoglu M, et al. Pancreatic neuropathy and neuropathic pain--a comprehensive pathomorphological study of 546 cases. Gastroenterology. 2009; 136:177–186.e171. [PubMed: 18992743]
150. Barreto SG, Saccone GTP. Pancreatic nociception--revisiting the physiology and pathophysiology. Pancreatology. 2012; 12:104–112. [PubMed: 22487519]
151. Mola, FFd, Sebastiano, Pd. Pain and pain generation in pancreatic cancer. Langenbecks Arch Surg. 2008; 393:919–922. [PubMed: 18193269]
152. Demir IE, Schorn S, Schremmer-Danninger E, et al. Perineural mast cells are specifically enriched in pancreatic neuritis and neuropathic pain in pancreatic cancer and chronic pancreatitis. PLoS ONE. 2013; 8:e60529. [PubMed: 23555989]
153. Lopes DM, Denk F, Chisholm KI, et al. Peripheral inflammatory pain sensitisation is independent of mast cell activation in male mice. Pain. 2017; 158:1314–1322. [PubMed: 28394852]
154. Wang Y, Lei J, Gupta M, et al. Electroacupuncture in conscious free-moving mice reduces pain by ameliorating peripheral and central nociceptive mechanisms. Scientific Reports. 2016; 6:34493. [PubMed: 27687125]
155. Talagas M, Lebonvallet N, Leschiera R, et al. What about physical contacts between epidermal keratinocytes and sensory neurons? Experimental dermatology. 2017
156. Bigliardi-Qi M, Sumanovski LT, Buchner S, et al. Mu-opiate receptor and Beta-endorphin expression in nerve endings and keratinocytes in human skin. Dermatology (Basel, Switzerland). 2004; 209:183–189.
157. Han L, Ma C, Liu Q, et al. A subpopulation of nociceptors specifically linked to itch. Nature neuroscience. 2013; 16:174–182. [PubMed: 23263443]
158. Baraniuk JN. Rise of the sensors: nociception and pruritus. Curr Allergy Asthma Rep. 2012; 12:104–114. [PubMed: 22367178]
159. LaMotte RH, Dong X, Ringkamp M. Sensory neurons and circuits mediating itch. Nat Rev Neurosci. 2014; 15:19–31. [PubMed: 24356071]
160. Basavaraj KH, Navya MA, Rashmi R. Stress and quality of life in psoriasis: an update. International Journal of Dermatology. 2011; 50:783–792. [PubMed: 21699511]
161. Szepietowski JC, Reich A. Pruritus in psoriasis: An update. Eur J Pain. 2016; 20:41–46. [PubMed: 26415584]
162. Arck PC, Slominski A, Theoharides TC, et al. Neuroimmunology of stress: skin takes center stage. The Journal of Investigative Dermatology. 2006; 126:1697–1704. [PubMed: 16845409]
163. Farber EM, Lanigan SW, Boer J. The role of cutaneous sensory nerves in the maintenance of psoriasis. International Journal of Dermatology. 1990; 29:418–420. [PubMed: 2397964]
164. Naukkarinen A, Nickoloff BJ, Farber EM. Quantification of cutaneous sensory nerves and their substance P content in psoriasis. The Journal of Investigative Dermatology. 1989; 92:126–129. [PubMed: 2462593]

165. Eedy DJ, Johnston CF, Shaw C, et al. Neuropeptides in psoriasis: an immunocytochemical and radioimmunoassay study. The Journal of Investigative Dermatology. 1991; 96:434–438. [PubMed: 2007781]

166. Amatya B, El-Nour H, Holst M, et al. Expression of tachykinins and their receptors in plaque psoriasis with pruritus. The British Journal of Dermatology. 2011; 164:1023–1029. [PubMed: 21299544]

167. Naukkarinen A, Harvima IT, Aalto ML, et al. Quantitative analysis of contact sites between mast cells and sensory nerves in cutaneous psoriasis and lichen planus based on a histochemical double staining technique. Archives of Dermatological Research. 1991; 283:433–437. [PubMed: 1724896]

168. Naukkarinen A, Harvima I, Paukkonen K, et al. Immunohistochemical analysis of sensory nerves and neuropeptides, and their contacts with mast cells in developing and mature psoriatic lesions. Archives of Dermatological Research. 1993; 285:341–346. [PubMed: 7692828]

169. Naukkarinen A, Järvikallio A, Lakkakorpi J, et al. Quantitative histochemical analysis of mast cells and sensory nerves in psoriatic skin. The Journal of Pathology. 1996; 180:200–205. [PubMed: 8976881]

170. Harvima IT, Nilsson G, Suttle M-M, et al. Is there a role for mast cells in psoriasis? Archives of Dermatological Research. 2008; 300:461–478. [PubMed: 18719932]

171. Caughey GH, Leidig F, Viro NF, et al. Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. J Pharmacol Exp Ther. 1988; 244:133–137. [PubMed: 2447273]

172. Franconi GM, Graf PD, Lazarus SC, et al. Mast cell tryptase and chymase reverse airway smooth muscle relaxation induced by vasoactive intestinal peptide in the ferret. J Pharmacol Exp Ther. 1989; 248:947–951. [PubMed: 2495355]

173. Walls AF, Brain SD, Desai A, et al. Human mast cell tryptase attenuates the vasodilator activity of calcitonin gene-related peptide. Biochemical Pharmacology. 1992; 43:1243–1248. [PubMed: 1562277]

174. Carvalho, RFdS, Nilsson, G., Harvima, IT. Increased mast cell expression of PAR-2 in skin inflammatory diseases and release of IL-8 upon PAR-2 activation. Experimental Dermatology. 2010; 19:117–122. [PubMed: 19889021]

175. Amadesi S, Nie J, Vergnolle N, et al. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neurosci. 2004; 24:4300–4312. [PubMed: 15128844]

176. Ständer S, Moormann C, Schumacher M, et al. Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. Experimental Dermatology. 2004; 13:129–139. [PubMed: 14987252]

177. Ohsawa Y, Hirasawa N. The role of histamine H1 and H4 receptors in atopic dermatitis: from basic research to clinical study. Allergology International: Official Journal of the Japanese Society of Allergology. 2014; 63:533–542. [PubMed: 25249063]

178. Krogstad AL, Lönnroth P, Larson G, et al. Increased interstitial histamine concentration in the psoriatic plaque. The Journal of Investigative Dermatology. 1997; 109:632–635. [PubMed: 9347790]

179. Pestelli E, Floriani I, Fabbri P, et al. Cetirizine modulates adhesion molecule expression in a double-blind controlled study conducted in psoriatic patients. International Journal of Tissue Reactions. 2003; 25:1–8. [PubMed: 12854881]

180. Kim JE, Cho DH, Kim HS, et al. Expression of the corticotropin-releasing hormone-proopiomelanocortin axis in the various clinical types of psoriasis. Experimental Dermatology. 2007; 16:104–109. [PubMed: 17222223]

181. Chang SE, Han SS, Jung HJ, et al. Neuropeptides and their receptors in psoriatic skin in relation to pruritus. The British Journal of Dermatology. 2007; 156:1272–1277. [PubMed: 17535226]

182. Kou K, Nakamura F, Aihara M, et al. Decreased expression of semaphorin-3A, a neurite-collapsing factor, is associated with itch in psoriatic skin. Acta Derm Venereol. 2012; 92:521–528. [PubMed: 22565412]

183. Nakamura M, Toyoda M, Morohashi M. Pruritogenic mediators in psoriasis vulgaris: comparative evaluation of itch-associated cutaneous factors. The British Journal of Dermatology. 2003; 149:718–730. [PubMed: 14616362]

184. Taneda K, Tominaga M, Negi O, et al. Evaluation of epidermal nerve density and opioid receptor levels in psoriatic itch. The British Journal of Dermatology. 2011; 165:277–284. [PubMed: 21457210]

185. Kupczyk P, Reich A, Hołysz M, et al. Opioid Receptors in Psoriatic Skin: Relationship with Itch. Acta Derm Venereol. 2017; 97:564–570. [PubMed: 27958613]

186. Nigam R, El-Nour H, Amatya B, et al. GABA and GABA(A) receptor expression on immune cells in psoriasis: a pathophysiological role. Archives of Dermatological Research. 2010; 302:507–515. [PubMed: 20455067]

187. Tominaga M, Takamori K. Itch and nerve fibers with special reference to atopic dermatitis: therapeutic implications. The Journal of Dermatology. 2014; 41:205–212. [PubMed: 24628070]

188. Suárez AL, Feramisco JD, Koo J, et al. Psychoneuroimmunology of psychological stress and atopic dermatitis: pathophysiologic and therapeutic updates. Acta Derm Venereol. 2012; 92:7–15. [PubMed: 22101513]

189. Järvikallio A, Naukkarinen A, Harvima IT, et al. Quantitative analysis of tryptase- and chymase-containing mast cells in atopic dermatitis and nummular eczema. The British Journal of Dermatology. 1997; 136:871–877. [PubMed: 9217819]

190. Ilves T, Harvima IT. Decrease in chymase activity is associated with increase in IL-6 expression in mast cells in atopic dermatitis. Acta Derm Venereol. 2015; 95:411–416. [PubMed: 25270893]

191. Saarinen JV, Harvima RJ, Naukkarinen A, et al. The release of histamine is associated with the inactivation of mast cell chymase during immediate allergic wheal reaction in the skin. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2001; 31:593–601. [PubMed: 11359428]

192. Zhao W, Oskeritzian CA, Pozez AL, et al. Cytokine production by skin-derived mast cells: endogenous proteases are responsible for degradation of cytokines. J Immunol. 2005; 175:2635–2642. [PubMed: 16081839]

193. Tunon de Lara JM, Okayama Y, McEuen AR, et al. Release and inactivation of interleukin-4 by mast cells. Annals of the New York Academy of Sciences. 1994; 725:50–58. [PubMed: 8031009]

194. Horsmanheimo L, Harvima IT, Järvikallio A, et al. Mast cells are one major source of interleukin-4 in atopic dermatitis. The British Journal of Dermatology. 1994; 131:348–353. [PubMed: 7918008]

195. Ackermann L, Harvima IT. Mast cells of psoriatic and atopic dermatitis skin are positive for TNF-alpha and their degranulation is associated with expression of ICAM-1 in the epidermis. Archives of Dermatological Research. 1998; 290:353–359. [PubMed: 9749989]

196. Fischer M, Harvima IT, Carvalho RFS, et al. Mast cell CD30 ligand is upregulated in cutaneous inflammation and mediates degranulation-independent chemokine secretion. J Clin Invest. 2006; 116:2748–2756. [PubMed: 16964309]

197. Saarinen JV, Harvima RJ, Naukkarinen A, et al. Interleukin-4-positive mast cells are highly associated with the extent of immediate allergic wheal reaction in the skin. Allergy. 2001; 56:58–64. [PubMed: 11167353]

198. Ribbing C, Engblom C, Lappalainen J, et al. Mast cells generated from patients with atopic eczema have enhanced levels of granule mediators and an impaired Dectin-1 expression. Allergy. 2011; 66:110–119. [PubMed: 20608913]

199. Ilves T, Tiitu V, Suttle M-M, et al. Epidermal Expression of Filaggrin/Profilaggrin Is Decreased in Atopic Dermatitis: Reverse Association With Mast Cell Tryptase and IL-6 but Not With Clinical Severity. Dermatitis: Contact, Atopic, Occupational, Drug. 2015; 26:260–267.

200. Tominaga M, Tengara S, Kamo A, et al. Psoralen-ultraviolet A therapy alters epidermal Sema3A and NGF levels and modulates epidermal innervation in atopic dermatitis. Journal of Dermatological Science. 2009; 55:40–46. [PubMed: 19443185]

201. Urashima R, Mihara M. Cutaneous nerves in atopic dermatitis. A histological, immunohistochemical and electron microscopic study. Virchows Archiv: An International Journal of Pathology. 1998; 432:363–370. [PubMed: 9565347]

202. Tobin D, Nabarro G, Baart de la Faille H, et al. Increased number of immunoreactive nerve fibers in atopic dermatitis. J Allergy Clin Immunol. 1992; 90:613–622. [PubMed: 1383306]

203. Kubanov AA, Katunina OR, Chikin VV. Expression of Neuropeptides, Neurotrophins, and Neurotransmitters in the Skin of Patients with Atopic Dermatitis and Psoriasis. Bulletin of Experimental Biology and Medicine. 2015; 159:318–322. [PubMed: 26201903]

204. Järvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. Archives of Dermatological Research. 2003; 295:2–7. [PubMed: 12709813]

205. Xiang Z, Nilsson G. IgE receptor-mediated release of nerve growth factor by mast cells. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2000; 30:1379–1386. [PubMed: 10998013]

206. Nilsson G, Forsberg-Nilsson K, Xiang Z, et al. Human mast cells express functional TrkA and are a source of nerve growth factor. Eur J Immunol. 1997; 27:2295–2301. [PubMed: 9341772]

207. Groneberg DA, Serowka F, Peckenschneider N, et al. Gene expression and regulation of nerve growth factor in atopic dermatitis mast cells and the human mast cell line-1. J Neuroimmunol. 2005; 161:87–92. [PubMed: 15748947]

208. Peters EMJ, Michenko A, Kupfer J, et al. Mental stress in atopic dermatitis--neuronal plasticity and the cholinergic system are affected in atopic dermatitis and in response to acute experimental mental stress in a randomized controlled pilot study. PLoS ONE. 2014; 9:e113552. [PubMed: 25464511]

209. Kimata H. Enhancement of allergic skin wheal responses in patients with atopic eczema/dermatitis syndrome by playing video games or by a frequently ringing mobile phone. European Journal of Clinical Investigation. 2003; 33:513–517. [PubMed: 12795649]

210. Steinhoff M, Neisius U, Ikoma A, et al. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci. 2003; 23:6176–6180. [PubMed: 12867500]

211. Kempkes, C., Buddenkotte, J., Cevikbas, F., et al. Role of PAR-2 in Neuroimmune Communication and Itch. In: Carstens, E., Akiyama, T., editors. Itch: Mechanisms and Treatment. Boca Raton (FL): CRC Press/Taylor & Francis; 2014.

212. Sonkoly E, Muller A, Lauerma AI, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol. 2006; 117:411–417. [PubMed: 16461142]

213. Cevikbas F, Wang X, Akiyama T, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. J Allergy Clin Immunol. 2014; 133:448–460. [PubMed: 24373353]

214. Hawro T, Saluja R, Weller K, et al. Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. Allergy. 2014; 69:113–117. [PubMed: 24251414]

215. Feld M, Garcia R, Buddenkotte J, et al. The pruritus- and TH2-associated cytokine IL-31 promotes growth of sensory nerves. J Allergy Clin Immunol. 2016; 138:500–508.e524. [PubMed: 27212086]

216. Nobbe S, Dziunycz P, Mühleisen B, et al. IL-31 expression by inflammatory cells is preferentially elevated in atopic dermatitis. Acta Derm Venereol. 2012; 92:24–28. [PubMed: 22041865]

217. Niyonsaba F, Ushio H, Hara M, et al. Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. J Immunol. 2010; 184:3526–3534. [PubMed: 20190140]

218. MacQueen G, Marshall J, Perdue M, et al. Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. Science. 1989; 243:83–85. [PubMed: 2911721]

219. Esposito P, Chandler N, Kandere K, et al. Corticotropin-releasing hormone and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. J Pharmacol Exp Ther. 2002; 303:1061–1066. [PubMed: 12438528]

220. Singh LK, Pang X, Alexacos N, et al. Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing hormone, neurotensin, and substance P: A link to neurogenic skin disorders. Brain, Behavior, and Immunity. 1999; 13:225–239.

221. Peters EMJ, Kuhlmei A, Tobin DJ, et al. Stress exposure modulates peptidergic innervation and degranulates mast cells in murine skin. Brain, Behavior, and Immunity. 2005; 19:252–262.

222. Pavlovic S, Daniltchenko M, Tobin DJ, et al. Further exploring the brain-skin connection: stress worsens dermatitis via substance P-dependent neurogenic inflammation in mice. The Journal of Investigative Dermatology. 2008; 128:434–446. [PubMed: 17914449]

223. Peters EMJ, Liezmann C, Spatz K, et al. Nerve growth factor partially recovers inflamed skin from stress-induced worsening in allergic inflammation. The Journal of Investigative Dermatology. 2011; 131:735–743. [PubMed: 21085186]

224. Hagiyama M, Inoue T, Furuno T, et al. Increased expression of cell adhesion molecule 1 by mast cells as a cause of enhanced nerve-mast cell interaction in a hapten-induced mouse model of atopic dermatitis. The British Journal of Dermatology. 2013; 168:771–778. [PubMed: 23106683]

225. Oh M-H, Oh SY, Lu J, et al. TRPA1-dependent pruritus in IL-13-induced chronic atopic dermatitis. J Immunol. 2013; 191:5371–5382. [PubMed: 24140646]

226. Andoh T, Maki T, Li S, et al. β2-Microglobulin elicits itch-related responses in mice through the direct activation of primary afferent neurons expressing transient receptor potential vanilloid 1. Eur J Pharmacol. 2017; 810:134–140. [PubMed: 28687195]

227. Patricio ES, Costa R, Figueiredo CP, et al. Mechanisms Underlying the Scratching Behavior Induced by the Activation of Proteinase-Activated Receptor-4 in Mice. The Journal of Investigative Dermatology. 2015; 135:2484–2491. [PubMed: 25955385]

228. Yamaguchi J, Nakamura F, Aihara M, et al. Semaphorin3A alleviates skin lesions and scratching behavior in NC/Nga mice, an atopic dermatitis model. The Journal of Investigative Dermatology. 2008; 128:2842–2849. [PubMed: 18615113]

229. Wang J, Tran J, Wang H, et al. mTOR Inhibition improves anaemia and reduces organ damage in a murine model of sickle cell disease. Br J Haematol. 2016; 174:461–469. [PubMed: 27030515]

230. Gao W, Zan Y, Wang Z-JJ, et al. Quercetin ameliorates paclitaxel-induced neuropathic pain by stabilizing mast cells, and subsequently blocking PKCε-dependent activation of TRPV1. Acta Pharmacol Sin. 2016; 37:1166–1177. [PubMed: 27498772]

231. Fewtrell CMS, Gomperts BD. Effect of flavone inhibitors of transport ATPases on histamine secretion from rat mast cells. Nature. 1977; 265:635–636. [PubMed: 67562]

232. Martin MW, O'Sullivan AJ, Gomperts BD. Inhibition by cromoglycate and some flavonoids of nucleoside diphosphate kinase and of exocytosis from permeabilized mast cells. Br J Pharmacol. 1995; 115:1080–1086. [PubMed: 7582506]

233. Huang X, Zhao W, Hu D, et al. Resveratrol efficiently improves pulmonary function via stabilizing mast cells in a rat intestinal injury model. Life Sciences. 2017; 185:30–37. [PubMed: 28733146]

234. Zhao W, Zhou S, Yao W, et al. Propofol prevents lung injury after intestinal ischemia–reperfusion by inhibiting the interaction between mast cell activation and oxidative stress. Life Sciences. 2014; 108:80–87. [PubMed: 24878149]

235. Theoharides TC, Sieghart W, Greengard P, et al. Antiallergic drug cromolyn may inhibit histamine secretion by regulating phosphorylation of a mast cell protein. Science. 1980; 207:80–82. [PubMed: 6153130]

236. Shishibori T, Oyama Y, Matsushita O, et al. Three distinct anti-allergic drugs, amlexanox, cromolyn and tranilast, bind to S100A12 and S100A13 of the S100 protein family. Biochemical Journal. 1999; 338:583–589. [PubMed: 10051426]

237. Hojo M, Maghni K, Issekutz TB, et al. Involvement of α-4 Integrins in Allergic Airway Responses and Mast Cell Degranulation In Vivo. Am J Respir Crit Care Med. 1998; 158:1127–1133. [PubMed: 9769271]

238. Pardanani A, Elliott M, Reeder T, et al. Imatinib for systemic mast-cell disease. The Lancet. 2003; 362:535–537.

239. Goldsmith P, McGarity B, Walls AF, et al. Corticosteroid treatment reduces mast cell numbers in inflammatory bowel disease. Dig Dis Sci. 1990; 35:1409–1413. [PubMed: 1977567]

240. An intranasal Syk-kinase inhibitor (R112) improves the symptoms of seasonal allergic rhinitis in a park environment - ScienceDirect.

241. Price MM, Oskeritzian CA, Falanga YT, et al. A specific sphingosine kinase 1 inhibitor attenuates airway hyperresponsiveness and inflammation in a mast cell–dependent murine model of allergic

asthma. Journal of Allergy and Clinical Immunology. 2013; 131:501–511.e501. [PubMed: 22939756]

242. Lucio J, D'Brot J, Guo CB, et al. Immunologic mast cell-mediated responses and histamine release are attenuated by heparin. J Appl Physiol. 1992; 73:1093–1101. [PubMed: 1383185]
243. Wang YH, Taché Y, Harris AG, et al. Desloratadine prevents compound 48/80-induced mast cell degranulation: visualization using a vital fluorescent dye technique. Allergy. 2005; 60:117–124. [PubMed: 15575942]
244. Genovese A, Patella V, De Crescenzo G, et al. Loratadine and desethoxylcarbonyl-loratadine inhibit the immunological release of mediators from human FcεRI+cells. Clinical & Experimental Allergy. 1997; 27:559–567. [PubMed: 9179431]
245. Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. Br J Pharmacol. 1987; 90:421–429. [PubMed: 2435353]
246. Simpson AJ, Bood JR, Anderson SD, et al. A standard, single dose of inhaled terbutaline attenuates hyperpnea-induced bronchoconstriction and mast cell activation in athletes. Journal of Applied Physiology. 2016; 120:1011–1017. [PubMed: 26846550]
247. Sahid MNA, Liu S, Kiyoi T, et al. Inhibition of the mevalonate pathway by simvastatin interferes with mast cell degranulation by disrupting the interaction between Rab27a and double C2 alpha proteins. Eur J Pharmacol.
248. Tan JW, Israf DA, Md Hashim NF, et al. LAT is essential for the mast cell stabilising effect of tHGA in IgE-mediated mast cell activation. Biochemical Pharmacology. 2017
249. Chakraborty S, Kar N, Kumari L, et al. Inhibitory effect of a new orally active cedrol-loaded nanostructured lipid carrier on compound 48/80-induced mast cell degranulation and anaphylactic shock in mice. Int J Nanomedicine. 2017; 12:4849–4868. [PubMed: 28744120]
250. Li X, Lee YJ, Jin F, et al. Sirt1 negatively regulates FcεRI-mediated mast cell activation through AMPK- and PTP1B-dependent processes. Scientific Reports. 2017; 7:6444. [PubMed: 28744004]
251. Nam ST, Park YH, Kim HW, et al. Suppression of IgE-mediated mast cell activation and mouse anaphylaxis via inhibition of Syk activation by 8-formyl-7-hydroxy-4-methylcoumarin, 4μ8C. Toxicology and Applied Pharmacology. 2017; 332:25–31. [PubMed: 28736076]
252. Säfholm J, Manson ML, Bood J, et al. Prostaglandin E2 inhibits mast cell-dependent bronchoconstriction in human small airways through the E prostanoid subtype 2 receptor. J Allergy Clin Immunol. 2015; 136:1232–1239.e1231. [PubMed: 25962903]
253. De Filippis D, Luongo L, Cipriano M, et al. Palmitoylethanolamide reduces granuloma-induced hyperalgesia by modulation of mast cell activation in rats. Mol Pain. 2011; 7:3. [PubMed: 21219627]
254. El-Agamy DS. Anti-allergic effects of nilotinib on mast cell-mediated anaphylaxis like reactions. Eur J Pharmacol. 2012; 680:115–121. [PubMed: 22329898]

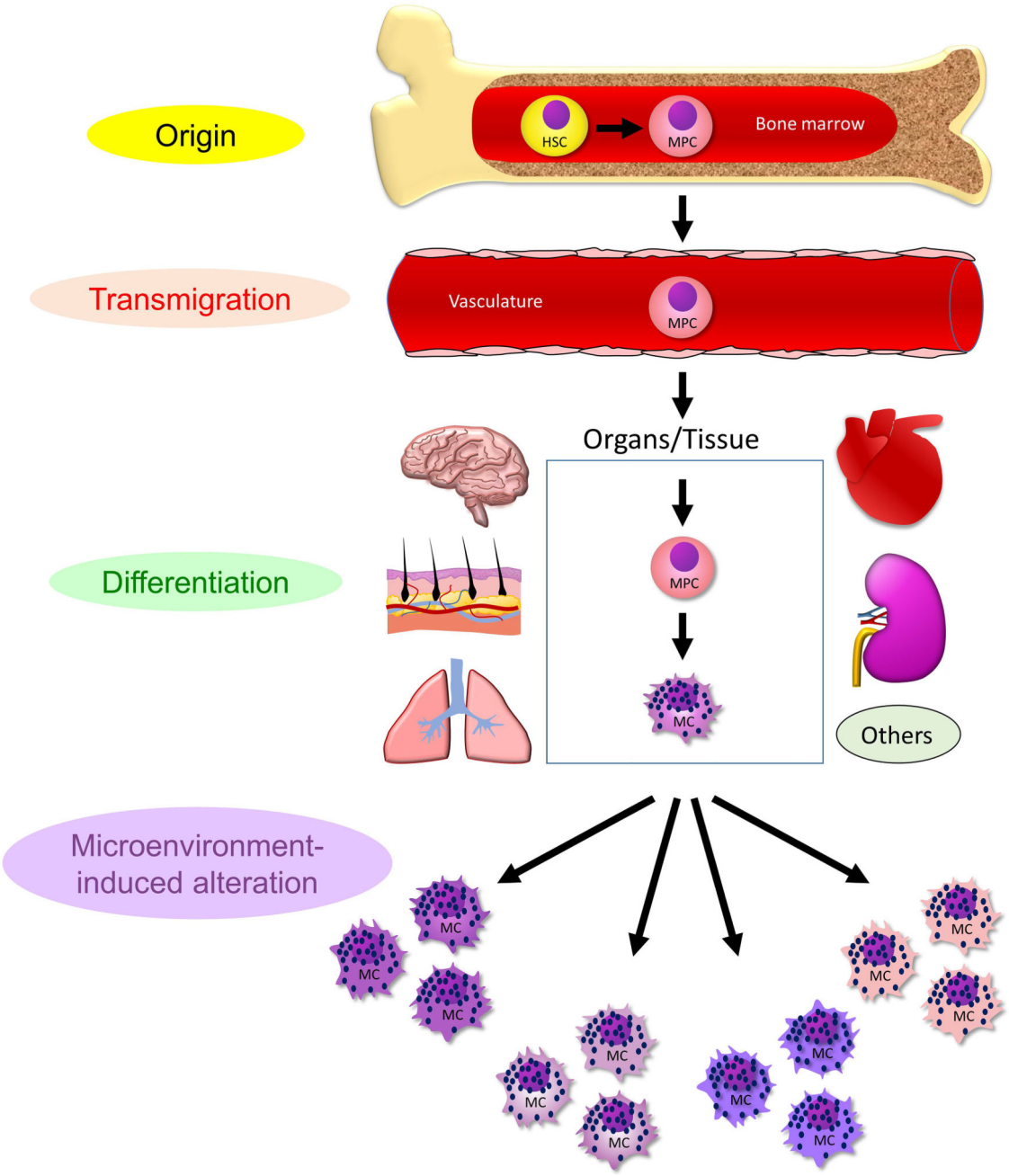

Figure 1. Origin and differentiation of mast cells

Mast cells are tissue-resident myeloid cells, which originate from hematopoietic stem cells (HSC) in the bone marrow. HSC differentiate into early or mature myeloid progenitor cells (MPC) in the bone marrow and transmigrate to different tissues via circulation. In the tissues MPCs differentiate into mast cells and undergo maturation. During maturation, remarkable heterogeneity occurs depending upon the tissue, microenvironment, and pathological condition. In the same species, mast cells from different organs may vary significantly. In the same tissue, heterogeneity may occur between the pathological and normal condition.

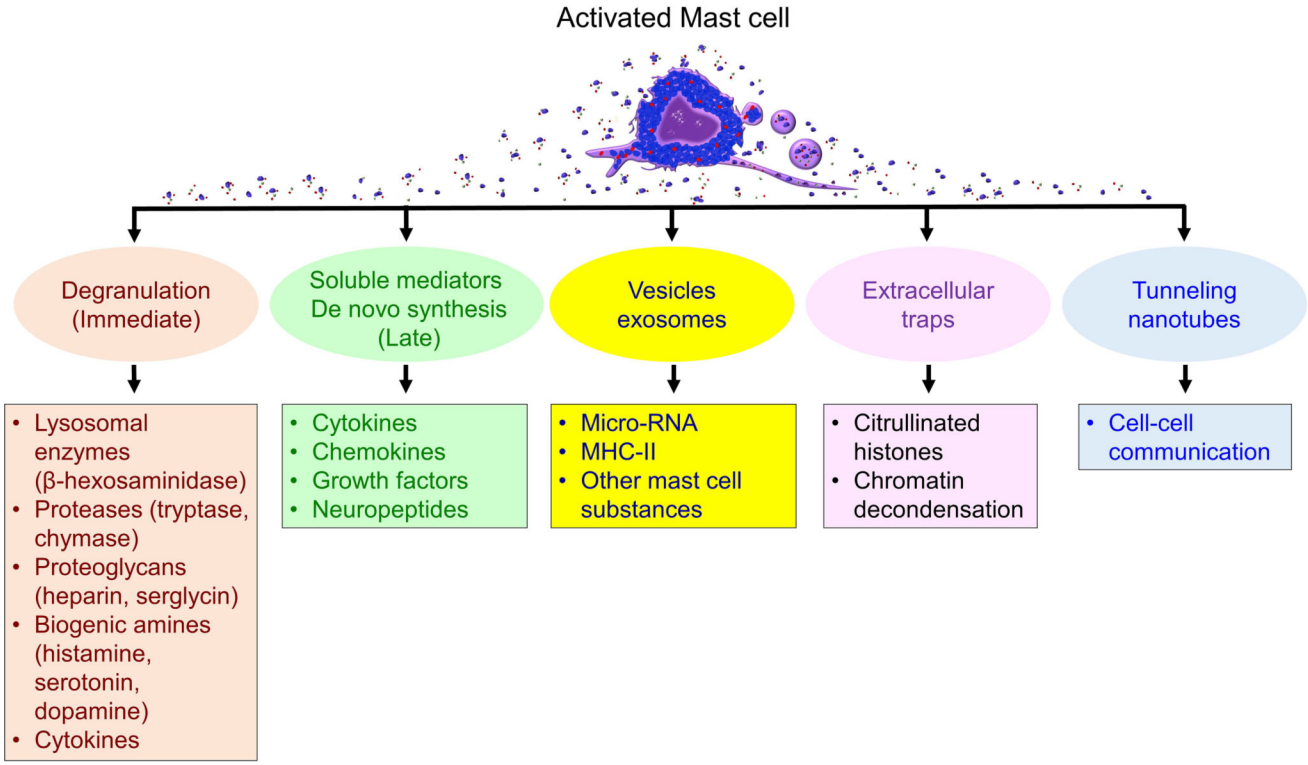

Figure 2. Different modes of mast cell activation

Mast cell activation involves diverse processes involving the release of mediators and structural alterations for dissemination of mediators and cell-cell communication. The mode of activation is highly heterogeneous and depends upon location and chemical and anatomical stimuli in the surrounding microenvironment and pathological condition.

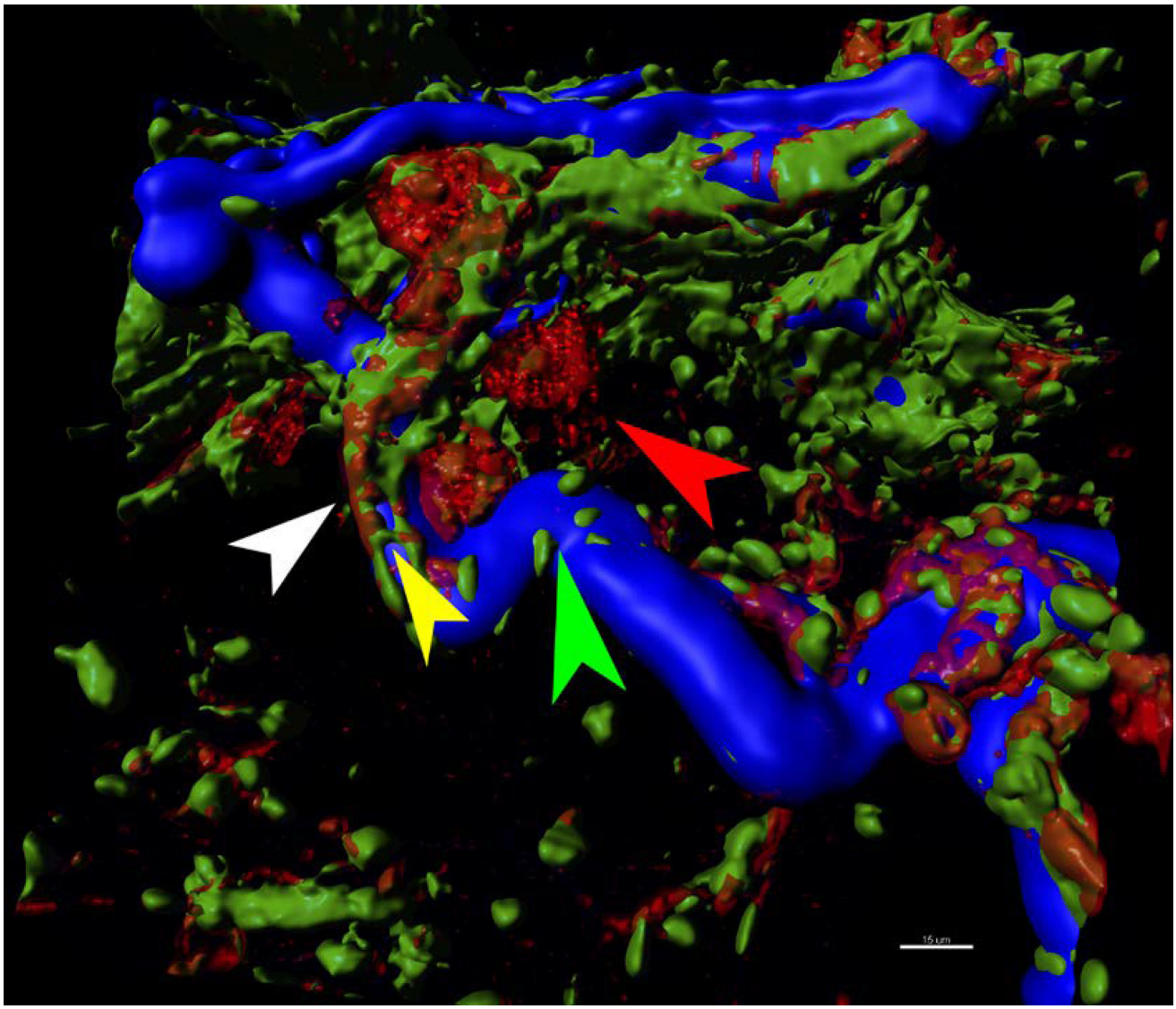

Figure 3. Activated mast cells surround nerve fibers in the skin of sickle mice

Laser scanning confocal microscopy (LSCM) of 100 um-thick dorsal skin sections immunostained with 1:200 rabbit anti-c-*kit* (Santa Cruz Biotech., sc-5535), 1:200 goat anti-tryptase (Santa Cruz Biotech., sc-32473), and 1:1000 chicken anti-Neurofilament H-200 (Abcam, ab72996). Sections were subsequently labeled with the following secondary antibodies: 1:400 donkey cy2 anti-rabbit (711-225-152), 1:400 donkey cy3 anti-goat (705-165-147), and 1:400 donkey cy5 anti-chicken (703-175-155) (Jackson Immunoresearch). Z-stacks of 4 um thickness each were sequentially acquired using an Olympus FluoView FV1000 BX2 Upright Confocal microscope at 60× magnification. Rendition of 3D reconstruction of original LSCM image was performed using Imaris 7.5.2 software (Bitplane Inc., Zurich, Switzerland). Image represents reproducible images from 5 different ~5-month old male transgenic HbSS-BERK sickle mice. Note the blue axonal nerve fiber surrounded by degranulating mast cells (green) loaded with tryptase (red). Several degranulating mast cells (red arrow) are nesting in the nerve plexus (blue), which

may be responsible for disruption of the nerve plexus observed in this mouse model earlier. In addition to degranulation, mast cells are extending pseudopodia clutching the nerve fiber (white arrow) and interconnecting mast cells around the axon (yellow arrow). Multiple structures appear like vesicle of different sizes (green arrow) suggestive of exosomes (<150 nm), microvesicles (100–1,000 nm), and large oncosomes (1,000–5,000 nm). Thin sprouting nerve fibers are seen emerging between the highly dense degranulating mast cells. This inflammatory pathology caused by mast cell activation may underlie neuropathic pain as a result of neural injury in SCD.

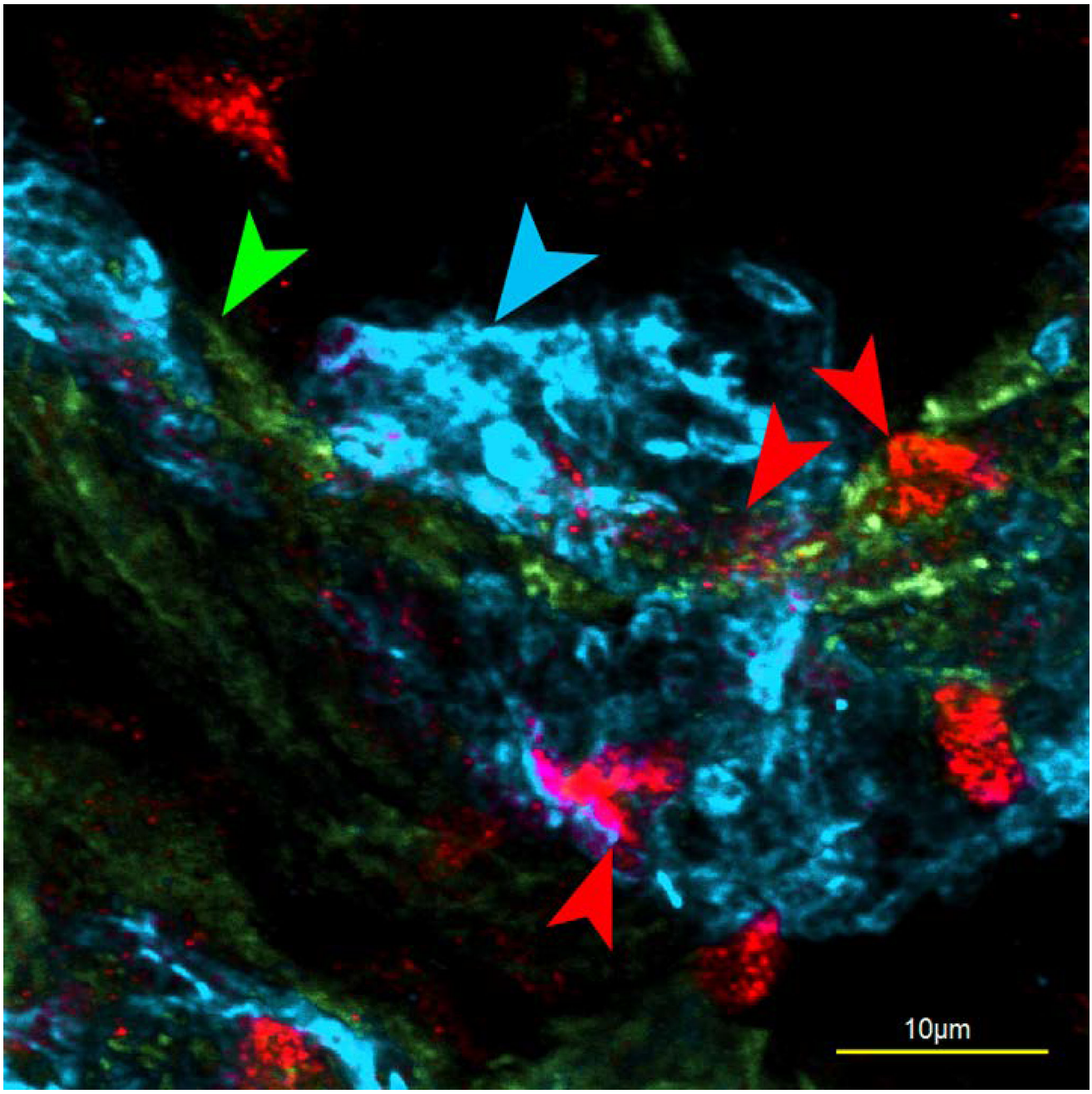

Figure 4. Mast cell extracellular traps (MCETS) surround cutaneous vasculature in sickle mice

Laser scanning confocal microscopy of 100 um-thick dorsal skin sections were immunostained with rabbit anti-Histone H3 (red), a marker of chromatin modification in traps (citrulline R2 + R8 + R17; 1:1000; Abcam, ab5103), goat anti-F_cεRI (turquoise), a marker of mast cells, and a rat anti-CD31 (green), a marker of blood vessels (both1:200; Santa Cruz, sc-18916). Secondary antibodies used were from Jackson Immunoresearch: 1:400 donkey cy3 anti-rabbit (711-165-152), 1:400 donkey cy2 anti-goat (705-225-147), and 1:400 donkey cy5 anti-rat (712-175-153). Images were acquired in *z*-sections of 1 um/slice, using an Olympus FluoView FV1000 BX2 Upright Confocal microscope at 240×

magnification. Image represents reproducible images from 5 different ~5-month-old male transgenic HbSS-BERK sickle mice. Note red aggregates and spread out arrays (red arrows) exhibiting MCETs due to citrullination of histones shooting out from the turquoise $F_c\varepsilon$RI-stained mast cells (turquoise arrows). Mast cells in turquoise surround the green blood vessel (green arrow), and their extravasating citrullinated arrays of traps appear to be penetrating into the lumen. Sickle cell disease (SCD) is characterized by vascular dysfunction, vasculopathy, and vascular leakage. This intimate association of degranulating mast cells and MCETs suggest that mast cell activation may contribute to vascular dysfunction in SCD.

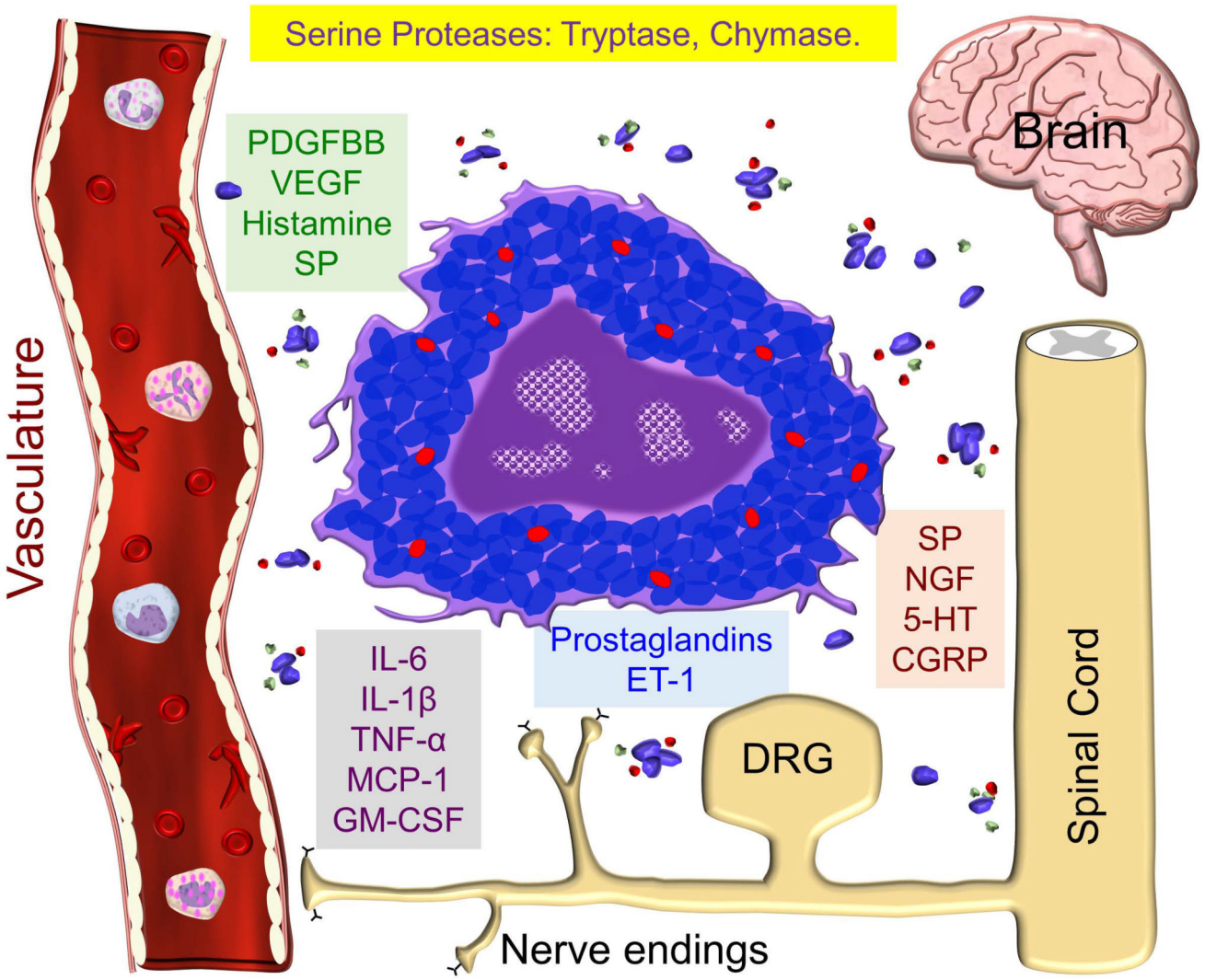

Figure 5. Proposed function of mast cells in neurovascular interactions underlying painor itch

Mast cells release a plethora of substances upon activation that can have direct effects on the vasculature, nerve fibers in the periphery, spinal cord, and brain. Inflammatory cytokines, endothelin, histamine, proteases, prostaglandins, and neurotransmitters released from mast cells can directly activate nociceptors or pruriceptors and microglial cells; this contributes to neuronal sensitization and leads to pain or itch. In parallel, these substances influence vascular function by increasing vascular permeability, remodeling, blood flow, and coagulation; these substances also increase inflammation by releasing cytokines and neuropeptides and by recruiting and activating neutrophils and other pro-inflammatory cells. In turn, activated nerve fibers, endothelial cells, and pro-inflammatory cells release several substances including neuropeptides, which may have a paracrine feedback effect on mast cell activation. Many substances released by mast cells may act in an autocrine manner, thus leading to their continuous activation and sustained direct and indirect effects on vascular and nervous systems. Substances, such as neuropeptides released by nerve fibers, increase vascular permeability and plasma extravasation and lead to neurogenic inflammation

accompanied by pain or itch. Together, mast cell activation orchestrates a vicious cycle of inflammation, neurogenic inflammation, and pain/itch.

Table 1

Therapeutic strategies targeting mast cells.

	Target	Pathology	Reference
Acupuncture		SCD	(229)
Quercitin	PKCε-dependent activation of TRPV1	Chemo induced neuropathy	(230)
Natural flavonoids		Stimulated mast cell	(231, 232)
Resveratrol	Oxidative stress	Intestinal ischemia/reperfusion (IIR)-induced acute lung injury	(233)
Propofol	Oxidative stress	Intestinal ischemia/reperfusion (IIR)-induced acute lung injury	(234)
Cromolyn	Mast cell protein	SCD, stimulated mast cell	(7, 235, 236)
anti-VLA-4 mAbs	β1 integrin	allergic airway responses	(237)
Imatinib	tyrosine kinase enzymes	Systemic mast cell disease	(238)
Corticosteroids	Depletion of mast cell	Inflammatory bowel disease	(239)
R112	IgE-FcεRI signaling pathway	seasonal allergic rhinitis	(240)
SK1-I	specific SphK1 inhibitor	murine model of allergic asthma	(241)
Heparin	inhibitor of inositol 1,4,5-triphosphate (InsP3) receptors	immediate cutaneous reaction (ICR) and acute bronchoconstrictor response (ABR)	(242)
Loratadine and Desloratadine	H_1-receptor antagonist	allergic rhinitis and chronic idiopathic urticaria seasonal and perennial rhinitis and some allergic skin disorders	(243, 244)
salbutamol	beta-adrenoceptor agonist	stimulated mast cell	(245)
terbutaline	beta-adrenoceptor agonist	exercise-induced bronchoconstriction	(246)
simvastatin	Mevalonate pathway	stimulated mast cell	(247)
tHGA	LAT axis pathway	stimulated mast cell	(248)
cedrol		stimulated mast cell	(249)
Sirt1	AMPK- and PTP1B-dependent processes	stimulated mast cell	(250)
4μ8C	Lyn and Fyn	stimulated mast cell and passive cutaneous anaphylaxis	(251)
Prostaglandin E2	E prostanoid subtype 2 receptor	asthmatic responses	(252)
CP55,940	cannabinoid receptor agonist	SCD	(130)
Palmitoylethanolamide	PPAR-α and GPR55	chronic granulomatous inflammation	(253)
AT-200	nociceptin receptor agonist	SCD	(144)
Nilotinib	tyrosine kinase inhibitor	anaphylactic allergic reaction	(254)