Cell Host & Microbe
Forum

Pain and Itch: Beneficial or Harmful to Antimicrobial Defense?

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Pain and itch are unpleasant sensations accompanying many microbial infections. Recent studies demonstrate that pain- and itch-mediating somatosensory neurons are able to directly detect pathogens, triggering neuronal activation and subsequent regulation of immune responses. We discuss whether pain and/or itch during infection is beneficial or harmful to host antimicrobial defense.

Introduction

The mammalian somatosensory nervous system densely innervates barrier tissues, including the skin, genitourinary tract, gastrointestinal tract, and respiratory tract. Pain and itch are mediated by peripheral somatosensory neurons termed nociceptors and pruriceptors, respectively. Microbial infections by different types of pathogens often exhibit intense pain or itch. For example, bacterial infections of the skin (e.g., *Staphylococcus aureus*, *Streptococcus pyogenes*), oral cavity (e.g., *Streptococcus mutans*), and urinary tract (e.g., *E. coli*) produce significant pain. Parasitic skin infections (e.g., *Onchocerca volvulus*) and fungal skin infections (e.g., *Tinea pedis*, *Candida albicans*) are characterized by intense itch. The molecular and cellular mechanisms leading to the activation of nociceptors or pruriceptors during infection are not well understood. We discuss how the sensory nervous system could play a critical role in mammalian host defense.

Sensory Neuron Detection of Microbes

Pain and itch play a critical role in protecting organisms from sources of danger such as extreme temperatures, reactive chemicals, and tissue injury. Pain leads to motor reflexes and behavioral avoidance of these dangers. Similarly, itch leads to scratch-mediated removal of insects and harmful substances from the skin surface. Nociceptor neurons express distinct molecular transducers of pain at their peripheral nerve terminals, such as transient receptor potential (TRP) ion channels, which are activated by noxious stimuli including heat, cold, and reactive chemicals (Basbaum et al., 2009). Pruriceptor neurons express G protein-coupled receptors and cytokine receptors that sense inflammatory mediators from the immune system. Upon activation, action potentials are transduced to the dorsal horn of the spinal cord and then relayed to the brain, where these signals are processed as pain or itch.

Bacterial, viral, and fungal pathogens constitute another major source of organismic danger. Recent findings show that sensory neurons developed specific molecular mechanisms to detect pathogens and their derived molecules (Figure 1A). Neuronal activation downstream of pathogen sensing implicates a role for the nervous system in host detection of and defense against pathogens. Like the immune system, one mode of sensory neuron pathogen recognition is through pattern recognition receptors including toll-like receptors (TLRs). Mouse nociceptor and pruriceptor neurons express TLR3 (recognizing double-stranded RNA), TLR4 (recognizing lipopolysaccharides [LPSs]), TLR5 (recognizing flagellin), TLR7 (recognizing single-stranded RNA), and TLR9 (recognizing unmethylated CpG) (Liu et al., 2010, 2012; Qi et al., 2011; Ferraz et al., 2011, Xu et al., 2015).

Neuronal recognition of TLR ligands leads to neuronal sensitization and induction of pain and itch behavior in mice. TLR3, TLR7, or TLR9 ligands augment sensory neuron expression of the heat-sensing ion channel TRPV1 and neuronal production of prostaglandin E2 (PGE2), two molecular mechanisms that can induce increased neuronal firing and pain (Qi et al., 2011). LPS from the oral bacteria *Porphyromonas gingivalis* sensitizes nociceptor neurons through TLR4, leading to increased excitability and release of the sensory neuropeptide CGRP (Ferraz et al., 2011). LPS also directly gates the opening of the nociceptive ion channel TRPA1 and subsequent neuronal firing (Meseguer et al., 2014). The lipid A moiety of LPS mediates this TRPA1 activation, and *Trpa1*$^{-/-}$ mice show abolition of LPS-mediated pain behaviors. Flagellin, a major component of bacterial flagella, was recently found to activate Aβ-fiber neurons involved in neuropathic pain through neuronal TLR5 (Xu et al., 2015). Pruriceptor activation and itch also result from neuronal recognition of distinct TLR ligands. Ji and colleagues found that TLR7 is expressed by pruriceptors, and synthetic TLR7 ligands imiquimod and loxoribine produce TLR7-dependent itch behavior in mice (Liu et al., 2010). TLR3 is also expressed by pruriceptors, and PolyI:C, a ligand for TLR3, induces neuronal activation and itch (Liu et al., 2012). Viral double-stranded RNA and single-stranded RNA are known pathogen-derived ligands for TLR3 and TLR7, respectively, but it remains to be determined whether these viral ligands produce itch during infection through direct recognition by pruriceptor neurons. Furthermore, in contrast to the extensive characterization of TLR signaling in immune cells, less is known about neuronal signaling downstream of pathogen activation, an area of future investigation. The fungal pathogen *Candida albicans* and its cell wall component zymosan, for example, directly activate TRPV1-expressing nociceptor neurons (Kashem et al., 2015), but the molecular mechanisms mediating this neuronal activation are not well understood.

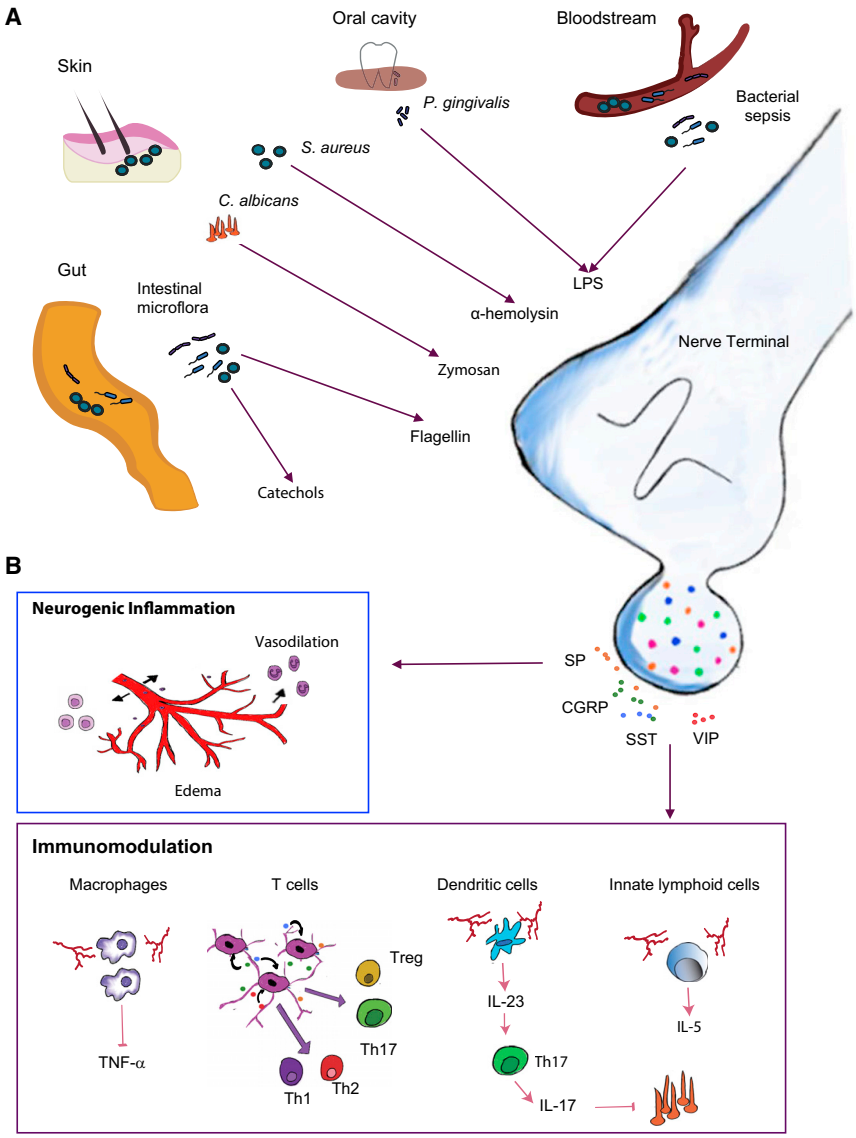

Figure 1. Microbial Detection by Sensory Neurons and Neuroimmune Crosstalk

Sensory neurons directly recognize pathogens to produce neuronal activation, pain, and itch. In turn, these neurons release mediators that crosstalk with innate and adaptive immune cells to modulate their function.

(A) Nociceptor and pruriceptor neurons respond to a variety of conserved molecular structures from pathogenic microbes at different barrier sites. Specific mechanisms of neuronal activation include detection of lipolysaccharides (LPS), flagellin, catechols, *S. aureus* pore-forming toxin α-hemolysin, and *C. albicans*-derived zymosan.

(B) Upon calcium influx, sensory neurons release molecular mediators from their peripheral nerve terminals, including the neuropeptides substance P (SP), CGRP, SST, and VIP. These neuropeptides drive neurogenic inflammation (vasodilation, edema) and immunomodulation. Neuronal modulation of immune cell functions includes suppression of macrophage TNF-α production, alteration of T cell differentiation into distinct subtypes, mediation of dendritic cell production of IL-23 and downstream IL-17 signaling, and activation of type 2 innate lymphoid cells (ILC2).

In addition to TLR ligands, somatosensory neurons also detect pathogens through other molecular mechanisms. *S. aureus* skin infection induces significant mechanical and thermal hypersensitivity in mice (Chiu et al., 2013). This increased pain sensitivity is not due to immune cells; rather, it occurs through direct neuronal depolarization by N-formylated peptides through FPR1 receptors and by the *S. aureus* pore-forming toxin α-hemolysin (Chiu et al., 2013). N-formylated peptides are metabolic byproducts of all bacteria due to their expression of the formylase enzyme. Pore-forming toxins are critical virulence determinants for many bacterial pathogens, and their capacity to induce ion flux and depolarize cell membranes suggests a wider mechanism of neuronal activation. It remains to be determined whether nociceptors recognize other bacterial pathogens through similar molecular mechanisms to produce pain.

Another intriguing question is whether mammalian commensal microbiota also interact with neurons. Recent work has shown gut microflora produce metabolites and neurotransmitters (e.g., catechols) that directly modulate enteric nervous system (ENS) and CNS function. The role of resident microbes in regulating nociceptors and pruriceptors is less understood. One interesting study found that germ-free mice showed significantly less pain in response to inflammatory stimuli than those raised in conventional conditions (Amaral et al., 2008). Future work will be needed to understand the role of homeostatic interactions between sensory neurons and resident microflora in pain and itch.

Sensory Neuron Crosstalk with Immune Cells

Neuroimmune communication is bidirectional in pain and itch. Nociceptor and pruriceptor neurons are sensitized by immune cell-derived cytokines (e.g., IL-1β, TNF- α), chemicals (e.g., histamine, bradykinin), and lipid mediators (e.g., prostaglandins), which act through phosphorylation of neuronal ion channels and other mechanisms to lower the threshold of action potential firing, thus leading to increased pain and itch. Neurons, in turn, release mediators, including neuropeptides, that are able to modulate immune cell function. Typical antimicrobial host defense mechanisms, such as cytokine/chemokine production, immune cell recruitment, phagocytosis, and production of antimicrobial peptides, are initiated only minutes to hours after infection. Neurons, by contrast, are able to respond within milliseconds of encountering the presence of danger. Since nociceptor and pruriceptor neurons are able to directly sense pathogens, the sensory nervous system may be a first responder to pathogen invasion and key orchestrator of downstream innate and adaptive immunity.

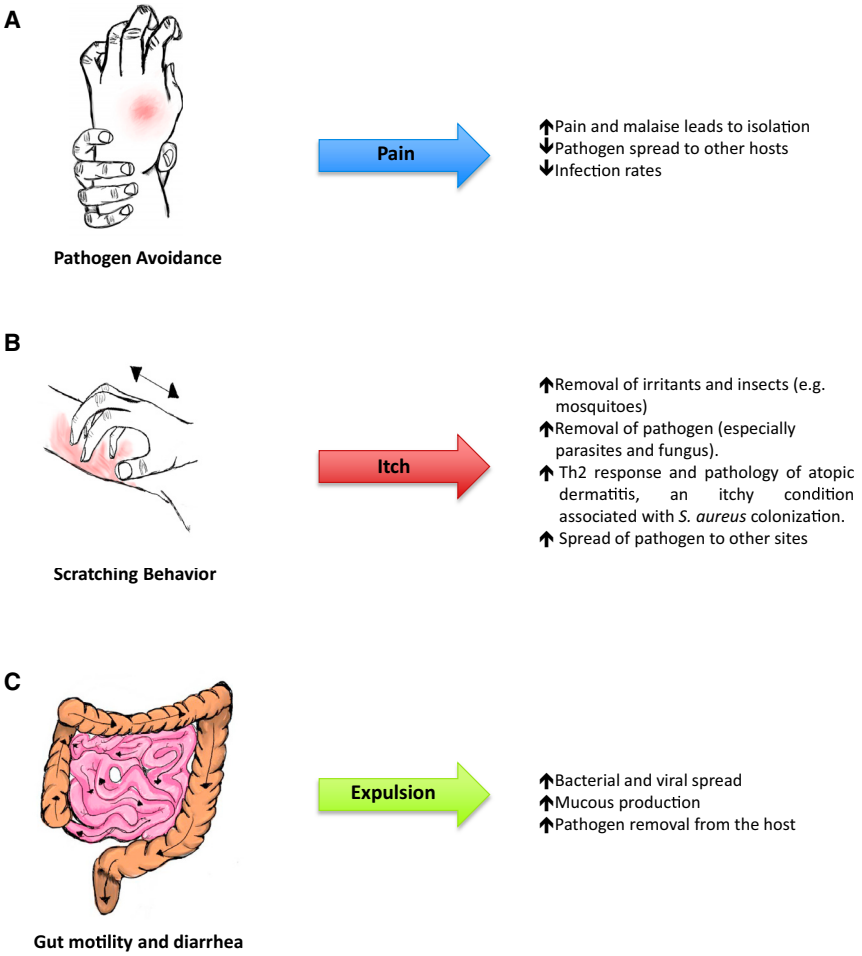

Figure 2. Sensory Neurons Drive Behaviors that Affect Host Defense and Pathogen Spread
Nociceptor and pruriceptor sensory neurons initiate behavioral responses that protect the host from infection and alter spread of pathogens to the environment.
(A) Pain and avoidance behavior during infection minimizes exposure to pathogens and pathogenic spread to other hosts.
(B) Pruriceptors mediate itch and scratch behavior, leading to physical removal of ectoparasites (e.g., mosquitoes) and microorganisms (e.g., fungi, parasites, or *S. aureus* in atopic dermatitis). This event could also facilitate dissemination of pathogens to other anatomical sites and hosts.
(C) Gastrointestinal tract-innervating nociceptor neurons mediate pain and nausea. Their crosstalk with enteric neurons and epithelial cells drives peristalsis and diarrhea, outcomes which are beneficial to the host for pathogen removal, but may in turn facilitate pathogen spread to the environment.

Innate Immunity

We discuss how neuroimmune regulation may be an integral part of host-pathogen defense (Figure 1B). Neuropeptides are an important mechanism by which sensory neurons crosstalk with immune cells. Neuropeptides are expressed by CNS and peripheral nervous system (PNS) neuronal subtypes and released from peripheral nerve terminals to mediate cell-cell communication. Neuropeptides have potent immunomodulatory effects on innate immune cell types. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) inhibit macrophage production of cytokines and nitric oxide through a cAMP-dependent pathway. These neuropeptides protect mice from LPS-induced endotoxic shock through inhibition of TNF-α and IL-6. In *S. aureus* infection, nociceptors suppressed the recruitment of monocytes and neutrophils to the site of infection and draining lymph node hypertrophy (Chiu et al., 2013). Calcitonin-gene-related peptide (CGRP) mediated the suppression of *S. aureus*-induced lymphadenopathy and downregulation of macrophage TNF-α production. Bacterial and viral pathogens may exploit these neuron-driven immunosuppressive pathways to facilitate their survival within the host.

Adaptive Immunity

Nociceptors and their mediators could play an important role in the differentiation and function of adaptive immune cells during host defense (Figure 1B). Recent evidence demonstrates nociceptors can regulate Th17 cell-driven immunity (Kashem et al., 2015; Riol-Blanco et al., 2014) and type 2 immune cell-driven allergic responses mediated by Th2 cells and type 2 innate lymphoid cells (ILC2) (Talbot et al., 2015). $TRPV1^+$ nociceptor neurons exhibit host resistance to candidiasis via production of CGRP, which regulates dermal dendritic cells (DCs) to facilitate production of IL-23, thus driving IL-17A-mediated host protection against *C. albicans* (Kashem et al., 2015). Th2 and ILC2 cells play an important role in driving host defenses against parasites, and it remains to be determined whether nociceptor neurons modulate these cells during host defense against helminthes or other parasites. It is possible that distinct types of neuropeptides could drive the polarization of T cell subtypes (Th1, Th2, Th17, or T regulatory cells) during infection through regulation of antigen-presentation cells or direct communication with these lymphocytes. Another area of interest would be whether B cells and antigen-driven antibody responses to pathogens are regulated by sensory neurons. Neuroimmune regulation could depend on the nature of the pathogen (intracellular versus extracellular) and type of immune response (e.g., type 1 versus type 2 responses).

Sensory Neuron Modification of Behavioral Outcomes

Pain and Avoidance

Physical avoidance of hostile environments is a key aspect of pain behavior and is regarded as an important protective mechanism against dangers. Avoidance may also be a critical mechanism of host defense against pathogens (Figure 2A). In *C. elegans*, avoidance of pathogenic bacteria occurs through acute or learned behavior responses. For acute (or innate) behavioral avoidance, a subset of sensory neurons, BAG, detects CO_2 produced by microbial respiration through TOL-1, a TLR, alerting *C. elegans* to the presence of pathogenic *Serratia marcescens* (Brandt

and Ringstad, 2015). For learned behavioral avoidance, chemosensory neurons direct the aversive learning of odors from pathogenic bacteria, while these neurons directed chemoattraction toward odors from nonpathogenic bacteria. Does this type of behavior also occur in mammalian hosts? Chemosensory neurons that express bitter taste receptors in mammalian airways have been shown to sense acyl-homoserine lactone produced by Gram-negative pathogens (Tizzano et al., 2010). This early detection of bacterial products triggers an innate immune response, restricting bacterial survival and formation of biofilms (Tizzano et al., 2010). It remains to be determined whether direct olfaction or gustation of pathogens leads to their future avoidance by mammals. In addition, bacterial and viral infections in mammals leading to pain regulated sickness behaviors such as anorexia, nausea, lethargy, fever, social withdrawal, and isolation—all of these phenomena may collectively facilitate elimination of the microbial burden and decrease the spread of pathogens from host to host. It is intriguing to note some pathogens such as *M. ulcerans*, *M. leprae*, and *Treponema pallidum* (causative of syphilis) produce painless lesions. *M. ulcerans* blocks pain by producing a mycolactone that hyperpolarizes nociceptor neurons via type 2 angiotensin receptors (Marion et al., 2014). Silencing of neurons may give these pathogens an advantage through immunomodulation or behavioral modification.

Itch and Scratch

Mosquitoes, fleas, and other ectoparasites trigger itch through injection of their salivary secretions or irritation of epidermal pruriceptor nerve fibers. Itch and scratch behavior are also major features of many infectious diseases; in particular, parasitic and fungal skin infections. One functional role of itch may be scratch-mediated removal of these irritating pathogens. Pathogens may also take advantage of this sensation for their spread from host to host (Figure 2B), including itch-inducing parasites such as *Onchocerca volvulus*, which need to exit the host as part of their life cycle; scratching could expose their presence to insects and other vectors. Scratching could also induce damage to tissue blood vessels, allowing entry of pathogens into the circulation. Bacterial pathogens could also take advantage of itch/scratch for their spread. Atopic dermatitis, a skin inflammatory condition characterized by chronic itch, is characterized by colonization of its lesions by the skin pathogen *Staphylococcus aureus*. It is possible chronic itch and scratching of the skin could facilitate the spread of *S. aureus* from host to host through fingernails and skin. The molecular mechanisms responsible for itch production in all of these pathogenic infections are not well understood, and may be mediated by direct triggering of pruriceptor neurons by pathogens or inflammatory mechanisms through mast cells and other cells.

Gastrointestinal Expulsion

The upper and lower gastrointestinal tract is densely innervated by nociceptor neurons originating in dorsal root ganglia, which mediate pain, as well as nodose/jugular ganglia neurons, which mediate nausea and other physiological functions. These neurons can crosstalk with gut-resident enteric neurons to regulate gut motility and epithelial cells to regulate gastric secretions (Figure 2C). Nociceptor neurons are hypersensitive in conditions such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), leading to chronic pain sensitivity. However, the molecular and cellular mechanisms of nociceptor crosstalk with gut-intrinsic cells and pathogens are not well understood. There is increasing interest in determining the mechanisms by which gastrointestinal flora, such as resident commensal bacteria and invading pathogens, interact with the sensory nervous system. Vagal nociceptors send electrical signals to the brain after sensing microbes or their toxins, which eventually generates motor reflexes that regulate antimicrobial expulsive phenomena, including vomiting (through this, the host can expel a significant number of pathogens), mucus production (facilitating mucociliary clearance of pathogens), and increased GI motility and diarrhea (reduces microbial numbers in the intestine). These responses may be important for both host survival and the spread of certain types of pathogens.

Concluding Remarks

Pain and itch are integral parts of inflammation. In the first century AD, Celsus originally defined pain as one of the four cardinal signs of inflammation. The finding that somatosensory neurons can directly detect bacterial and fungal ligands implicates a role for the nervous system in pathogen detection and defense. The types and characteristics of pathogen-derived molecules and the mechanisms of their detection by the nervous system are only beginning to be elucidated. It is increasingly clear that pain and itch are not just symptoms of inflammation, but rather that nociceptor and pruriceptor neurons play direct roles in driving or suppressing the immune response and host defense. It is possible that certain types of host defenses are coupled to distinct subsets of sensory neurons. Uncovering the molecular links between specific branches of the sensory nervous system and immune system is an important future step. Sensory neuron-driven effector functions relevant to host defenses are not limited to cellular interactions with immune cells, but also extend to behavioral modifications including avoidance, scratch, cough, and gastrointestinal expulsion. The complex role of the nervous system in host-pathogen interactions will serve as the basis for many future molecular, cellular, behavioral, and epidemiological studies.

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