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Feature Review

# Setting the tone: nociceptors as conductors of immune responses

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**Nociceptors have emerged as master regulators of immune responses in both homeostatic and pathologic settings; however, their seemingly contradictory effects on the functions of different immune cell subsets have been a source of confusion. Nevertheless, work by many groups in recent years has begun to identify patterns of the modalities and consequences of nociceptor-immune system communication. Here, we review recent findings of how nociceptors affect immunity and propose an integrated concept whereby nociceptors are neither inherently pro- nor anti-inflammatory. Rather, we propose that nociceptors have the role of a rheostat that, in a context-dependent manner, favors tissue homeostasis and fine-tunes immunity by preventing excessive histotoxic inflammation, promoting tissue repair, and potentiating anticipatory and adaptive immune responses.**

## Highlights

Interactions between nociceptors and mammalian immune cells are complex; however, most promote tissue repair and homeostasis, adaptive immune responses, or Type 2 immunity and inflammation.

Nociceptors inhibit histotoxic, collateral damage-causing immune responses and promote tissue repair chiefly through the secretion of the calcitonin gene-related peptide (CGRP) neuropeptide.

Nociceptors promote adaptive immunity by fine-tuning dendritic cell functions, through innervation of secondary lymphoid organs, and by communicating with B lymphocytes.

Nociceptors can regulate Type 2 immunity inflammation by secreting neuropeptides or glutamate.

While the primary role of nociceptors is to maintain tissue homeostasis, their actions can promote pathological conditions if unchecked.

Nociceptor functions are themselves modulated by immune cell actions, forming numerous feed-forward and feed-back loops.

## Significance

The peripheral nervous system is being increasingly recognized as a regulator of immune responses at steady-state and in response to infections, cancer, tissue injury, and other challenges. In particular, nociceptors, a population of somatosensory neurons that confer the sensation of itch or pain, can exert both pro- and anti-inflammatory effects. Developing a better understanding of nociceptors as conductors of immune responses is at the forefront of neuroimmunology research.

## The peripheral nervous system as a controller of immunity

The peripheral nervous system (PNS) has risen to prominence in recent years as a sophisticated and multifaceted regulator of mammalian immune responses at steady-state and in response to infections, cancer, tissue injury, and other challenges [1]. Multiple PNS components have been implicated, including sympathetic and parasympathetic [2] as well as somatosensory fibers [3]. While the autonomic nervous system determines the overall systemic immunological tone throughout the body, local neuroimmune interactions are thought to be critical for the defense of peripheral barrier tissues (the skin and mucosal surfaces), which are prone to infection and injury. Here, the timely detection and appropriate response to dangerous stimuli are vital to the survival of the organism, while inappropriate responses to innocuous environmental stimuli need to be avoided. Consequently, barrier tissues harbor a diverse arsenal of specialized immune cells and are also densely innervated by somatosensory fibers, particularly nociceptors [3].

Nociceptors represent a distinct evolutionarily ancient afferent branch of the PNS that responds to noxious stimuli, such as physical trauma, extremes of temperature, pressure, and toxic or irritant chemicals [4]. Various nociceptor subsets exist, mainly defined by their morphology, **neuropeptide** (see Glossary) content, and the expression of distinct ion channels, which are often present in partially overlapping patterns, and lend each subset its specificity [5]. The afferent signals transmitted by nociceptors to the brain are typically experienced as itch or pain. Consequently, depending on the nature of the stimulus, the type of nociceptive fibers, and the anatomic location, nociceptors may activate a withdrawal reflex (pain) [4] or facilitate the removal (itch) [6] or expulsion (cough/sneeze reflex) of offending irritants [7,8]. In addition to eliciting such behavioral defenses, nociceptors also emit efferent signals to control immune responses that may be either pro- or anti-inflammatory, depending on the pathophysiological context and the responsive immune cell type (Table 1) [3]. The multitude of apparently conflicting immunological effects of nociceptors that can result in either the promotion or inhibition of inflammation has been a major conundrum in the field. Here, we focus on recent advances in our understanding of the nociceptor–immune cell dialog, review and integrate

the evidence of how this dialog shapes immune cell functions, and propose a model to reconcile and unify ostensibly confusing findings.

## Nociceptors: pro- or anti-inflammatory?

To reconcile the plethora of seemingly contradictory observations of nociceptor-mediated pro- versus anti-inflammatory effects, we propose that the biological activities of nociceptors ultimately serve a single-minded purpose: to foster the restoration of injured tissues while minimizing bystander damage. This concept is rooted in the premise that the electrical activity of nociceptors (pain) is an immediate and inevitable consequence of any physical, chemical, or biological event that triggers the destruction or structural damage of barrier tissue elements. The associated loss of barrier integrity and resulting increased risk of infection necessitates the rapid recruitment of myeloid leukocytes, which exert potent antimicrobial effects by generating reactive oxygen species (ROS) and releasing proteolytic enzymes, chromatin (**NETosis**), cytokines, and other proinflammatory factors that are essential for the timely elimination of invading pathogens. However, continued exposure to these histotoxic agents can exacerbate tissue damage and prevent wound healing and tissue regeneration.

We propose that nociceptor control of immune cell functions may have evolved to achieve three goals: (i) to mitigate inflammatory activities that exacerbate collateral tissue damage; (ii) to promote the development of adaptive immunity and establishment of long-lived protective humoral and cellular immune memory; and (iii) to favor **T helper (Th) 2 immune responses** aimed at expelling or removing parasites (Figure 1, Key figure). Inevitably, this nociceptor-driven skewing of immunity carries the inherent risk that neuroimmune communications may misfire to inadvertently promote pathological states. For example, inhibiting the functions of activated neutrophils may not only protect tissues from inflammation-induced pathology, but also impair the clearance of pathogens. Similarly, promoting adaptive immune responses may not only boost protection against microbial (re-)infections, but also trigger and/or exacerbate autoimmune disorders. Finally, the induction of Type 2 responses confers protection against helminth infections, but may drive allergic diseases and asthma. Thus, although the pathological sequelae of nociceptor activity that arise in certain settings are both real and relevant, these negative manifestations of neuroimmune interactions often obscure rather than illuminate the physiological benefits.

## Immunomodulation by nociceptor-derived CGRP

Although nociceptors have at their disposal multiple modalities to communicate with immune cells, their ability to dampen inflammation can be attributed mainly to the neuropeptide, **calcitonin gene-related peptide (CGRP)** (Figure 2) expressed by most nociceptor subsets [5]. The CGRP receptor is expressed by most immune cells and constitutes a heterodimer comprising two subunits: receptor activity-modifying protein 1 (RAMP1), which lends the complex its specificity, and calcitonin receptor-like receptor (Calcrl), a G-protein-coupled receptor responsible for signal transmission. Multiple classes of G proteins can couple with the CGRP receptor, likely in a cell type- and context-dependent manner, and, as a result, CGRP receptor signaling exhibits a significant degree of complexity [9]. Nonetheless, the 'canonical' CGRP receptor pathway responsible for anti-inflammatory effects observed in most leukocytes has been well established and relies on adenylyl cyclase-dependent activation of protein kinase A (PKA), which triggers the cAMP early repressor (ICER), blocking NF-κB activity and preventing upregulation of proinflammatory mediators, such as tumor necrosis factor (TNF)α [10]. Indeed, CGRP-mediated immunosuppression has been described in many pathological scenarios, sometimes with detrimental consequences. For example, in mouse models of *Pseudomonas aeruginosa* infection of the eye [11], *Escherichia coli* urinary tract infection [12], *Staphylococcus aureus* pneumonia [13], skin infection with *Streptococcus pyogenes* [14], and Streptococcal

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meningitis [15], ablation of **TRPV1$^+$/NaV1.8$^+$** nociceptors or pharmacological inhibition of CGRP can be protective and improve bacterial clearance. Functionally, CGRP signaling in meningeal macrophages, for example, reduces the production of chemokines (CCL2, CCL3, and CXCL10) and subsequent recruitment of neutrophils into the tissue [15]. Additionally, neutrophils themselves can be affected by CGRP, which attenuates their bactericidal abilities, due, at least in part, to inhibiting the activity of **myeloperoxidase (MPO)** [14]. The immunomodulatory effects of CGRP have also been described in dendritic cells (DCs) [16,17]. However, many of the early studies relied on DC cultures derived *in vitro* from bone marrow progenitors under the influence of granulocyte-monocyte colony-stimulating factor (GM-CSF) [16,17]. Such cultures contain a significant proportion of macrophages [18], which may obfuscate the results. Indeed, recent studies show more nuanced effects of CGRP on *bona fide* mouse DCs (see later) [19,20]. Finally, lymphoid cells are also susceptible to the effects of CGRP. Specifically, CGRP treatment restricted the proliferation and interleukin (IL)-13 secretion of **type 2 innate lymphoid cells (ILC2s)** and, accordingly, genetic ablation of the neuropeptide (*Calca*$^{-/-}$) or its receptor (*Ramp1*$^{-/-}$) resulted in improved helminth expulsion during murine infection with *Nippostrongylus brasiliensis* [21,22]. On the flip side, in some cases, infectious organisms appear to have evolved to activate nociceptors [23,24] to take advantage of their immunomodulatory effects to promote microbial dissemination and avoid detection [25]. Moreover, in the B16F10 mouse model of melanoma, TRPV1$^+$ nociceptor-derived CGRP promoted an '**exhausted phenotype**' in tumor-infiltrating CD8$^+$ T cells and attenuated their activity. Accordingly, *in vivo* ablation of nociceptors (*Trpv1*$^{cre}$*::DTA*$^{fl/WT}$ or *Nav1.8*$^{cre}$*::DTA*$^{fl/WT}$) or the CGRP receptor (*Ramp1*$^{-/-}$) in CD8$^+$ T cells ameliorated the disease and decreased tumor growth [26], suggesting that tumors can also subvert the immunosuppressive CGRP axis to avoid antitumor immunity.

Despite such apparent high fitness costs, the immunosuppressive CGRP axis has been maintained throughout mammalian evolution, suggesting an important role in other context(s). Indeed, the crosstalk between the commensal microbiota and nociceptors confers important benefits to the host. In the mouse skin, microbiota-induced CGRP promotes tissue homeostasis at the steady-state by curbing pathological antimicrobial T cell responses and, in its absence, aberrant inflammatory responses ensue [27]. Upon injury, however, IL-17A derived from microbiota-specific **Th17** cells, can signal via the IL-17 receptor on injured sensory neurons and directly promote their regeneration [28]. In the mouse gut, NaV1.8$^+$ nociceptor-derived CGRP promotes mucus secretion from **goblet cells** [29] and regulates **microfold (M) cell** density, thus limiting the invasion of *Salmonella enterica* serovar Typhimurium [30]. Similarly, another neuropeptide, **Substance P (SP)**, derived from TPRV1$^+$ nociceptors, promotes tissue homeostasis in the gut by preventing dysbiosis and ameliorating inflammation in the dextran sodium sulfate (DSS)-induced colitis mouse model [31]. Taken together, the interplay between microbiota and nociceptors suggests that the latter not only respond to acute insults, but also establish and maintain an immune setpoint to enable the symbiotic coexistence of the host with diverse microbiota.

Finally, the immunosuppressive CGRP axis can prevent tissue damage and directly promote repair after injury. Specifically, the inhibitory effect of CGRP derived from TRPV1$^+$ nociceptors on ILC2s helped alleviate experimental allergic inflammation in mouse airways elicited by the exposure to the aeroallergen *Alternaria alternata* [32]. Additionally, CGRP released from activated NaV1.8$^+$ nociceptors in injured mouse skin and skeletal muscle enabled tissue repair by inducing the expression of **thrombospondin-1 (TSP1)** in macrophages and neutrophils [33]. In turn, TSP1 inhibited inflammation, induced a protissue repair phenotype in macrophages characterized by the upregulation of Arginase 1 and CD206 [33], and prevented **nociceptor sensitization**, a potentially pathological state in which the excitability of nociceptors is chronically increased [34]

## Glossary

**Adrenomedullin:** nociceptive neuropeptide of the CGRP family.
**Amphiregulin:** growth factor of the EGF family involved in tissue protection and repair.
**Calcitonin gene-related peptide (CGRP):** nociceptive neuropeptide of the CGRP family.
**CD301b$^+$ DCs:** subset of classical DCs present in barrier tissues, including the skin; mainly associated with inducing Th2 responses.
**Cholinergic neurons:** neurons that use acetylcholine as a neurotransmitter.
**Class switch:** process through which antibodies switch from IgM to other classes (IgA, IgE, or IgG).
**Exhausted phenotype:** observed in effector T cells; associated with reduced functional capacity and characterized by the upregulation of exhaustion markers; common in tumor-infiltrating lymphocytes and chronic virus-specific CD8$^+$ T cells.
**GABAergic neurons:** inhibitory interneurons of the CNS that use gamma-aminobutyric acid as a neurotransmitter.
**Germinal center response:** process through which high-affinity antigen-specific B cell clones are selected in secondary lymphoid tissues.
**Glutamatergic neurons:** excitatory interneurons of the CNS that use glutamate as a neurotransmitter.
**Goblet cells:** columnar epithelial cells that secrete mucins.
**Hapten:** chemically reactive small molecule that covalently attaches to larger molecules (e.g., proteins), making them immunogenic.
**Immune checkpoint molecules:** group of regulatory cell surface receptors that inhibit T cell activation when triggered by binding to their ligand(s); often inhibited in clinical practice (checkpoint blockade) to promote anti-tumor immune responses (e.g., PD-1).
**Intermedin:** nociceptive neuropeptide of the CGRP family.
**Langerhans cells (LCs):** tissue-resident, epidermal macrophages of embryonic origin with molecular and functional features of DCs.
**Mas-related G-protein-coupled receptor D (MrgprD):** G-protein-coupled receptor expressed by a subset of nociceptors; activated by pruritogens, such as β-alanine.
**Microfold (M) cells:** specialized intestinal epithelial cells imbued with the ability to acquire antigens from the

(Box 1). Accordingly, a chimeric CGRP peptide engineered to contain an extracellular matrix-binding motif to extend its half-life at the site of application has shown promise in the *Lepr*$^{db/db}$ preclinical mouse model of diabetic wound healing [33], suggesting the utility of CGRP agonists as therapeutic agents. Together, the available data implicate CGRP as a central communication signal through which nociceptors first attenuate the histotoxic actions of immune cells to prevent excessive immunopathology and, subsequently, promote tissue repair and regeneration.

## Nociceptor control of tissue homeostasis via CGRP-independent mechanisms

CGRP release is not the only modality through which nociceptors can inhibit inflammation and potentiate tissue repair. For example, TAFA4, a chemokine-like protein released from a subset of **nonpeptidergic nociceptors** marked by the expression of Gα$_i$-interacting protein (GINIP), promotes IL-10 production in macrophages following UV-induced skin damage [35]. In turn, IL-10 supports the survival of tissue repair-promoting, anti-inflammatory **TIM4$^+$ dermal macrophages**, facilitating tissue regeneration and preventing fibrosis, as evidenced by exacerbated inflammation and fibrosis in TAFA4-deficient mice (*Tafa4*$^{-/-}$) [35]. In addition to blunting histotoxic responses, nociceptors can also directly activate protissue repair responses. In particular, the activation of nociceptors by imiquimod (IMQ), an agonist of an ion channel expressed on nociceptors (**TRPA1**) and of **Toll-like receptor (TLR) 7** on DCs, promoted IL-23 secretion by dermal DCs [35]. IL-23 can then trigger the secretion of IL-17 and IL-22 by dermal γδT cells, thereby enhancing local tissue repair pathways [36]. Accordingly, genetic ablation of TRPA1 in nociceptors (*Trpa1*$^{-/-}$) or of IL-23 (*Il12b*$^{-/-}$) in DCs prevented this effect in mice [36]. It is not yet known whether the CGRP, TAFA4, and IL-17 pathways can act synergistically or are mutually exclusive and whether different subsets of nociceptors can induce different types of protissue repair environment in an insult- or tissue-specific manner.

Aside from CGRP, other nociceptor-derived neuropeptides can have immunosuppressive effects. These include **adrenomedullin** and **intermedin** [which utilize the same signaling subunit (Calcrl) as the CGRP receptor], as well as **vasoactive intestinal peptide (VIP)** and **pituitary adenylate-cyclase-activating polypeptide (PACAP)**, which were even dubbed 'macrophage inactivation factors' [37]. However, despite a plethora of compelling *in vitro* observations [37,38], there are few *in vivo* data demonstrating a role for these neuropeptides in nociceptor-mediated immunosuppression. Of note, food intake-induced VIP secretion in the gut was reported to act on ILCs; however, the findings appear contradictory because VIP inhibited cytokine production by ILCs in one study, while potentiating it in others [39–41]. Therefore, further work is necessary to reconcile these findings as well as to assess what, if any, effects these neuropeptides may have on immune responses in other contexts.

## A special relationship: nociceptors and DCs

Of all the immune cell types, DCs appear to have a particular affinity for nociceptors, which modulate their functions in a context-dependent manner by several molecularly distinct communication pathways (Figure 3). For example, even though CGRP generally dampens the functions of other immune cells, DCs mount a more nuanced response to this neuropeptide. *In vitro* work suggested that this is due, at least partly, to differential intracellular wiring of the CGRP receptor, whereby CGRP-induced adenylyl cyclase activation in DCs stimulates p38 kinase [19]. Whether this is in addition to, or instead of, the canonical pathway involving PKA remains to be established. Notwithstanding, rather than inhibiting DC functions, CGRP signaling induces an anticipatory sentinel phenotype, characterized by the upregulation of genes involved in antibacterial and antiviral responses (e.g., *Cfp*, *Clec10a*, *Cd300lf*, *Ifitm1*, *Ifitm2*, *Ifitm6*, and others) as well as the proform of the cytokine IL-1β (**pro-IL-1β**). However, CGRP signaling does not initiate an overt inflammatory response by DCs [19]. Similarly, through

intestinal lumen and transport them to submucosal lymphoid tissues.
**Myeloperoxidase (MPO):** neutrophil enzyme that catalyzes the reaction producing hypochlorous acid as an antimicrobial agent.
**NaV1.8:** voltage-gated sodium channel expressed by most nociceptors; often used to target nociceptors experimentally.
**NETosis:** release of neutrophil extracellular traps; modified chromatin decorated with bactericidal proteins.
**Neuropeptides:** group of short peptides, usually expressed as a part of larger proproteins, released as effector molecules from many neurons including nociceptors.
**Neuromedin U (NMU):** neuropeptide produced by neurons in the intestine, brain, and dorsal root ganglia.
**Nociceptor sensitization:** process that results in a decreased threshold of activation and enhanced response to suprathreshold stimuli in nociceptors.
**Nonpeptidergic nociceptors:** group of nociceptor subsets historically defined as not expressing neuropeptides. However, recent studies suggest that this is not always the case; by contrast, peptidergic nociceptors use neuropeptides as their effector molecules.
**Pattern recognition receptor (PRR):** class of innate immune receptors recognizing conserved pathogen- or danger-associated molecular patterns.
**Pituitary adenylate-cyclase-activating polypeptide (PACAP):** neuropeptide produced by nociceptors but also other types of neuron, primarily after neuronal injury.
**Pro-IL-1β:** biologically inactive proform of the cytokine IL-1β, which needs to be proteolytically processed and released, traditionally, in the process of pyroptosis.
**Substance P (SP):** nociceptive neuropeptide of the tachykinin family.
**T helper 2 (Th2) immune response:** immune response characterized by the production of 'type 2' cytokines, such as IL-4, IL-5, and IL-13.
**T helper 17 (Th17) immune response:** immune response characterized by the production of IL-17.
**Thrombospondin-1 (TSP1):** extracellular matrix glycoprotein with pleiotropic functions involved in, for example, immunosuppression, fibrotization, and angiogenesis.
**TIM4$^+$ dermal macrophages:** immunoregulatory subset of tissue-resident macrophages.

yet-to-be-defined molecular means, TRPV1$^+$ nociceptor activation in wounded skin stimulates IL-27 expression in **CD301b$^+$ DCs**. In turn, IL-27 can induce a local anticipatory antiviral response. Consequently, loss of nociceptor signaling in mice increases their susceptibility to herpes simplex virus 1 (HSV-1) infection [42]. Teleologically, these effects on DCs make sense because a painful stimulus triggering nociceptive activation (and subsequent CGRP release) might herald a barrier breach, but could also be the result of a sterile insult. Consequently, the induction of an anticipatory response in DCs without inducing inflammation represents a balanced reaction that fortifies barrier immunity without increasing the risk of immunopathology.

Nonetheless, chronic stimulation of nociceptors has long been known to induce neurogenic inflammation [43]. Accordingly, recent work showed that repeated optogenetic activation of cutaneous TRPV1$^+$ nociceptors in mice is sufficient to induce skin inflammation in a CGRP-dependent manner (based on CGRP antagonist experiments in these mice) [44]. Similarly, ectopic activation of TRPV1$^+$ nociceptors by an epineural device in mice potentiated Freund's adjuvant-induced immune responses, as evidenced by the increased inflammatory cell infiltrate noted at the site of nociceptive activation [45]. While the precise molecular and cellular mechanism remains to be defined, it is tempting to speculate that these proinflammatory effects are due to CGRP-induced overproduction of IL-1β by DCs, which may, in turn, induce further responses in an auto/paracrine manner. This would suggest that nociceptors not only induce an anticipatory response in DCs, but also promote DC-mediated inflammation if a nociceptive stimulus persists and/or occurs in combination with proinflammatory signals, such as TLR agonists (e.g., IMQ). Indeed, during cutaneous *Candida albicans* infection in mice, TRPV1$^+$ nociceptor-derived CGRP was necessary to induce IL-23 secretion from DCs, initiating a protective local Th17 response [20], suggesting infectious agents as one such persistent stimulus. Surprisingly, in the context of psoriasiform skin inflammation induced by repeated topical treatment with the TLR-7 agonist, IMQ, in mice, TRPV1$^+$ nociceptors also potentiated a similar IL-17 response, but in a CGRP-independent manner [46]. This implies that there are several modalities by which nociceptors can potentiate DC functions. Indeed, mechanistic studies in an *in vitro* co-culture system demonstrated direct electrical coupling of mouse DCs and nociceptors, whereby nociceptor firing induced a calcium influx into DCs and promoted their cytokine production after activation [19]. While such direct electrical connectivity between nociceptors and immune cells represents a novel paradigm in how the two systems communicate, evidence demonstrating the involvement of this mechanism *in vivo* is lacking. Notwithstanding, psoriasiform dermatitis induced by repeated IMQ treatments is akin to the neurogenic inflammation induced by chronic stimulation of nociceptors and thus, represents a pathological example of a response gone wrong, rather than a physiological system functioning 'as intended' [46]. Taken together, there is mounting evidence from diverse experimental systems that nociceptors can communicate with DCs using several molecular means to modulate DC functions, which is protective in the case of cutaneous infections, but may become maladaptive if the nociceptive stimulation persists.

## Nociceptors: conductors of adaptive immunity

In addition to directly affecting innate DC functions, nociceptors can also control DC migration from peripheral tissues to lymph nodes (LNs), thereby profoundly impacting the ability of DCs to instigate adaptive immune responses. Specifically, the nociceptor-derived chemokine, CCL2, serves as a retention signal for CCR2$^+$ DCs, preventing their premature egress from peripheral tissues into murine draining lymphatics [19]. Consequently, selective genetic ablation of CCL2 in mouse NaV1.8$^+$ nociceptors (*Ccl2-mCherry*$^{fl/fl}$ × *Scn10a*$^{Cre/+}$ mice) resulted in the decreased ability of DCs to activate adaptive immune responses against skin-derived antigens in a model of contact hypersensitivity (CHS) induced by the **hapten** DNFB (2,4-dinitrofluorbenzene)

**Toll-like receptor (TLR) 7:** endosomal TLR that is specific for viral single-strand RNAs; pharmacologically activatable by imidazoquinolines.
**TRPA1:** nociceptive nonselective cation channel activated by various chemical irritants, including the pungent compounds present in extracts from mustard seeds and wasabi.
**TRPV1:** nociceptive nonselective cation channel with preference for $Ca^{2+}$ ions activated by noxious heat and capsaicin; often used to target nociceptors experimentally.
**Type 2 innate lymphoid cells (ILC2s):** subset of ILCs (ILC2) representing an innate counterpart of Th2 cells and promoting type 2 responses.
**Vasoactive intestinal peptide (VIP):** neuropeptide of the glucagon/secretin superfamily; produced by not only nociceptors, but also other types of neuron.

Table 1. Summary of recently published effects of nociceptors on immune processes *in vivo* in the indicated mouse models[a,b] [4,11–15,19–22,26,27,29–33,35,36,39–41,44–47,52,53,55,58–60,62,64–67,69,74,90–93].

| Tissue | Physiological context/ experimental model | Target cell type | Molecular mechanism | Immune effect[b] | Refs |
|---|---|---|---|---|---|
| Skin | *Staphylococcus aureus* infection | n.d. | n.d. | Reduced bacterial clearance | [92] |
| | Direct optogenetic activation of nociceptors | n.d. | CGRP | Neurogenic/anticipatory inflammation | [44] |
| | Direct optogenetic activation of nociceptors during CFA immunization | n.d. | n.d. | Increased inflammatory response | [45] |
| | Topical imiquimod treatment | DCs | n.d. | **Local Th17 psoriasiform inflammation** | [46] |
| | *Candida albicans* infection | DCs | CGRP | Local antifungal Th17 response | [20] |
| | Allergen exposure (papain) | DCs | SP | **Th2 priming, allergic inflammation** | [69] |
| | Mechanical tissue damage | DCs | n.d. | Improved tissue regeneration | [36] |
| | Oxazalone-induced contact hypersensitivity | DCs | PACAP | **Improved T-cell priming** | [47] |
| | Topical capsaicin and imiquimod treatment, DNFB-induced contact hypersensitivity | DCs | CGRP, direct contact, CCL2 | **Anticipatory sentinel response, enhanced local cytokine production, improved T-cell priming** | [19] |
| | UV irradiation-induced tissue damage | Macrophages | TAFA4 | Reduced inflammatory damage, improved regeneration | [35] |
| | *Streptococcus pyogenes* infection | Neutrophils | CGRP | Reduced bacterial clearance | [14] |
| | HSV-1 infection | Neutrophils, monocytes | n.d. | **Reduced local inflammation and improved CD8 T-cell priming** | [52] |
| | Allergen exposure (HDM) | Mast cells | SP | **Allergic inflammation** | [67] |
| | MrgprD agonism, neuronal ablation | Mast cells | Glutamate | **Reduced allergic inflammation** | [74] |
| | Microbiota exposure | CD8 T cells | CGRP | **Reduced inflammation** | [27] |
| Skin, muscle | Mechanical tissue damage | Macrophages, neutrophils | CGRP | Reduced inflammation, improved regeneration | [33] |
| Skin, airways | Allergen exposure (HDM, calcipotriol) | B cells | Substance P | **Class switch to IgE and IgE secretion** | [65] |
| Skin, joints | Sciatic nerve injury, arthritis | n.d. | HMGB1 | Neurogenic inflammation, exacerbated arthritis | [91] |
| Lung/airways | *S. aureus* pneumonia | Neutrophils, γδT cells | CGRP | Reduced cytokine production and bacterial clearance | [13] |
| | Allergen exposure (OVA) | ILC2s, CD4 T cells | VIP | **Allergic inflammation** | [64] |
| | Allergen exposure (HDM) | ILC2 | NMU | Potentiation of alarmin-driven Th2 response | [60] |
| | Airway inflammation | ILC2 | CGRP | Inhibition of alarmin-driven Th2 response | [22] |
| | *Nippostrongylus brasiliensis* infection | ILC2 | CGRP | Suppression of Type 2 inflammation and helminth expulsion | [21] |
| | Allergen exposure (*Alternaria alternata*) | ILC2 | CGRP | **Suppression of allergic inflammation** | [4,32] |
| | Exposure to OVA:IgE immunocomplexes | CD4 T cells | SP | **Potentiation of Th2 inflammation** | [66] |
| Lung, Intestine | *N. brasiliensis* infection | ILC2 | NMU | Improved type 2 responses and helminth immunity | [58,59] |
| Intestine | Nociceptor silencing/ablation | n.d. | SP | Alterations in intestinal microbiota and dysbiosis | [31] |
| | Food intake | ILC3 | VIP | Inhibition of IL-22 production | [39] |
| | Food intake | ILC3 | VIP | Anticipatory IL-22 production | [40] |

Table 1. (continued)

| Tissue | Physiological context/ experimental model | Target cell type | Molecular mechanism | Immune effect[b] | Refs |
|---|---|---|---|---|---|
| | *Trichuris muris, Citrobacter rodentium* infection | ILC3, ILC2 | VIP | Potentiation of ILC2 and ILC3 response to IL-33 and IL-23 | [41] |
| | *T. muris* infection, DSS colitis | ILC2 | NMU | Improved helminth immunity and tissue protection | [62] |
| | *Salmonella enterica* serovar Typhimurium infection, segmented filamentous bacteria colonization | M-cells | CGRP | Decreased abundance of M cells, protection from *S. enterica* Typhimurium infection | [30] |
| | DSS colitis | Goblet cells | CGRP | Increased mucus production, tissue protection | [29] |
| Tumor | B16F10 melanoma | CD8 T cells | CGRP | **Induction of exhausted phenotype in CD8$^+$ T-cells** | [26] |
| Bone | *Candida* sp. osteomyelitis | Myeloid cells, osteoclasts | CGRP | Decreased osteoinflammation and osteoclast multinucleation | [93] |
| Meninges | *Streptococcus pneumoniae* and *Streptococcus agalactiae* meningitis | Macrophages, neutrophils | CGRP | Reduced bacterial clearance | [15] |
| Spleen | Immunization, influenza infection, dietary capsaicin | B cells | CGRP | **Potentiation of germinal center response and antibody response** | [55] |
| Lymph node | Direct optogenetic activation of nociceptors | n.d. | n.d. | Changes in transcriptome of various lymph node cells | [53] |
| Urinary tract | UPEC infection | Macrophages, neutrophils | CGRP | Reduced bacterial clearance | [12] |
| Cornea | *Pseudomonas aeruginosa* infection | Neutrophils | CGRP | Reduced bacterial clearance | [11] |
| Dorsal root ganglia | Peripheral nerve damage | Macrophages | miR-21-5p | **Pro-inflammatory macrophage polarization** | [90] |

[a]Abbreviations: CFA, complete Freund's adjuvants; DNFB, 2,4-dinitrofluorbenzene; DSS, dextran sodium sulfate; HDM, house dust mite; OVA, ovalbumin; n.d., not determined; SP, Substance P; UPEC, uropathogenic *E. coli*.
[b]Effects that can be broadly summarized as proinflammatory are in red and anti-inflammatory/prohomeostatic in green. Effects that are not inherently pro- or anti-inflammatory are in black. Bold font indicates more long-term/chronic effects; non-bold font indicates acute effects.

[19]. This hyporesponsiveness in the absence of nociceptor-derived CCL2 was because DCs prematurely departed from the sensitized skin without having acquired an adequate amount of antigen, as evidenced by accelerated disappearance of DCs from hapten-exposed epidermis [19]. Similarly, another study also reported a defect in a CHS response to another hapten, oxazolone, in TRPV1$^+$ nociceptor-ablated mice [via resiniferatoxin (RTX)-mediated denervation] [47]. In this setting, the CHS response was restored by local injection of a nociceptor neuropeptide, PACAP. *In vitro*, DC exposure to PACAP induced CCR7 expression, a chemokine receptor that allows DCs to migrate into lymphatics and reach the paracortex in draining LNs (dLNs), suggesting a possible mechanism of action for CHS responsiveness [47]. Of note, CCR7 upregulation in DCs is canonically induced during the process of DC maturation, most commonly after microbial stimulation through a **pattern recognition receptor (PRR)** and, accordingly, PACAP treatment also increased the expression of costimulatory molecules CD80 and CD86 in DCs [47]. This raises the possibility that a painful non-infectious stimulus results in nociceptor release of PACAP to trigger DC maturation and migration, perhaps eliciting immune responses in situations where PRR ligands may be scarce or absent. However, the oxazolone CHS model induces Th2-skewed inflammation [48] and earlier *in vitro* studies reported that PACAP enabled immature DCs to specifically promote Th2 responses while reducing the overall ability of LPS-activated DCs to stimulate T cell proliferation [49]. Consequently, it will be important to assess whether the *in vivo* effect of PACAP is also restricted to the initiation of Th2 responses.

## Key figure

### Nociceptors: the good and the bad

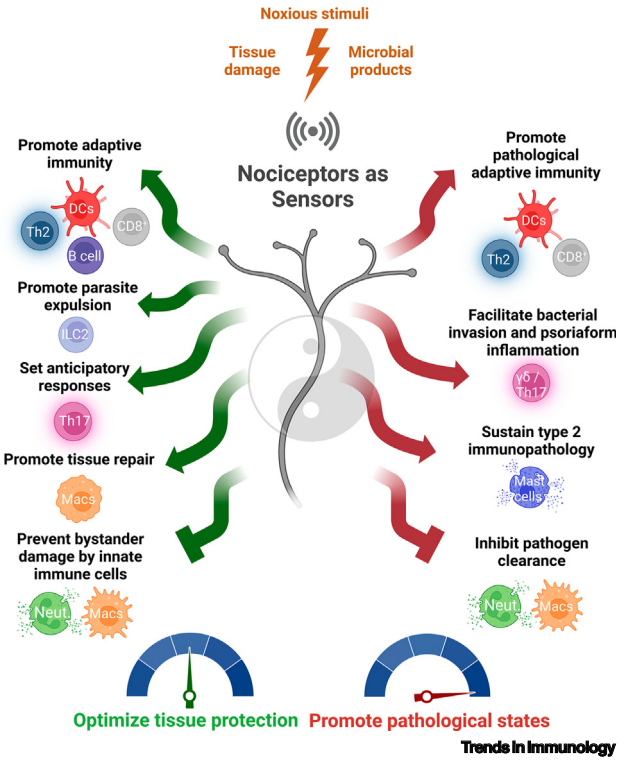


Figure 1. Nociceptors control the functions of the mammalian immune system to promote tissue protection and the maintenance of homeostasis (left). However, when triggered at the inappropriate moment, their actions can also promote and sustain pathological states (right). Abbreviations: DC, dendritic cell; ILC2, type 2 innate lymphoid cells; Macs, macrophages; Neut, neutrophils; Th, T helper. Figure created with BioRender (biorender.com).

Thus, rigorous *in vivo* testing will be required to validate these ideas. Nonetheless, the above data imply a two-step mechanism by which nociceptors may fine-tune the ability of DCs to carry immunological information from peripheral tissues to LNs. First, nociceptor-derived CCL2 can prevent premature egress of DCs from the periphery, allowing for sufficient uptake of antigens. Subsequently, PACAP can induce CCR7 upregulation, presumably with some delay, thus promoting efficient DC migration into, and within, LNs at the appropriate time. In addition, it is noteworthy that the enhancement of pro-IL-1β production by DCs may represent a third mechanism by which nociceptors promote adaptive immunity, given that IL-1β expression by DCs is important for the induction of durable T cell responses [50]. For example, preventing DC IL-1β release (i.e., *Nlrp3*$^{-/-}$ or *Casp1/11*$^{-/-}$ mice) or carrying out IL-1β neutralization impairs the induction of CD8$^{+}$ T cell-mediated antitumor immunity in B16OVA melanoma or LLC1 mouse models [51]. Indeed, in support of a model in which nociceptors are necessary for T cell immunity, a recent study showed that, during HSV-1 infection in mice, ablation of NaV1.8$^{+}$ nociceptors (*Nav1.8*$^{cre}$*::DTA*$^{fl/WT}$) resulted in defective induction of CD8$^{+}$ T cell responses, accompanied by a failure to resolve neutrophil-mediated inflammation and resulting in larger skin lesions [52].

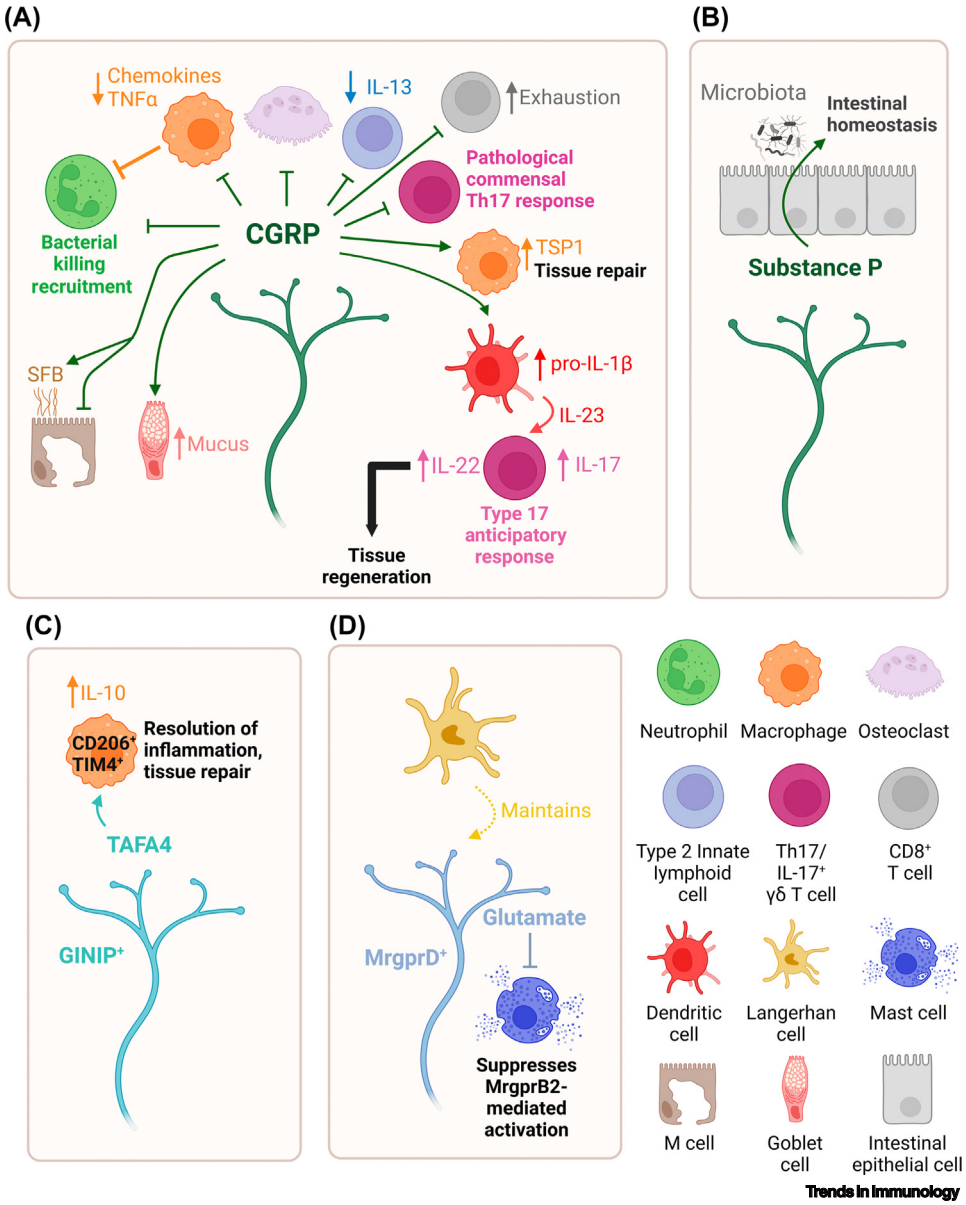


Figure 2. Nociceptors can promote the maintenance of homeostasis. (A) Mostly based on mouse models, nociceptors secrete the neuropeptide calcitonin gene-related peptide (CGRP), which inhibits histotoxic actions of, primarily, macrophages [12,15], neutrophils [11–14], and type 2 innate lymphoid cells (ILC2s) [21,22,32], while promoting a tissue repair phenotype in macrophages [33]. CGRP also induces a sentinel phenotype in dendritic cells (DCs) [19], an anticipatory [44] and a prorepair type 17 response [36], while curbing pathological responses to commensals [27] and maintaining the barrier functions of intestinal epithelial cells [29,30]. (B) Through Substance P (SP) release, nociceptors promote intestinal homeostasis [31]. (C) Nociceptors participate in the resolution of inflammation by promoting IL-10 secretion by macrophages through TAFA4 [35] and (D) suppressing mast cell activation through glutamate [74]. Figure created with BioRender (biorender.com). Abbreviations: GINIP, $G\alpha_i$-interacting protein; Mrgpr, Mas-related G-protein-coupled receptor; M cell, microfold cell; pro-IL-1β; proform interluekin-1β; SFB, segmentous filamentous bacteria; TNF, tumor necrosis factor; TSP1, thrombospondin-1.

Thus, even though some of the molecular mechanisms remain to be firmly established, it is becoming clear that the coordinated actions of nociceptors in nonlymphoid tissues can have a profound impact on the initiation and quality of T cell responses in dLNs.

Box 1. Effect of immune mediators on nociceptors: 'tuning the sensor'

In addition to sensing direct signs of damage and danger, nociceptors are also equipped to sense ongoing immune responses. For example, the prostaglandin $PGE_2$ sensitizes nociceptors. Indeed, inhibition of prostaglandin synthesis is the main mechanism through which nonsteroidal anti-inflammatory drugs (NSAIDs) alleviate pain [78]. Notably, proinflammatory cytokines, such as IL-1, IL-6, IL-17, and TNFα, similarly sensitize nociceptors and promote pain in various contexts [79], while IL-31 secretion from transforming growth factor (TGF)β-stimulated DCs induces itch during wound healing in mice [80]. Moreover, several pronociceptive roles of chemokines have also been described. For example, DC-derived CCL22 and CCL17 act on $CCR4^+$ sensory fibers to enhance their excitability, resulting in postoperative pain in mice [81], while CXCL5 acting on $CXCR2^+$ fibers can drive joint pain in a mouse model of gouty arthritis [82]. In addition, CCL2 signaling directly promotes inflammatory pain caused by complete Freund's adjuvant [83]. Conversely, the binding of PD-L1 to the **immune checkpoint molecule** PD-1 reduced itch in the imiquimod (IMQ)-induced psoriasis mouse model [84] and decreased neuronal hyperexcitability and neuropathic pain following nerve injury in mice [85]. The anti-inflammatory cytokine IL-10 can resolve nociceptive hypersensitivity in mice [86] and downregulate the expression of CCL2 in nociceptors [87]. This suggests that immune pathways can also provide negative signals to attenuate nociceptor activity. Similarly, TSP-1, in addition to promoting tissue repair, can signal through CD47 on nociceptors to inhibit $PGE_2$-mediated sensitization, at least *in vitro* [34]. Thus, nociceptors not only affect immune responses, but can also register, and functionally respond to, ongoing inflammation, forming the basis for feedback loops, some of which have been discussed in this review, and others that remain to be uncovered.

Finally, in many diseased settings, the functions of nociceptors can become abnormal, resulting in allodynia (activation by subthreshold stimuli) and hyperalgesia (exaggerated response to suprathreshold stimuli) [88]. It will be important to assess how the effects of nociceptors on the immune system differ in chronic versus acute pathologic conditions compared with steady-state, and to disentangle their physiological functions from their role in disease progression.

Beyond their ability to fine-tune the function and migration of DCs in barrier tissues, nociceptors also directly innervate LNs [53]. Single cell RNA sequencing (scRNAseq) analysis demonstrated that lymphatic endothelial cells (LECs) rather than immune cells may be the primary target of nociceptors in mouse LNs, at least based on the number of differentially regulated genes elicited by short-term optogenetic activation of $NaV1.8^+$ LN nociceptors [53]. While the homeostatic and/or pathophysiological relevance of the nociceptor-LEC dialog remains to be investigated, these data align with previous observations linking nociceptive activity in $TRPV1^-$ $NaV1.8^+$ fibers with the flow and retention of antigens in mouse peripheral LNs following KLH immunization [54]. Consequently, there may be yet another modality through which nociceptors regulate the development of antigen-specific immune responses, a possibility that certainly merits further attention.

Another secondary lymphoid organ, the spleen, was also recently shown to be innervated by $TRPV1^+$ nociceptors in mice (Figure 3) [55]. Based on its ability to activate nociceptors *in vitro,* prostaglandin $E_2$ ($PGE_2$) accumulation during NP-KLH immunization or influenza A virus (IAV) infection in mice was suggested to drive splenic TRPV1 nociceptor activation; however, direct *in vivo* evidence for such a mechanism is lacking [55]. Nevertheless, the activation of these splenic nociceptors resulted in the release of CGRP, which acted directly on B cells to promote the **germinal center response** and antibody production in mice [55]. Accordingly, mice that lacked CGRP receptor expression in B cells ($Cd19^{Cre/+}Calcrl^{fl/fl}$) exhibited decreased titers of antigen-specific antibodies and an increased susceptibility to IAV infection compared with wild-type controls [55]. Moreover, ingestion of capsaicin, an agonist of the TRPV1 ion channel expressed on most nociceptors, was sufficient to promote splenic B cell responses in mice (e.g., immunoglobulin titers and B cell proliferation). This was blocked following nociceptor ablation (via RTX denervation) in the left thoracic (T)8–13 dorsal root ganglia (DRGs), where spleen-innervating nociceptors reside, suggesting a direct effect [55]. However, how dietary capsaicin acts on spleen-innervating nociceptors and whether the activation of lymphoid organ-innervating nociceptors in general leads to any sensory outputs remain to be established.

Finally, nociceptors innervating peripheral tissues are also involved in the development of humoral responses: optogenetic activation of $TRPV1^+$ nociceptors at the time and location

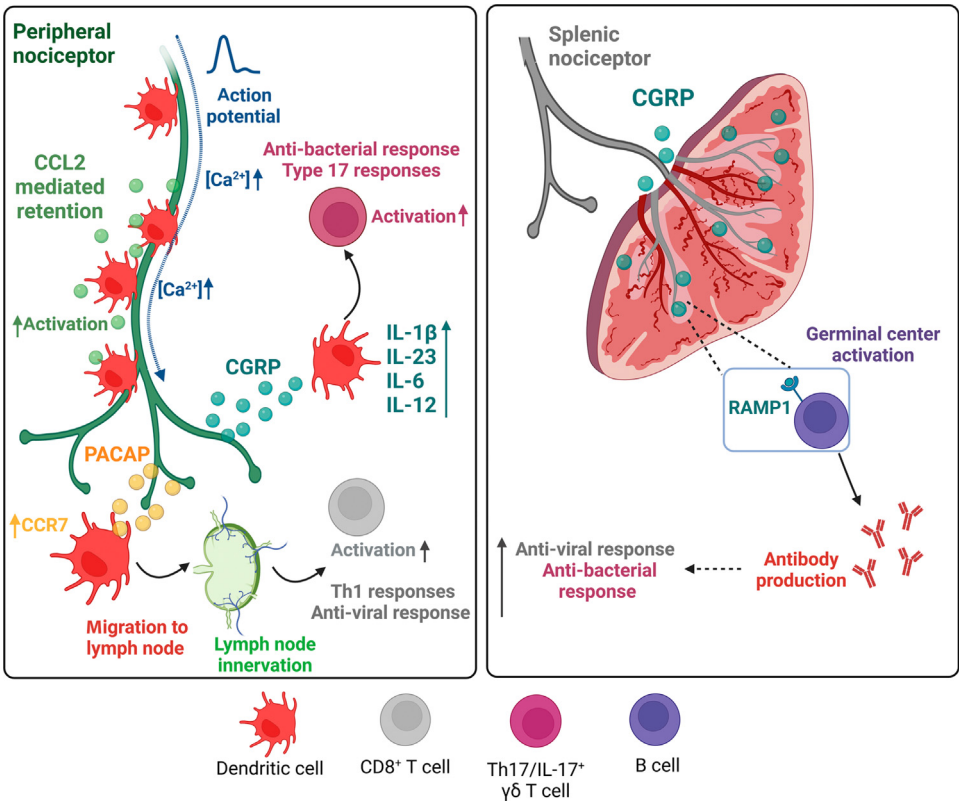


Figure 3. Nociceptors can potentiate dendritic cell (DC) functions and promote adaptive immunity. Mostly based on mouse models, nociceptors fine-tune DC migration from peripheral tissues to the lymph nodes (LNs), thus facilitating the development of adaptive T cell responses [19,47,52]. By inducing calcium flux in interacting DCs, nociceptors potentiate DC cytokine responses upon activation [19], while calcitonin gene-related peptide (CGRP) release induces an anticipatory response in steady-state DCs [19] and potentiates their ability to induce Type 17 inflammation [20,46] (left). Spleen-innervating nociceptors promote humoral adaptive immune responses by directly enhancing B cell germinal center responses in a CGRP-dependent manner [55] (right). Figure created with BioRender (biorender.com). Abbreviations: IL, interleukin; PACAP, pituitary adenylate-cyclase-activating polypeptide; RAMP1, receptor activity-modifying protein 1; Th, T helper.

of antigen encounter results in increased antibody titers in mice [56]. However, the underlying mechanisms remain to be identified. Nevertheless, this observation is relevant because understanding the molecular drivers might inform the future development of improved vaccines that generate optimal humoral responses. Taken together, the activation of nociceptors may help safeguard organismal homeostasis by curbing the nonspecific, potentially tissue-destructive, responses of inflammatory leukocytes, while inducing anticipatory responses and promoting the induction of both humoral and cellular adaptive immunity.

## Nociceptors: instigators of Th2 responses

Th2 responses are thought to have evolved primarily as a means to expel multicellular parasites, such as helminths, from a host. An analogous defense function can be attributed to itch, cough, and sneezing behaviors that are induced by nociceptors [6–8,57]. Therefore, the impact of nociceptors on Th2 responses could be viewed as a natural extension of their protective role against parasite infections. Indeed, nociceptors have a propensity for inducing, potentiating, and sensing Th2 responses, and at least three neuropeptides have been implicated in this process: SP, **Neuromedin U (NMU)**, and VIP (Figure 4). NMU, in particular, can act on ILC2s in

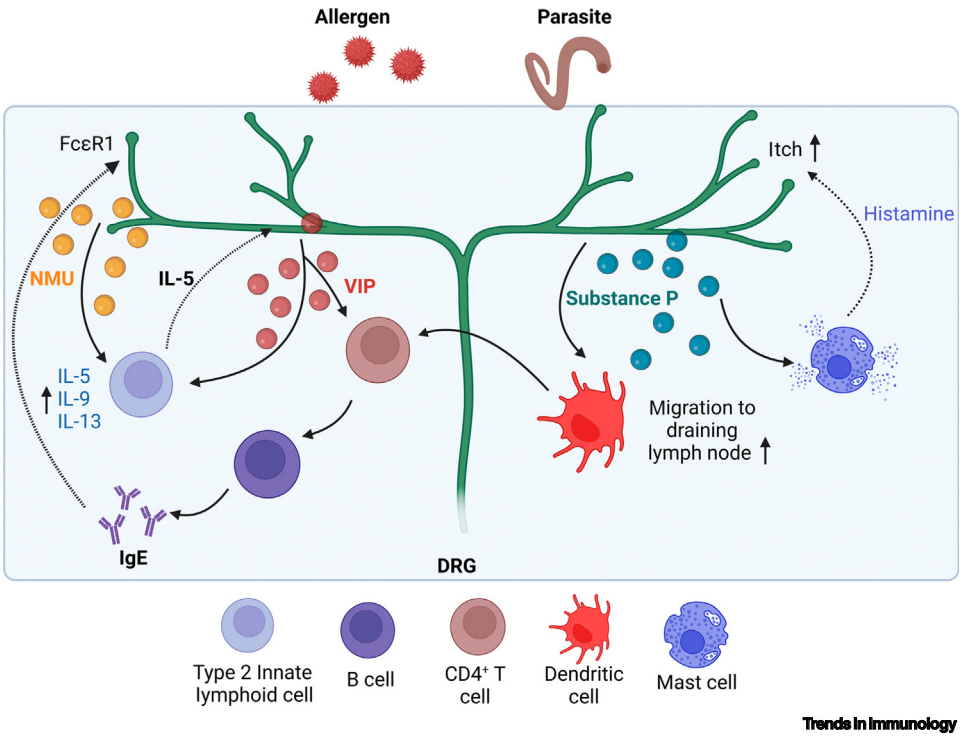


Figure 4. Nociceptors can elicit and enhance Type 2 inflammation. Mostly based on mouse models, nociceptors elicit T helper type 2 (Th2) responses by secreting the neuropeptides Substance P (SP) [66–69], vasoactive intestinal peptide (VIP) [64], and Neuromedin U (NMU) [58–62], which can promote the development of type 2 responses by exerting an effect on dendritic cells (DCs) [69] or mast cells [67,68]. Neuropeptides also directly potentiate type 2 inflammation by enhancing the functions of type 2 innate lymphoid cells (ILC2s) [58–62] and Th2 T cells [64] and inducing class switch to IgE in B cells [65]. Figure created with BioRender (biorender.com). Abbreviation: DRG, dorsal root ganglion.

mice [58–60] and humans [61] to promote the secretion of type 2 cytokines, including IL-5, IL-9, and IL-13, as well as **amphiregulin** [62]. Accordingly, genetic deletion of the NMU receptor (*Nmur1*$^{-/-}$) [58,59] or amphiregulin (*Nmur1*$^{iCre\text{-}Egfp}$::*Areg*$^{fl/-}$) in murine ILC2s [62] impaired the clearance of the helminths *N. brasiliensis* [58,59] and *Trichuris muris*, respectively [62]. However, in high-income countries, where helminth infections are generally rare, these interactions can drive allergic inflammation [60]. Consequently, an antagonist of a nociceptive ion channel, TRPA1, which showed promise in attenuating allergic airway inflammation in several preclinical studies, recently entered a Phase 1 randomized clinical trial (NCT03381144[i]) as a potential treatment for allergic asthma [63]. Even though the drug was found to prevent TRPA1 agonist-induced increase in dermal blood flow, pain, and itch in healthy volunteers, the trial was discontinued due to off-target toxicity. Another compound with improved selectivity for TRPA-1 has recently entered a multicenter, randomized, Phase 2a trial to test efficacy and safety in patients with chronic cough (NCT05660850[ii]). These aims further underscore the translational relevance of neuroimmune interactions in human health.

Of note, in an example of a feed-forward loop that can drive self-perpetuating allergic responses, at least in mice, NaV1.8$^{+}$ nociceptors in the airways can directly respond to the Th2 cytokine IL-5 by releasing VIP, a neuropeptide that acts on CD4$^{+}$ T cells and ILC2s to further promote type 2 responses and allergic inflammation [64]. Additionally, TRPV1$^{+}$ nociceptor-derived SP can also promote **class switching** in murine LPS+IL-4-activated B cells, to the IgE isotype [65]; this is relevant because nociceptors themselves express FcεRI and, when IgE–antigen complexes

bind the receptor, SP release ensues [66]. Additionally, TRPV1$^+$ nociceptors can also be directly activated by allergens with intrinsic protease activity to release SP and induce the degranulation of mast cells (as in the house dust mite-induced allergy mouse model or in human mast cell lines) [67,68]. SP can also induce the migration of CD301b$^+$ DCs to draining LNs; in turn, these events promote antigen-specific Th2 immune responses in the skin, as demonstrated in the papain-induced allergy mouse model [69]. At the same time, activation of mast cells and basophils also feeds back to nociceptors. Specifically, mast cell-derived histamine [70], as well as other factors, promote itch and neuropeptide secretion *in vitro* and *in vivo* in mouse and human models of allergic contact dermatitis [71]. Mouse and human activated basophils (e.g., in models of atopic dermatitis) can similarly trigger nociceptors to induce itch via the release of leukotrienes (LTs), and activating the LTC4-CysLTR2 signaling pathway in nociceptors [72]. Finally, immune cell-derived mediators may not only activate or sensitize nociceptors, but also promote nociceptor network remodeling. For example, recurrent infections with uropathogenic *E. coli* (UPEC) in mice resulted in the recruitment to the urinary bladder of mast cells and monocytes, which secrete nerve growth factor (NGF), in turn inducing axonal sprouting and pain sensations [73].

Perhaps to counterbalance the many pro-type 2 immunity feed-forward loops, a subset of polymodal, nonpeptidergic cutaneous sensory fibers expressing the **Mas-related G-protein-coupled receptor D (MrgprD)** can secrete glutamate, which induces transcriptional reprogramming of skin mast cells. Indeed, mice lacking MrgprD$^+$ neurons (*Mrgprd*$^{DTR}$) exhibit exacerbated croton oil-induced allergic dermatitis, but also show an improved ability to clear cutaneous *S. aureus* infection compared with controls [74]. MrgprD$^+$ fibers are themselves maintained by dermal **Langerhans cells (LCs)**, a specialized subset of tissue-resident macrophages. Indeed, sustained LC depletion (LC$^{DTA}$ mice) over at least 30 days resulted in the loss of MrgprD$^+$ neurons [74]; this suggested that the LC–MrgprD$^+$ neuronal axis sets an overall immune tone in the skin by suppressing mast cell (hyper)responsiveness. Of note, LC numbers were reported to be reduced in skin biopsies from patients with chronic allergic contact dermatitis compared with healthy controls [75]. This loss of LCs might have been due to mast cell activation, which is known to induce LC migration from the skin in both mice and humans [76]. Thus, although this idea remains to be formally tested, we propose that the prolonged activation of mast cells in allergic dermatitis leading to sustained local LC depletion and loss of inhibitory MrgprD$^+$ neurons might ultimately result in a permanent breakdown in skin homeostasis and contribute to chronic inflammation.

Of note, Th2 responses can also be regulated through context-dependent processing of nociceptive activity within the central nervous system (CNS). Recent work in mice showed that chronic pain led to the activation of inhibitory **GABAergic neurons** in the amygdala, while acute pain stimulated excitatory **glutamatergic neurons** in the somatosensory cortex [77]. The output of these two systems can then tune the activity of spleen-innervating **cholinergic neurons** originating in the the dorsal motor nucleus of the vagus to decrease or increase the proportion of splenic Th2 cells, respectively [77]. While the physiological impact of such central processing of painful stimuli remains to be established, it adds a new paradigm of how nociceptors may systemically affect the immune system beyond their direct local interactions with immune cells, further underscoring the complexity of neuroimmune interactions.

## Concluding remarks

Even though the immune and nervous systems were historically considered separate, it is now clear that they are inextricably linked to form an integrated defense system that appears to be geared toward promoting tissue homeostasis. The field of neuroimmunology is rapidly expanding, as is our understanding of the neuroimmune interactions between sensory neurons and the

## Outstanding questions

How is differential neuropeptide secretion regulated? Nociceptors control various immune cell functions by releasing specific neuropeptides. Yet, it remains unclear how such divergent neuropeptide release is mediated. It is possible that nociceptor subsets innervating distinct anatomical locations (e.g., skin versus airways) or fibers responsive to specific stimuli (e.g., microbial components versus protease activity) preferentially express specific neuropeptides. Nonetheless, existing scRNAseq data sets indicate that most individual nociceptors express more than one neuropeptide [89], suggesting that this cannot be the only explanation. It will be important to assess whether and how differential activation (combination of ion channels, cytokine receptor activation, etc.) leads to differential neuropeptide secretion.

Are neuroimmune interactions tissue specific? A significant heterogeneity exists among the types and numbers of immune cells residing in various tissues. Similarly, a 'division of labor' has been described for nociceptors, such that, for example, itch-specific fibers appear to be absent in viscera and localize exclusively to barrier tissues directly facing the environment (skin, nasal and oral mucosa, eyes, etc.) [57] where the appropriate physiological response (scratching) can be elicited. Such tissue-specific adaptation raises the possibility that there are also tissue-specific 'sets of rules' governing the communication between nociceptors and the immune system. Assessing the best approach to answer this question is needed to determine whether the observations made in one tissue can be extrapolated to others.

How do nociceptors control global immune responses? The context-dependent control of local immune responses by nociceptors has received the most attention to date. Nonetheless, nociceptive control over the induction of adaptive immune responses [19,52,55,56,65] and the CNS processing of pain stimuli [77] discussed in the main text demonstrate that the effects of nociceptor actions can be far-reaching. A more granular understanding of how these effects shape immune responses as well as discovering other

immune system in peripheral tissues. Nonetheless, major questions remain (see Outstanding questions). Furthermore, it is becoming increasingly apparent that neuroimmune interactions are probably not limited to individual cell–cell dialogs. On the contrary, their outcomes are likely an emergent property resulting from the integration of various signals across, and within, the nervous and immune systems. These signals include feed-forward and feed-back loops between the two systems, neuronal reflexes, as well as inputs from other systems, including the microbiota, host metabolism, and the CNS. Consequently, as the field matures and the list of known interactions increases, we will likely see more focus on integrating the individual interactions into functional networks (Figure 5). However, several challenges will need to be overcome. First, novel, more precise tools will be required. Nociceptor ablation using chemical or genetic tools has been instrumental to advance the field but, as discussed throughout this review, nociceptors can use multiple distinct molecular mechanisms to communicate with different immune cells, often within the same tissue. Thus, conditional ablation of individual modes of communication (e.g., neuropeptides) will be necessary to disentangle the effects of nociceptors on the immune system, and unambiguously define the underlying molecular mechanisms. Second, nociceptors themselves are not a single entity and include different types of neurons with distinct morphology, (electro)physiological and stimulus-response properties, molecular profiles, and peripheral targets [5]. Further work will be necessary to determine which nociceptive subsets are involved in which types of neuroimmune interaction. Finally, integration of all such data into functional networks with predictive power will likely benefit from the development of innovative computational strategies, including the use of machine learning algorithms.

means through which nociceptors can control global immune responses can further inform our understanding of neuroimmunology.

What other molecular means of communication do nociceptors use? Neuropeptides have historically been considered the main, if not the only, means of communication between nociceptors and the immune system. However, recent work indicates that other means of communication exist, including miRNA-laden exosomes [90], secreted proinflammatory proteins [91], chemokines [19], and direct physical contact with electrical coupling [19]. Further delineating underlying molecular mechanisms and identifying additional communication modalities will be essential to fully comprehend how nociceptors modulate the functions of the immune system.

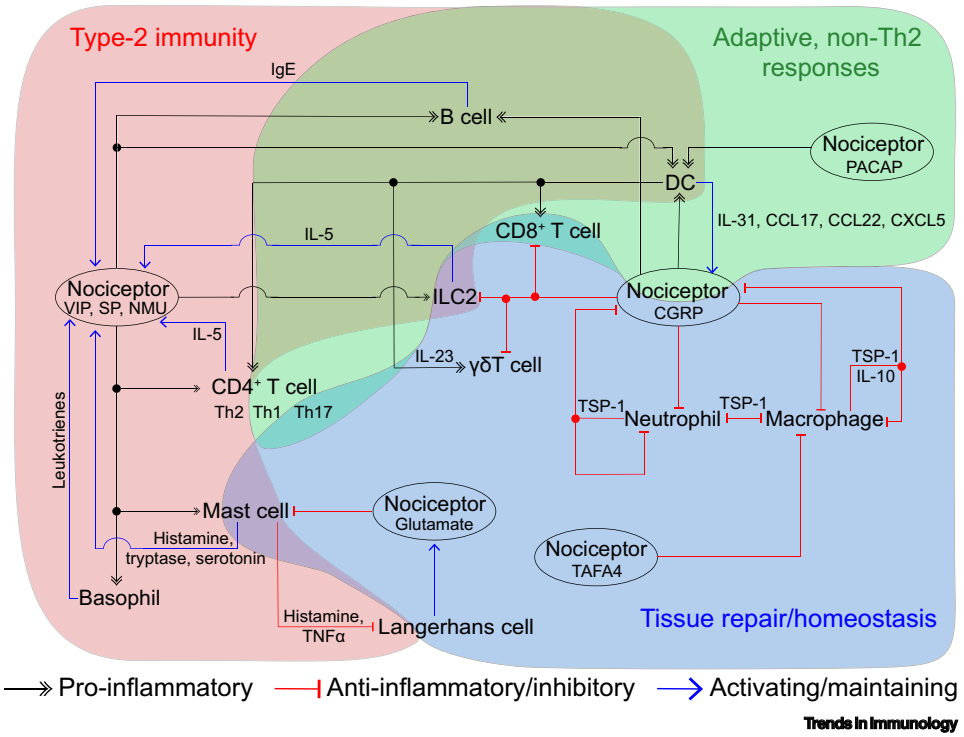


Figure 5. Wiring diagram of known interactions between nociceptors and the immune system. Nociceptors use various molecular means to promote or inhibit inflammation, engaging in numerous feed-forward and feed-back loops. However, most nociceptor actions can be categorized as promoting adaptive immune responses (green), tissue repair (blue), or T helper type 2 (Th2) responses (red). Abbreviations: CGRP, calcitonin gene-related peptide; DC, dendritic cell; IL, interleukin; NMU, Neuromedin U; SP, Substance P; TNF, tumor necrosis factor; TSP1, thrombospondin-1; VIP, vasoactive intestinal peptide.

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## Declaration of interests

U.H.v.A. is a paid consultant of AbbVie, Avenge Bio, Bluesphere Bio, Metaphore, Gate Biosciences, intrECate Biotherapeutics, Interon, Institute for Protein Innovation, Moderna, Morphic Therapeutics, MP Healthcare Venture, Shoreline, and Tessera. U.H.v.A holds stock/stock options at Avenge Bio, Beam, Bluesphere, Metaphore, IntrECate, Interon, Morphic, and Selecta. U.H.v.A is an inventor on the following related pending patent: Ziegler *et al.* 'Methods and composition for modulating immune response and immune homeostasis', Docket # BROD-4830US. U.H.v.A holds a leadership/fiduciary role on the Board of Directors of intrECate Biotherapeutics and as a Councilor of the American Association of Immunologists. The other authors have no interests to declare.

## Resources

[i]https://clinicaltrials.gov/study/NCT03381144

[ii]https://clinicaltrials.gov/study/NCT05660850

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