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REVIEW

The neuropathic pain triad: neurons, immune cells and glia

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Nociceptive pain results from the detection of intense or noxious stimuli by specialized high-threshold sensory neurons (nociceptors), a transfer of action potentials to the spinal cord, and onward transmission of the warning signal to the brain. In contrast, clinical pain such as pain after nerve injury (neuropathic pain) is characterized by pain in the absence of a stimulus and reduced nociceptive thresholds so that normally innocuous stimuli produce pain. The development of neuropathic pain involves not only neuronal pathways, but also Schwann cells, satellite cells in the dorsal root ganglia, components of the peripheral immune system, spinal microglia and astrocytes. As we increasingly appreciate that neuropathic pain has many features of a neuroimmune disorder, immunosuppression and blockade of the reciprocal signaling pathways between neuronal and non-neuronal cells offer new opportunities for disease modification and more successful management of pain.

Pain has long been regarded as the unpleasant sensory consequence of neuronal activity in specific nociceptive pathways that is triggered by noxious stimuli, inflammation, or damage to the nervous system. This is certainly true for acute nociceptive pain, such as the pain elicited by a pinprick or excessive heat. However, it is now clear that neurons are not the only players that drive the establishment and maintenance of common clinical pain states. In this review we focus on immune and glial cell responses to peripheral nerve injury and how they alter neuronal function in the peripheral and central nervous systems. Recognition of the critical involvement of immune cells and glia in the pathophysiological changes after nerve injury offers a completely new treatment approach, one that is certainly needed because most analgesic drugs lack satisfactory efficacy for neuropathic pain and produce undesirable side effects[1]. Neuropathic pain management is currently aimed only at reducing symptoms, generally by suppressing neuronal activity. In contrast, modulating the immune response to nerve injury and targeting glia may provide opportunities for disease modification by aborting neurobiological alterations that support the development of persistent pain.

Peripheral nerve injury provokes a reaction in peripheral immune cells and glia at several different anatomical locations: macrophages and Schwann cells facilitate the wallerian degeneration of axotomized nerve fibers distal to a nerve lesion; an immune response in the dorsal root ganglia (DRGs) is driven by macrophages, lymphocytes and satellite cells; activation of spinal microglia dominates the early glial response in the CNS to peripheral nerve injury, which is followed by activation and proliferation of astrocytes (**Fig. 1**). Macrophages, which are derived from circulating monocytes, and microglia, the resident mononuclear phagocytes of the CNS, share numerous similarities in their immunological and functional properties[2]. Microglial cells are generally considered to stem from a monocytic cellular lineage of mesodermal (myeloid) origin and enter the CNS during fetal development[3]. And although the satellite cells that surround the cell bodies of DRG neurons originate from the neural crest, they can be regarded as the peripheral equivalent of astroglia, because they provide trophic support for primary sensory neurons, express transporters that regulate neurotransmitter levels in the extracellular space and share some astroglial markers, such as glial fibrillary acidic protein (GFAP)[4].

Peripheral inflammatory reactions to nerve lesions

In contrast to primarily immune-mediated neuropathies, such as Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy, which are triggered by T cell activation, macrophages predominate in the initial inflammatory reaction to peripheral nerve injury. Neutrophil granulocytes participate in the very early immune response to nerve injury, potentially attracted by the release of nerve growth factor (NGF), chemokine (C-X-C motif) ligand 1 (CXCL1) and leukotriene-B4. Although neutrophil infiltration is limited to the immediate vicinity of the lesion site and relatively short-lived[5], chemoattractants and cytokines released from neutrophils may play an important role by reinforcing the recruitment of macrophages, particularly during the first 24 h after injury[5].

Immediately after nerve injury, resident macrophages, which account for up to 9% of the cell population in intact peripheral nerves, rush to the lesion site like a rapid-response team[6]. The recruitment and activation of resident macrophages and the invasion of further monocytes from the peripheral blood are orchestrated by the chemokine (C-C motif) ligands (CCLs) 2 and 3 acting on the receptors CCR2, CCR1 and CCR5, respectively[7]. Activated macrophages and denervated Schwann cells secrete matrix metalloproteases that attack the basal lamina of endoneurial blood vessels, leading to an interruption of the blood-nerve barrier[8]. Vasoactive mediators including calcitonin gene–related peptide (CGRP), substance P, bradykinin and nitric oxide are released from injured axons to cause hyperemia and swelling[9]. These

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vascular changes support the invasion of circulating immune cells, so that within two days of injury a dense cellular infiltrate, mainly composed of macrophages, T lymphocytes and mast cells, forms at the lesion site (**Figs. 1b** and **2a**). Upregulation of lysosomal markers and an abundant inclusion of lipid droplets indicate that macrophages transform into active phagocytes after nerve injury. Removal of distal degenerating axons and myelin debris by phagocytosis enables a reorganization of Schwann cells and lays the foundation for the regrowth of injured axons[10].

Within minutes of a nerve lesion, neuregulin, a growth and differentiation factor present on the axonal membrane, induces activation of the tyrosine kinase receptor ERBB2, which is constitutively expressed on Schwann cells[11] (**Fig. 2b**). Blocking this early neuregulin–ERBB2 signaling pathway disrupts demyelination. Later in the course of wallerian degeneration, ERBB2 and ERBB3 receptor upregulation and activation are associated with Schwann cell proliferation[12]. In the reverse direction, Schwann cells release chemical signals that promote axonal growth and remyelination[13]. These include NGF and glial cell line–derived neurotrophic factor (GDNF), which are retrogradely transported to the cell bodies of primary sensory neurons where they act as potent regulators of gene expression. NGF and GDNF also directly activate and sensitize nociceptors[14], contributing to the initiation of pain in response to nerve injury (**Fig. 2b**).

Signaling pathways between primary sensory neurons, Schwann cells and immune cells are highly intertwined, and cytokines and chemokines are central components in this complex network. Schwann cells, active resident and infiltrating macrophages, neutrophil granulocytes, and mast cells release prostaglandins[15], proinflammatory cytokines—including the interleukins (ILs) 1β, 6, 12 and 18, interferon-γ, tumor necrosis factor (TNF) and leukemia inhibitory factor (LIF)[16]—and cytokines with anti-inflammatory or regulatory function such as IL-10 and transforming growth factor-β1 (TGF-β1) (**Fig. 2a**,**b**). Chemokine receptors are present on Schwann cells and satellite glia. Subpopulations of sensory neurons also express the chemokine receptors CCR1, CCR4, CCR5 and CXCR4 and are activated by CCL3 (ref. 17), CCL5 and CCL22 (ref. 18). In sensory neuropathies associated with human immunodeficiency virus (HIV), HIV may use CCR5 and CXCR4 to enter nerve cells and produce direct axonal damage[19].

Proinflammatory cytokines contribute to axonal damage[20], but they also modulate spontaneous nociceptor activity and stimulus sensitivity[21–23]. Activation of TNF receptors in sensory neurons and recruitment of TNF receptor–associated factors (TRAFs), an important group

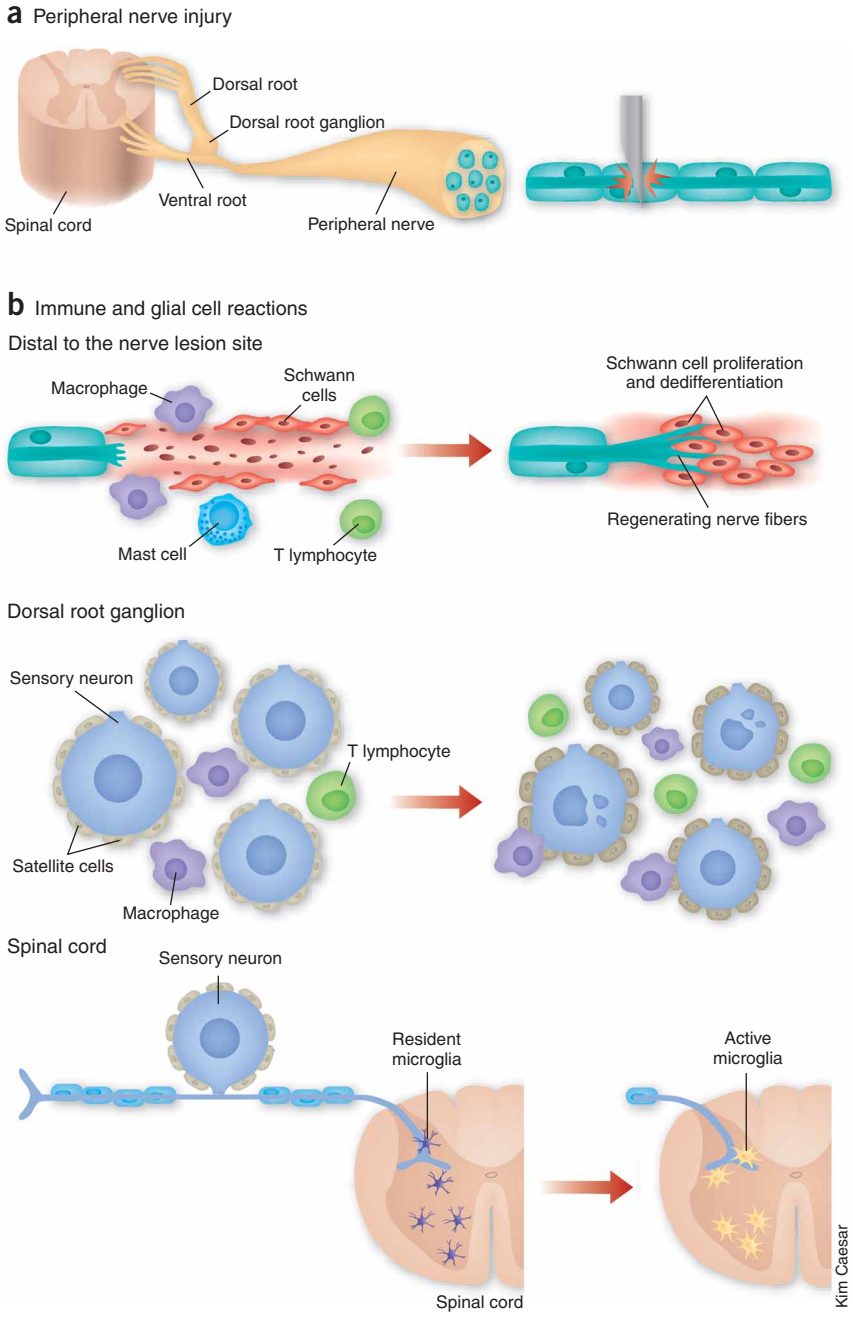

Figure 1 Immune and glial cell responses to peripheral nerve injury. (**a**) Nerve injury provokes recruitment and activation of immune cells at the site of a nerve lesion, in the DRG, and in the ventral and dorsal horns of the spinal cord. (**b**) Top, macrophages, T lymphocytes and mast cells invade the lesion site and spread around the distal stumps of injured nerve fibers. Schwann cells begin to proliferate, dedifferentiate and form bands of Büngner, which serve as guiding tubes for regenerating axons. Middle, macrophages and a few T lymphocytes reside in the DRG before injury. Their numbers increase sharply after injury. Macrophages also move within the sheath that satellite cells form around the cell bodies of primary sensory neurons. Satellite cells begin to proliferate and increase the expression of glial fibrillary acidic protein. Bottom, one week after nerve injury, dense clusters of microglial cells occur in the ventral horn of the spinal cord, surrounding the cell bodies of motor neurons. Massive microglial activation is also found in the dorsal horn, in the projection territories of the central terminals of injured primary afferent fibers.

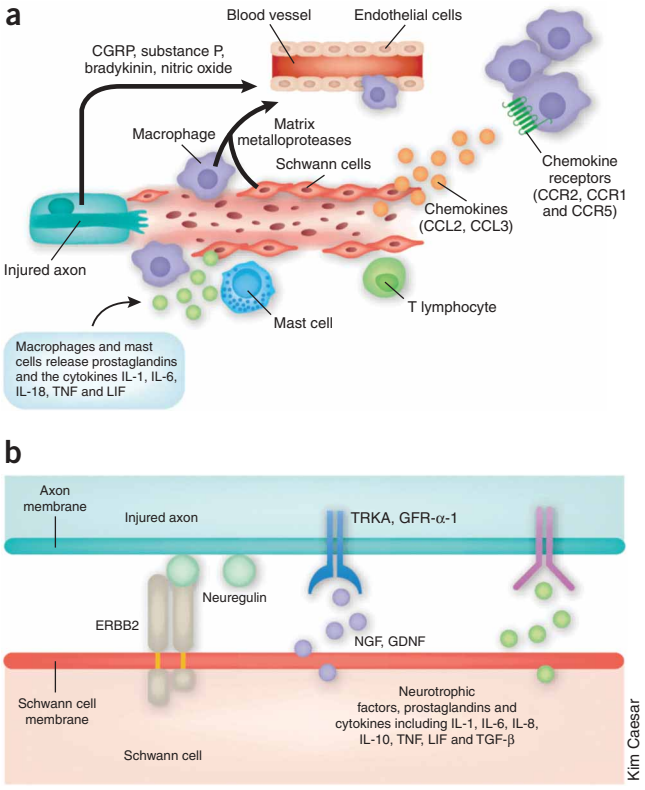

Figure 2 Inflammatory changes associated with wallerian degeneration. (**a**) Macrophages and Schwann cells produce matrix metalloproteases that interrupt the blood-nerve barrier. CGRP, substance P, bradykinin and nitric oxide released from the proximal stumps of injured nerve fibers induce hyperemia and swelling, promoting the invasion of further monocytes and T lymphocytes. The chemokines CCL2 and CCL3 attract and guide monocytes to the lesion site. Macrophages and mast cells release prostaglandins and the cytokines IL-1β, IL-6, IL-18, TNF and LIF. TNF has an autocrine effect on macrophages that is mediated through TNFR1 activation and enhances cytokine synthesis and release. TNF also promotes further macrophage infiltration. (**b**) Within minutes of the injury, neuregulin, a growth factor constitutively expressed on the axonal membrane, binds to a heteromeric receptor composed of ERBB2 and presumably ERBB3 on Schwann cells. Early ERBB2 activation is involved in demyelination, whereas late signaling through ERBB2 and ERBB3 supports Schwann cell proliferation. In the reverse direction, Schwann cells release the neurotrophic factors NGF and GDNF, prostaglandins, and cytokines; these sensitize nociceptors and modulate sensory neuron gene expression.

of intracellular adaptor proteins, leads to phosphorylation of the mitogen-activated protein (MAP) kinases p38 (ref. 24) and Jun N-terminal kinase (JNK), potentially activating the nuclear factor-κB (NF-κB) and Jun oncogene transcription pathways[25]. In macrophages, TRAF recruitment stimulates the synthesis and release of both pro- and anti-inflammatory cytokines[26]. TNF-mediated signaling also promotes further macrophage invasion by inducing the release of proteases and upregulation of adhesion molecules. IL-1 regulating the increase in NGF synthesis and release by Schwann cells[27] is another example illustrating the network of signaling pathways among immune cells, peripheral glia and primary sensory neurons. Communication among these cells favors axonal growth and survival (adaptation) but also triggers the development of persistent pain (maladaptation). Distinguishing the signals necessary for regeneration from those involved in the establishment of neuropathic pain will provide important clues for the development of drugs that reduce maladaptive changes in response to nerve injury without necessarily impairing the adaptive ones.

Mutant C57BL/6OlaHsd-Wld mice express a chimeric nuclear protein that interferes with ubiquitination and proteolysis and protects from wallerian degeneration[28]. These mice show a delayed immune response to nerve injury at the lesion site with a decreased and slowed upregulation of TNF and IL-1β in macrophages and Schwann cells[29]. Their pain responsiveness after nerve injury is reduced[29], highlighting the importance of the inflammatory reaction intrinsic to wallerian degeneration for the early onset of peripheral neuropathic pain.

Macrophage and T lymphocyte invasion of the DRG

Macrophages and a few T lymphocytes are normally present in the DRG, alongside satellite glial cells. Though remote from the actual lesion site, these resident immune and glial cells in the DRG react to nerve injury, and their response is reinforced by invading macrophages and T cells. In contrast, neutrophil granulocytes are only found in the DRG in experimental nerve lesions that involve inflammation, such as the chronic constriction injury model. Injury-induced macrophage invasion appears to be triggered by a release of chemokine (C-X3-C motif) ligand 1 (fractalkine)[30] and the chemokine CCL2 from DRG neurons[31–33]. CCL2 may also act directly in a paracrine fashion on subpopulations of DRG neurons that begin to express CCR2 after injury[32].

The density of macrophages immunoreactive for major histocompatibility complex II increases in the DRG 1 week after a nerve transection and remains elevated for at least 3 months. By that time, macrophages move from an initially rather diffuse distribution in the DRG to surround the cell bodies of injured sensory neurons (**Figs. 1b** and **3**). Two months after a peripheral nerve transection, a substantial proportion of macrophages in the DRG turn into active phagocytes, presumably removing debris from injured sensory neurons[34], many of which begin to degenerate after axotomy. In the rat, a decrease, predominantly in small unmyelinated neurons, is detectable 8 weeks after nerve transection[35]. In the mouse, 24% of DRG neurons are lost within 7 d of a nerve injury; after 4 weeks, their number is decreased by more than 50% (ref. 36). Ongoing loss of sensory neurons may therefore be one factor responsible for the sustained increase in the number of macrophages and T lymphocytes in the DRG. The persistence of the immune response in the DRG certainly contrasts with the inflammatory reaction distal to the nerve lesion site, which essentially ends with the removal of myelin debris during wallerian degeneration[34].

A marked upregulation of genes in the DRG that are related to immune cell function reflects the extent of both the recruitment and activity of macrophages and T cells and underscores the substantial changes that occur in the local environment of primary sensory neurons after axonal injury[37]. Increased synthesis and release of cytokines including IL-1, IL-6 and TNF can directly modulate neuronal activity and elicit spontaneous action potential discharges. Deletion of the type I IL-1 receptor or transgenic overexpression of the endogenous IL-1 receptor antagonist, for example, inhibits the development of spontaneous (ectopic) sensory neuron firing[23], and blocking IL-1- or IL-6-mediated signaling attenuates neuropathic pain-like behavior[23,38]. TNF has an enhanced direct effect on sensory neurons after nerve lesions, because both injured and neighboring uninjured nerve fibers become more sensitive to this cytokine[22]. Through a signaling pathway that involves TNF receptor 1 (TNFR1) and p38 MAP kinase, TNF acts to increase the density of tetrodotoxin (TTX)-resistant sodium channel currents in nociceptors[39] (**Fig. 3a**). In addition, cytokines such as LIF and IL-6 modulate the synthesis of neuropeptide transmitters. The

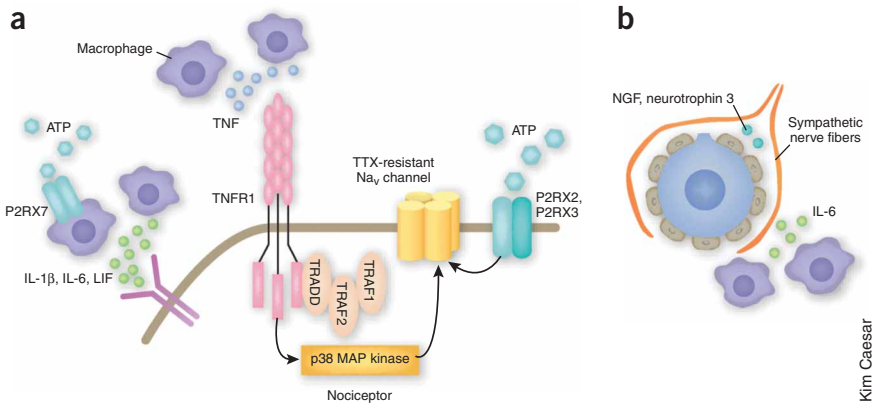

Figure 3 Immune response in the DRG. (**a**) Macrophages potentiate TTX-resistant voltage-gated sodium channel currents through a signaling pathway that involves TNF acting on TNFR1 and, further downstream, phosphorylation of p38 MAP kinase. Nociceptor activity is further modulated through activation of the purinergic P2RX2 and P2RX3 dimer. Activation of P2RX7 expressed by macrophages regulates the release of the cytokines IL-1β, IL-6 and LIF. (**b**) IL-6 is a macrophage-derived signal that triggers the sprouting of sympathetic nerve fibers into the DRG. The formation of noradrenergic fiber baskets around predominantly large-diameter sensory neurons is also dependent on NGF and neurotrophin-3, which are released from satellite cells.

resultant change in the phenotype of sensory neurons is likely to alter the efficacy of their synaptic input to the spinal cord.

A signaling pathway that modulates both neuronal and macrophage function in the DRG involves purinergic P2 receptors. Homo- or heteromers of P2RX subunits form unselective cation channels with a low affinity (in the micromolar range) for ATP but fast activation. In contrast, P2Y receptors are slow G protein–coupled receptors with a high affinity (in the nanomolar range) for ATP that trigger a variety of second-messenger signaling pathways, resulting in a prolonged signal duration[40,41]. Nociceptors predominantly express P2RX2 and P2RX3 subunits[42], whereas only few primary sensory neurons express P2Y receptors[43]. Cells of myeloid origin, including macrophages and B lymphocytes, express P2RX7 and several P2Y receptor subunits such as P2RY1, P2RY2 and P2RY4. T lymphocytes express P2RX1, P2RX4 and P2RX7 but lack P2Y receptors[44]. Satellite cells are immunoreactive for P2RX2 and P2RX7 (ref. 45) and express P2RY12 and P2RY14 receptor subunits[43], indicating that ATP may directly modulate their activity. Passive release of ATP from severed nerve fibers and surrounding damaged tissue will activate the purinergic receptors expressed by sensory neurons and immune cells, but intact primary sensory neurons themselves also release ATP upon stimulation. Involvement of P2RX2 and P2RX3 (refs. 46,47), P2RX4 (ref. 48) and P2RX7 (refs. 49,50) in the development of nerve injury–induced mechanical hypersensitivity has been demonstrated in several animal models. P2RX (and P2RY) agonists provoke an increase in nociceptor activity and sensitivity that is mediated in part by the TTX-resistant voltage-gated sodium channel $Na_V1.9$ (ref. 51) (**Fig. 3a**). Blocking P2RX2 and P2RX3 reduces Aδ and C fiber activity after nerve injury[52]. Mice deficient in P2RX7 have a substantially blunted hypersensitivity to mechanical and thermal stimuli in models of neuropathic pain, presumably because of a reduced release of inflammatory cytokines, including IL-1β, from macrophages and microglia[49,50] (**Fig. 3a**).

Several months after nerve injury, macrophages and T cells are also closely associated with sympathetic nerve fiber terminals that sprout into basket-like structures around large-diameter sensory neurons. Sprouting of noradrenergic terminals is reduced in mice lacking IL-6, indicating that cytokine release by macrophages is one of the signals that trigger the sympathetic nerve fiber invasion[53]. The formation of sympathetic fiber baskets furthermore depends on satellite cell–derived NGF and neurotrophin-3, suggesting coordinated communication between macrophages and satellite cells (**Fig. 3b**). In addition, satellite cells may be the origin of neural crest progenitors for neurons and Schwann cells that could help repopulate the DRG after injury[54].

Microglial and astrocyte activation in the spinal cord

Microglial cells have a key role in the response to direct injuries of the central nervous system inflicted by trauma or ischemia, in autoimmune diseases such as multiple sclerosis, and in neurodegenerative disorders. Given that microglial cells share a myeloid lineage and many functional features of peripheral macrophages, it is not too surprising that after a nerve lesion, microglial cells form dense clusters around the cell bodies of injured motor neurons in the ventral horn of the spinal cord, similar to the macrophages that surround injured sensory neurons in the DRG[55]. However, it is perhaps far less expected to find a massive recruitment and activation of microglia in the dorsal horn in vicinity of the central terminals of injured sensory nerve fibers[56–59] (**Fig. 1b**). Nerve injury-induced microglial activation is characterized by phosphorylation (activation) of p38 MAP kinase, extracellular signal-related kinase (ERK) isoforms 1 and 2, and the Src-family kinases (Src, Lck and Lyn)[60–63]. Spinal microglial activation in both dorsal and ventral horns peaks 1 week after injury, followed by a slow decline over several weeks. This temporal pattern differs from that of the very early inflammatory reaction distal to a nerve lesion site and the sustained infiltration of macrophages and lymphocytes in the DRG, suggesting that the central immune response to peripheral nerve injury is independently organized and has distinct functional consequences.

Three signaling pathways mediate the recruitment of resident spinal microglia and probably also circulating monocytes to the dorsal horn. These involve the chemokine fractalkine acting on the CX3CR1 receptor[64], CCL2 signaling through CCR2 (ref. 65), and Toll-like receptors[66,67] (**Fig. 4a**).

Fractalkine is a neuronal transmembrane glycoprotein from which a soluble chemokine domain can be cleaved by proteolysis; the chemokine is, however, active in both its membrane-bound and soluble form. As microglial cells[64] and astrocytes[68] express CX3CR1, signaling through fractalkine could help explain the topographic specificity of microglial recruitment and astrocyte proliferation in the territory of injured afferent fiber terminals (**Fig. 4a**). Fractalkine-mediated signaling between neurons and glia seems to contribute to the development of neuropathic pain. Intrathecal injection of fractalkine produces mechanical allodynia and thermal hyperalgesia[30,69], whereas administration of a neutralizing antibody against CX3CR1 delays the development of mechanical allodynia after chronic constriction of the sciatic nerve and spinal nerve ligation[30,69].

CCL2 is expressed by primary sensory neurons and Schwann cells after peripheral nerve injury[33] and chronic compression of the DRG, a model of spinal stenosis[32]. Nerve injury does not induce CCL2 expression in dorsal horn neurons, but CCL2 is transported to the central terminals of primary afferents in the dorsal horn[33]. Microglial

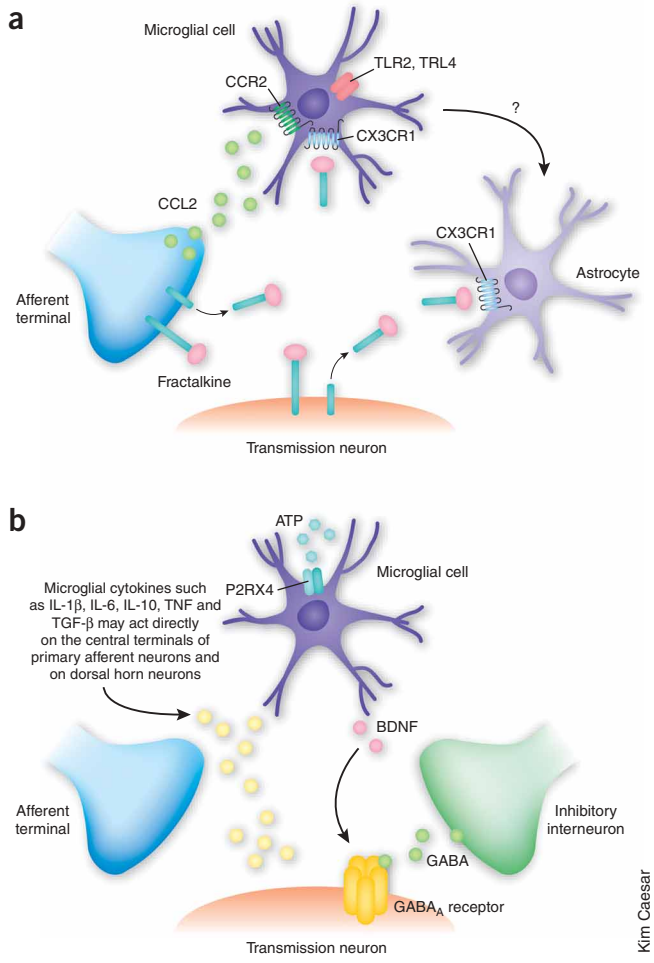

Figure 4 Recruitment and activation of spinal microglia and astrocytes. (**a**) Microglial recruitment depends on signaling pathways involving TLR2 and TLR4, and on the chemokine CCL2 acting on CCR2. The neuronal protein fractalkine has a chemokine domain that can be cleaved from its membrane-bound portion. Both bound and soluble fractalkine have chemokine function and may attract microglia as well as astrocytes by acting on CX3CR1. Because the microglial response to nerve injury precedes the proliferation of astrocytes, a direct path of communication may exist between these two glial cell types to coordinate their sequential temporal patterns of activation. (**b**) ATP binding to the purinergic receptor P2RX4 triggers microglial activation after nerve injury. Active microglia releases BDNF, which induces in a subpopulation of dorsal horn lamina I neurons an inversion of inhibitory GABAergic currents. In addition, microglial cytokines are likely to act directly on the central terminals of primary sensory afferents and on dorsal horn neurons.

cells express CCR2, the receptor for CCL2 (ref. 70), suggesting a potential pathway of direct communication between injured primary afferent fiber terminals and dorsal horn microglia (**Fig. 4a**); it is, however, unknown if (and how) CCL2 is released from afferent terminals. Mice lacking CCR2 show substantially less hypersensitivity to mechanical stimulation after partial sciatic nerve ligation[70], but this reduction of neuropathic pain-like behavior is not necessarily mediated by the absence of CCR2 in microglia. Instead, prevention of peripheral macrophage and lymphocyte recruitment to the nerve lesion site and the DRG may partly explain the effect of CCR2 knockout[70]. Furthermore, because primary sensory neurons start to express CCR2 and are depolarized by CCL2 after injury[32], CCR2 knockout interrupts signaling among sensory neurons, between denervated Schwann cells and sensory neurons, and potentially also between sensory neurons and microglia.

Mammalian Toll-like receptors (TLRs) are a family of twelve evolutionarily conserved membrane proteins that are fundamental in the initiation of innate immunity against invading pathogens[71]. TLRs recognize lipid, carbohydrate, peptide and nucleic acid structures expressed by different groups of microorganisms. TLR signaling involves five adaptor proteins that bind to downstream protein kinases including p38 MAP kinase and JNK. Ultimately, TLR signaling leads to activation of the transcription factor NF-κB, upregulation of interferons and increased expression of proinflammatory cytokines[71]. Recent findings support a link between neuropathic pain and the innate immune response mediated through TLR activation (**Fig. 4a**). In mice lacking TLR2 (ref. 66) or TLR4 (ref. 67), and rats treated with antisense oligonucleotides to produce TLR4 knockdown[67], microglial activation and the induction of proinflammatory cytokines after a peripheral nerve lesion are substantially diminished. Moreover, these animals show less neuropathic pain-like behavior[66,67]. Although microglial cells express both TLR2 and TLR4, the endogenous ligands that activate these TLRs are unclear. TLRs recognize nucleic acids and proteins released after cell damage—for example, the heat-shock proteins 60 and 70 (ref. 72)—so that they may be activated by cellular debris from the degenerating central terminals of injured afferents or in response to the nerve injury–induced transsynaptic apoptosis of dorsal horn neurons[73]. However, the extent of central terminal degeneration seems insufficient to explain the scale of microglial activation after nerve injury.

Microglial recruitment and activation in the dorsal horn is accompanied by an invasion of T lymphocytes[55]. Nerve injury also leads to increased proliferation and activation of astrocytes in the ipsilateral spinal cord. Compared with the microglial response, astrocyte proliferation begins relatively late and progresses slowly, but is sustained for a longer period (more than 5 months)[33,63,74]. The signal(s) that trigger and sustain astrocyte proliferation and activation are unknown, and it is intriguing to speculate that the astroglial response occurs secondary to the microglial activation (**Fig. 4a**).

Talk to me, listen to me

Recruitment and activation of different glial cells in complex temporal patterns requires well organized reciprocal communication between neurons and glia and among glial cells themselves. A prominent signaling pathway in the development of neuropathic pain involves ATP acting on microglial purinergic receptors. Microglial cells express P2RX4, P2RX7, P2RY2, P2RY6 and P2RY12 (refs. 40,43,58), and ATP is a potent stimulator of microglia *in vitro*[48]. When injected intrathecally, ATP and ATP-stimulated microglia provoke sustained mechanical hypersensitivity in the rat[48,75]. Tonic stimulation of spinal microglia with ATP causes a P2RX-mediated release of brain-derived neurotrophic factor (BDNF), which produces a depolarizing shift in the anion reversal potential of dorsal horn lamina I neurons[75] (**Fig. 4b**). This shift prompts an inversion of inhibitory GABA currents that contributes to mechanical allodynia after nerve injury[76]. On the other hand, not all GABAergic effects are inverted after peripheral nerve injury[73], and this change in GABA signaling may be limited to a subpopulation of lamina I neurons projecting to the brain. Peripheral nerve injury leads to an increase in microglial P2RX4 expression, and pharmacological blockade of spinal P2RX4 reduces mechanical allodynia after nerve injury without affecting acute pain-responsive behavior in uninjured rats[48]. However, the source of ATP in the dorsal horn

is unknown. Potentially, ATP is actively released from injured primary afferents and dorsal horn neurons, or increases as primary afferent terminals degenerate. Elevated ATP may not only stimulate microglia but also modulate synaptic transmission among neurons, as presynaptic P2X receptors are present on primary afferent terminals and inhibitory interneurons, and postsynaptic P2X receptors on dorsal horn neurons[41]. In slice preparations of the spinal cord, activation of P2X receptors enhances spontaneous and evoked excitatory postsynaptic currents and glutamate release in the dorsal horn[77].

Microglial activation leads to increased synthesis of the lysosomal cysteine protease cathepsin S (ref. 78) and the cytokines IL-1β, IL-6, IL-10, TNF and TGF-β. Direct modulation of dorsal horn neuron activity by these cytokines may be involved in the development of neuropathic pain[79] (**Fig. 4b**). However, cytokines also provide important autocrine feedback signals to microglial cells themselves. In the brain, IL-1β, IL-6, TNF and interferon-γ induce components of the complement cascade in microglia. We have recently identified several complement factors and receptors as some of the most prominently regulated genes in the dorsal horn across several distinct animal models of neuropathic pain[80]. These include complement-5 (C5) and its receptor, both of which are upregulated in microglial cells within 3 d of peripheral nerve injury. C5 is cleaved and activated by C3, a central complement component that represents the point of convergence for three different pathways of complement activation. Whereas intrathecal injection of a synthetic peptide equivalent to active C5 induces cold hypersensitivity in naive rats, C5 receptor blockade reduces cold-evoked pain-like behavior in rats after spared nerve injury[80], indicating that complement signaling converging on C5 activation modifies the painful response to cold. The C5 receptor is exclusively expressed in microglia; therefore, signaling pathways between microglia and neurons are required for mediating the effect of its activation on pain-like behavior. *In vitro*, C5 induces expression of C5 receptor on microglial cells, indicating that this complement factor is also involved in a feedforward mechanism that enhances microglial sensitivity to C5.

Immune and glial modulation: new treatment opportunities

Current therapeutic strategies for neuropathic pain aim to reduce the excitability of neurons in the peripheral nervous system or the CNS by modulating the activity of ion channels (gabapentin, pregabalin, carbamazepine, lidocaine and capsaicin) or by mimicking and enhancing endogenous inhibitory mechanism (tricyclic antidepressants, duloxetine and opioids). Considering that the involvement of immune cells and glia in the development of neuropathic pain is now well established, and given the enormous need for therapeutic progress, surprisingly few clinical studies have tested immunosuppressive drugs or drugs interfering with glial functions for neuropathic pain. Epidural and intrathecal corticosteroid injections to prevent or treat postherpetic neuralgia have been used based on the assumption that inflammation during the reactivation of herpes zoster virus contributes to persistent pain in this condition[81,82]. Epidural corticosteroid injections are commonly used to treat sciatica, a condition of mixed etiology including inflammation at the site of an intervertebral disc prolapse and facet joint arthritis; they generally provide short-term pain relief but not sustained improvement[83].

Preclinical studies have explored several routes of immune and glial modulation[84]. Global inhibitors of glial metabolism such as fluorocitrate[85], propentofylline[86], minocycline[87,88] and teriflunomide[89] reduce cytokine release and attenuate pain-responsive behavior in several animal models of neuropathic pain. Fluorocitrate inhibits aconitase, which leads to blockade of the citric acid cycle. Although fluorocitrate preferentially acts on glial cells, concerns about its toxicity prevent its clinical use[90]. Propentofylline reduces proliferation and activity of both microglia and astrocytes by inhibiting extracellular adenosine transporters and phosphodiesterases, which results in an increase in cyclic nucleotides, including cyclic AMP (ref. 91). However, trials examining the treatment effect of propentofylline in Alzheimer's disease, which also involves microglial activation, were unsuccessful. Minocycline is a member of the tetracycline class of broad-spectrum antibiotics that diffuses into the central nervous system. Apart from its antibiotic properties, minocycline inhibits matrix metalloproteases, reduces microglial activity by suppressing the expression of inducible nitric oxide synthase (iNOS) and the phosphorylation of p38 MAP kinase, and has neuroprotective function as an inhibitor of neuronal necrosis and apoptosis[92]. Minocycline is now under clinical investigation as a treatment for multiple sclerosis and amyotrophic lateral sclerosis. Teriflunomide is the active metabolite of leflunomide, an immunosuppressive drug approved for the treatment of rheumatoid arthritis. Leflunomide and teriflunomide block the *de novo* synthesis of pyrimidines in rapidly dividing cells such as lymphocytes by binding to dihydroorotate dehydrogenase; leflunomide and teriflunomide also inhibit antigen presentation. Prolonged treatment with leflunomide is, however, associated with an increased risk of sensory and motor neuropathy[93], limiting its use for immunomodulation in neuropathic pain.

More targeted interventions have been aimed at purinoceptors, cannabinoid receptors, MAP kinases, TNF and interleukins. Recently developed selective antagonists of P2RX2 and P2RX3 heteromultimers and inhibitors of P2RX3 (refs. 52,94) and P2RX7 (refs. 50,95) reduce spontaneous discharges and evoked responses of primary sensory neurons, decrease cytokine release and attenuate mechanical hypersensitivity after nerve injury. Type 2 cannabinoid receptors (CB2) are primarily expressed by peripheral immune cells, including macrophages and lymphocytes, and by microglia and astrocytes in the central nervous system[96]. CB2-selective agonists reduce pain-like behavior in animal models of peripheral nerve injury[97].

The prominent involvement of p38, JNK and ERK in the activation of microglia and astrocytes makes MAP kinases promising targets[60,62,63]. However, to specifically block spinal microglial activation, a p38 inhibitor would have to be directed against the β-isoform of this MAP kinase, sparing the α-isoform that is present in dorsal horn neurons[62]. Furthermore, MAP kinases are important in many cellular processes, such as proliferation and differentiation, stress response, and apoptosis. They are involved in development, learning and memory, so that systemic application of such inhibitors carries the risk of interfering with these important functions. Nevertheless p38 MAP kinase inhibitors are being evaluated for treatment of rheumatoid arthritis and may well be useful for neuropathic pain management.

Etanercept, a soluble TNF receptor fusion protein, and anakinra, a recombinant form of human IL-1 receptor antagonist, have been tested in animal models of peripheral nerve injury and reduce neuropathic pain-like behavior[24,79]. Induced expression of anti-inflammatory IL-10 in the DRG and spinal meninges provides a prolonged analgesic effect in rats after chronic constriction of the sciatic nerve[98]. Thalidomide, which primarily inhibits TNF synthesis but also modulates the expression of other cytokines, including IL-10 (ref. 99), decreases mechanical and thermal hypersensitivity in rats after nerve injury when the treatment starts at the time of the injury; thalidomide treatment has no analgesic effect once neuropathic pain hypersensitivity is established[100]. Likewise, minocycline does not reverse existing hypersensitivity after nerve injury[88], perhaps indicating that peripheral immune cells and microglia have an important but transient role in

the development of neuropathic pain. In contrast, propentofylline, which inhibits microglial and astrocyte activation, attenuates pain-responsive behavior when administered early after nerve injury and also decreases established hypersensitivity[86].

Conclusion

The contribution of immune cells and glia to the development and the persistence of pain after nerve injury challenges conventional concepts that are biased toward neurons being responsible for the pathophysiological changes that drive neuropathic pain. Yet this shift in our understanding provides an exceptional opportunity to progress to a new disease-modifying therapeutic approach. Therapeutic interventions must, however, take into account the different temporal patterns of immune and glial cell activation at the nerve lesion site, in the DRG, and in the spinal cord. Furthermore, we need to determine the extent to which the peripheral immune reaction and the central glial response are linked, and identify the pathways of such a connection. Finally, just as the participation of peripheral immune cells in wallerian degeneration facilitates the regeneration and healing of injured axons after a nerve lesion, the highly coordinated spinal glial response to nerve injury may not be exclusively maladaptive. Differentiating between the good, the bad and the ugly aspects of immune and glial responses to nerve injury will be essential for developing targeted new treatment strategies for neuropathic pain.

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