ROLE OF THE IMMUNE SYSTEM IN CHRONIC PAIN

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Abstract | During the past two decades, an important focus of pain research has been the study of chronic pain mechanisms, particularly the processes that lead to the abnormal sensitivity — spontaneous pain and hyperalgesia — that is associated with these states. For some time it has been recognized that inflammatory mediators released from immune cells can contribute to these persistent pain states. However, it has only recently become clear that immune cell products might have a crucial role not just in inflammatory pain, but also in neuropathic pain caused by damage to peripheral nerves or to the CNS.

PAIN
Pain has been defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage, or described in terms of such damage.

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Open almost any neuroscience or neurology textbook, and it will describe how noxious stimuli (stimuli that damage or threaten to damage tissues of the body) activate a system that begins with the peripheral terminals of primary sensory neurons, continues with relays through spinal and supraspinal nuclei, and leads to the activation of a matrix of cortical areas that is associated with the multiple conscious dimensions of PAIN. Our knowledge of this system dates back several hundred years, and is now considerable. Many of the attempts to provide relief to those in pain have sought to surgically or pharmacologically target this neuronal system at one level or another, mostly with only limited success. One reason for this failure is that the model is incomplete. Not only does it make no allowance for the context in which pain is usually perceived and the modifying influences of fear, anxiety, anticipation and pain history, but it is also neurobiologically incomplete — that is, some important features of neuronal processing have been overlooked or ignored in the traditional depiction of the model.

In this review, we discuss the role of non-neuronal cells in the processing of pain-related information by the nervous system, and, specifically, the idea that cells of the immune system might strongly influence neuronal function in many persistent or chronic pain states. This idea is not new, as the activation of inflammatory cells is classically associated with pain (dolor), heat (calor), redness (rubor), swelling (tumour) and loss of function (functio laesa). However, new research from several laboratories indicates that immune cells have an important role as pain modulators not just in inflamed tissues, but also in damaged peripheral nerves and in the CNS. We review these actions and discuss several persistent pain states in which immune cells are activated, considering how this arises, what mediators such cells release and how these mediators alter pain processing.

No immune cell involvement in acute pain

Strong stimulation of most tissues of the body elicits pain. This 'physiological' pain has a protective role in driving the behaviours that remove or minimize the threat. Much of the pain that is elicited by strong stimuli arises through the direct transduction of the stimulus into action potentials in specialized primary sensory neurons, nociceptors. These nociceptors are equipped with a range of receptors that render them sensitive to thermal, mechanical and some chemical stimuli (FIG. 1). Several transient receptor potential (TRP) channels (of which TRPV1 is the most extensively studied) are temperature sensitive and, when activated, depolarize nociceptor terminals[1]. The molecular nature of mechanical transduction is not well understood, but some of the ion channels that are expressed by nociceptors seem to respond to such stimuli[2]. Some chemical irritants act on ion channels or G-protein-coupled receptors to activate nociceptive endings. Acute tissue-damaging

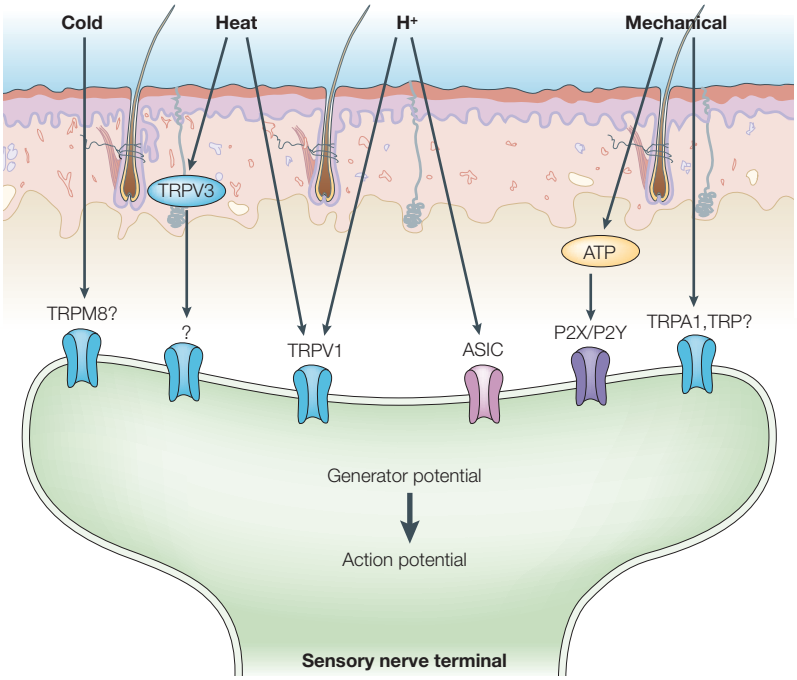

Figure 1 | **Physiological pain.** Nociceptors respond to acute tissue-damaging stimuli (such as heating the skin), either directly, through transduction of the stimulus energy by receptors (such as the transient receptor potential (TRP) channel TRPV1) on nerve terminals, or indirectly, through activation of TRP channels on keratinocytes (for example, TRPV3) and/or the release of intermediate molecules (such as ATP), which, in turn, act on sensory neuron receptors. Immune cells seem to have little, if any, part in this process. ASIC, amiloride-sensitive cation channel 2; P2X, ionotropic purinoceptor; P2Y, G-protein-coupled pyrimidinergic receptor; TRPA1, TRPM8, TRPV3, TRP channels.

stimuli can also indirectly activate nociceptors. Many nociceptors express ionotropic (P2X purinoceptors, notably P2X3) and G-protein-coupled (P2Y, pyrimidinergic) receptors that are responsive to ATP. All cells in the body contain millimolar concentrations of ATP, which is released if cells are lysed during injury and can then activate nociceptors[3]. In these acute responses to noxious stimuli, immune cells are either absent or silent bystanders. However, as discussed below, they are active players in a wide variety of persistent pain states.

Peripheral roles of immune cells in pain

Inflammatory pain. The signs and symptoms of inflammation include cell migration, oedema, fever, erythema, pain and hyperalgesia. Various events precipitate these inflammatory reactions, including exposure to microbial cell wall fragments or toxins (lipopolysaccharide (LPS) from bacteria and zymosan from yeast cell walls), irritant chemicals (such as carrageenan) and autoimmune reactions. A family of 'Toll-like' receptors is activated by these stimuli. As inflammation develops, spontaneous pain often evolves and inflamed tissue can show hyperalgesia — increased pain on stimulation, which includes both increased pain to noxious stimuli and a lowered pain threshold (the latter might more strictly be referred to as allodynia).

The types of immune cell that contribute to inflammatory pain depend on the inflammatory condition, but, in general, various cell types will be recruited and will contribute to abnormal pain sensitivity, albeit to different degrees. MAST CELLS, which are resident in peripheral tissues (BOX 1), contain a range of inflammatory mediators. Some of these are stored in cytoplasmic granules, whereas others (notably CYTOKINES) exist as precursors in the cell (or attached to the cell surface) and are activated and released following cleavage[4]. Activation of mast cells in human skin with compound 48/80, a basic polyamine that causes degranulation, produces an intense flare (erythema), a profound itch and marked hyperalgesia in response to heat[5]. In animal models of inflammation, the involvement of mast cells in pain processing has been assessed using repeated 48/80 treatment (to deplete mast cell mediators), the mast cell stabilizer sodium cromoglycate, mast-cell-deficient mice or histamine receptor antagonists. The results of these studies indicate that mast cells contribute to pain in many experimental inflammatory pain states[5–9]. The role of mast cells in pain processing has also been implicated in the clinical conditions of interstitial cystitis[10] and chronic pancreatitis, in which patients with pain show a 3.5-fold increase in the number of mast cells compared with patients without pain[8].

Activated macrophages — either resident or recruited from the blood (BOX 1) by chemotactic cytokines — have been reported to contribute to experimental pain states. They can release many inflammatory mediators, notably pro-inflammatory cytokines (particularly tumour necrosis factor-α (TNFα) and interleukin-1β (IL-1β)), nerve growth factor (NGF), nitric oxide (NO) and prostanoids (FIG. 2). When macrophages have been depleted from or recruited to peripheral tissues experimentally, they have been shown to contribute significantly to the inflammatory pain that is produced by zymosan and the irritant acetic acid[7]. The supernatant from macrophages that have been activated *in vitro* by LPS is hyperalgesic because of its cytokine content[11]. In addition, macrophage activation seems to have a marked effect on the subsequent recruitment and activation of other cell types (neutrophils) to the site of inflammation[12].

Neutrophils — the earliest inflammatory cell type to infiltrate tissues from the blood (BOX 2) — dominate the acute and early inflammatory responses. They can produce various inflammatory factors, including lipoxygenase products, nitric oxide, cytokines and CHEMOKINES. In inflammatory conditions, they also express opioids and might, in some circumstances, have anti-nociceptive effects[13]. The role of neutrophils has been studied by depleting them from the blood with cytotoxic agents or by increasing their numbers with granulocyte-colony stimulating factor (GCSF). When GCSF is injected into human skin, pain is a common complication[14]. Experimentally, the prevention of neutrophil accumulation in inflammation that is produced by carrageenan reduces the associated inflammatory pain[15]. NGF is a well-established inflammatory mediator[16], the hyperalgesic actions of which seem to depend, in part, on neutrophil accumulation[17,18].

MAST CELL
A multigranular cell that functions as a store for several key inflammatory/pain mediators (including NGF, TNFα, chemokines and histamine).

CYTOKINES
Small, secreted proteins that mediate and regulate immunity, inflammation and haematopoiesis. They act as intercellular mediators by binding to specific membrane receptors, which then signal through second messengers — often tyrosine kinases — to alter the target cell's behaviour.

CHEMOKINES
Small polypeptide cytokines that can attract leukocyte subsets.

Box 1 | Leukocytes and their recruitment to other tissues

There are three main types of leukocyte in the peripheral blood: granulocytes, monocytes and lymphocytes. Their numbers can vary markedly between individuals. In several pathologies, leukocytosis (an abnormally high number of leukocytes) occurs through the rapid differentiation of specific precursors in the bone marrow. Granulocytes include neutrophils, eosinophils and basophils, and are the first subset of leukocytes to be mobilized in response to injury and inflammation.

There are two subsets of monocytes: the fractalkine receptor $(CX_3CR1)^{high}$–chemokine (C–C motif) ligand 2 receptor $(CCR2)^{low}$ subset, which is constitutively able to extravasate into tissue sites (for example, serosal cavities), generating the resident tissue macrophages that act as a sort of sentinel against xenobiotic invasion; and the CX_3CR1^{low}–$CCR2^{+}$ subset, which is more specifically pro-inflammatory and is recruited to the site of inflammation after injury or infection[141]. The latter subset gives rise to the inflammatory macrophages, which help to resolve the host response to the inciting insult, leading to tissue repair and healing. In the physiological status, a lymphocyte subset characterized by high expression of CD62L freely circulates across tissues and rejoins the blood stream from the lymphatic microcirculation. These macrophages undertake immune surveillance: on encountering an antigen-presenting dendritic cell, they become activated and mount the adaptive immune response.

Lymphocytes are the larger group of circulating leukocytes, comprising T cells (which orchestrate the cellular immune response), B cells (which are antibody producers) and natural killer cells.

The process of physiological recruitment maintains the pools of resident cells in several tissues. In the CNS, monocytic precursors are recruited before closure of the blood–brain barrier, and differentiate first into amoeboid microglia (with an appearance closer to that of the macrophage) and then into ramified microglia. Mast cells are largely found in close contact with nerves. Tissue mast cells are found in close proximity with peripheral neurons and Schwann cells, and differentiate *in situ* from bone marrow precursors, rather than from circulating basophils as was previously thought.

The role of T and B cells in experimental pain models has been less well-studied. However, these cells can also generate inflammatory cytokines and chemokines, and contribute to innate immune responses. The elimination of subgroups of these cells in animals has confirmed their central role in acquired immunity and autoimmune diseases[19].

Which immune mediators alter pain processing? It is clear from the foregoing discussion that many potentially algogenic substances are released from immune cells into inflamed tissues. For TNFα, IL-1β, NGF and prostaglandin E_2 (PGE_2), there is considerable evidence that: they are present in inflammatory exudates; they can produce pain or hyperalgesia when exogenously administered to animals and humans; and, most importantly, antagonism or neutralization of these factors reduces pain and hyperalgesia in many animal models of inflammation (for a review, see REF. 20). There is also considerable evidence for the involvement of other mediators in inflammatory conditions, including lipoxygenase products (leukotriene B_4), bradykinin, serotonin, cytokines (IL-6 and leukaemia inhibitory factor (LIF)) and chemokines (CCL2, CXCL8 and GCSF)[20]. There is strong evidence that nitric oxide is an important mediator of hyperalgesia in the CNS (see below), but evidence for a peripheral action is less clear. Nitric oxide is induced in inflamed tissues, probably through both inducible and neuronal nitric oxide synthase (iNOS and nNOS, respectively)[21]. Nitric oxide donors can induce pain in humans[22] and NOS inhibitors can reduce inflammatory hyperalgesia in a PGE_2-dependent manner[23]. Surprisingly, antagonism of each of the mediators described above produces substantial anti-hyperalgesia — often approaching 100%.

In recent years, the importance of TNFα has been underscored by the tremendous success of TNFα antibodies or neutralizing reagents for the treatment of rheumatoid arthritis. These drugs, which do not cross the blood–brain barrier and, therefore, have a peripheral site of action, can modify symptoms and disease progression. In most patients, they have proved effective against a large number of endpoints, although their pain-relieving effects have not been extensively detailed (for reviews, see REFS 24,25). The importance of TNFα in pain relief is supported by recent clinical trials that have shown the efficacy of anti-TNFα therapy for many autoimmune disorders, including psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease.

How do inflammatory mediators produce pain and hyperalgesia? Many of the mediators act directly on nociceptive terminals that innervate the inflamed tissues. These neurons express receptors for TNFα, IL-1β, NGF, IL-6, LIF, histamine, bradykinin and prostanoids[20], and the post-receptor signalling cascades have been partially elucidated (FIG. 2). For example, bradykinin acts on B2 receptors on nociceptors and, through a diacylglycerol (DAG)–protein kinase C (PKC) pathway, opens cation-permeable ion channels. TRPV1 channels are also sensitized, which might account for bradykinin-induced heat sensitization. Inflammatory mediators induce the expression of B1 receptors in several cell types, which contributes indirectly to inflammatory hyperalgesia. Prostaglandins that are secreted by cyclooxygenases 1 and 2 (COX1 and COX2, respectively) act on a series of prostanoid receptors (EP, DP and IP) on nociceptors. They activate adenylate cyclase in these neurons, elevating the concentration of cyclic AMP (cAMP), which sensitizes nerve terminals, at least in part by increasing sodium currents[20]. Congruently, EP3- and IP-knockout mice show reduced LPS-induced hyperalgesia. The importance of these prostanoids is strengthened by the clinical data associated with cyclooxygenase inhibitors, which have good analgesic properties in inflammatory pain.

The algesic effects of NGF have also been extensively studied[16]. The high-affinity NGF receptors (tyrosine kinase receptor A, TrkA) are expressed by about 50% of nociceptors and their activation leads to phosphorylation and sensitization of TRPV1 receptors, which might account for NGF-induced heat hyperalgesia. However, the intracellular signalling pathways remain controversial (for a discussion, see REF. 26). Another important action of NGF is its modulation of nociceptor gene expression (such as *TRPV1*, *P2X3*, Nav 1.8, brain-derived neurotrophic factor (*BDNF*) and substance P) after retrograde transport of NGF–TrkA to the nucleus, which might underlie increases in long-term nociceptor sensitivity. As well as inducing other mediators in inflamed

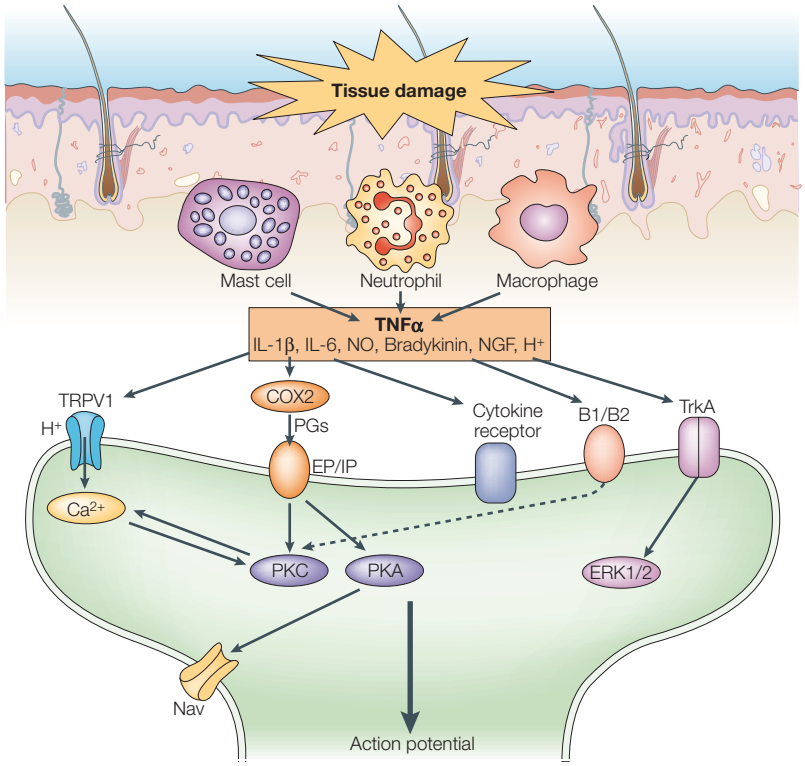

Figure 2 | **Inflammatory pain.** After tissue damage, mast cells and macrophages are activated and some blood-borne immune cells, including neutrophils, may be recruited. Various immune mediators are released (such as tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), nitric oxide (NO), bradykinin, nerve growth factor (NGF) and protons), which exert their algesic effects by acting directly on nociceptors or indirectly through the release of other mediators, most notably prostanoids. There is increasing knowledge of the intracellular cascades that are activated in nociceptors by these mediators, which ultimately either activate or sensitize these neurons. COX2, cyclooxygenase 2; B1/B2, bradykinin receptor; EP/IP, prostanoid receptor; ERK1/2, extracellular signal-regulated kinase 1/2; Nav, voltage-activated sodium channel; PGs, prostaglandins; PKA/PKC, protein kinase A/C; TrkA, tyrosine receptor kinase A; TRPV1, transient receptor potential channel.

NEUROPATHIC PAIN
This has been defined by the International Association for the Study of Pain as pain that is initiated or caused by a primary lesion or dysfunction in the nervous system.

tissue (for example, NGF, nitric oxide and PGE_2), TNFα might act directly on TNF receptors that are expressed by nociceptors to produce sensitization, although the associated intracellular mechanism is less clear. Lipoxygenase products (such as leukotriene B_4 and 8R,15S-diHETE) can sensitize nociceptors indirectly through neutrophils, and probably also directly, because they can open TRPV1 channels in isolated dorsal root ganglion (DRG) neurons[27]. Recently, the identification of protease-activated receptors (PARs) in sensory neurons indicated a new mechanism of nociceptor sensitization. Mast cell-derived tryptase can cleave specific PAR residues in the amino (N)-terminal domain, thereby exposing a novel N terminus that functions as a tethered ligand to activate the receptor. When activated, the PAR signalling pathway — through the inositol-1,4,5-triphosphate ($Ins(1,4,5)P_3$) pathway and probably other intracellular cascades — can not only induce the production of more classical algogens such as PGE_2, but also sensitize nociceptive terminals by acting on TRPV1 channels[28]. The mechanisms of chemokine-induced hyperalgesia are less clear, but many of these molecules can induce short-latency increases in calcium concentrations in isolated DRG neurons[29], which indicates direct receptor-mediated actions.

There is an increasing awareness that anti-inflammatory pathways might be important in limiting the duration of inflammatory responses. The resolution phase of inflammation is increasingly recognized as an active process that might be exploited to treat inflammatory pain (BOX 3).

Pain associated with peripheral neuropathy. Damage to peripheral nerves can precipitate NEUROPATHIC PAIN. Such pain is often persistent and is poorly treated by existing therapies[30] because distinct mechanisms are triggered by neuronal injury. Neuropathic pain arises as a result of many forms of nerve damage, including diabetic neuropathy, HIV neuropathy, post-herpetic neuralgia, drug-induced neuropathy and traumatic nerve injury. There are several animal models of neuropathic pain, almost all of which involve partial injury of the sciatic nerve of rodents[31]. An important feature of these models is that both lesioned and unlesioned axons have highly abnormal properties as a consequence of nerve damage, including dysregulated gene expression and the generation of ectopic (spontaneous) discharge. These abnormal activities, which later impinge on the CNS, seem to be crucial in generating neuropathic pain[32]. It is clear that abnormal peripheral inputs feed into a spinal processing system that is disrupted in these neuropathic states (see below). What is surprising is that activation of the immune system seems to have a crucial role in both peripheral and central abnormal sensory processing.

Which types of immune cell contribute to peripheral neuropathy? Several immune cell types have been implicated in neuropathic pain, but their relative contribution and the timing of their effects have not been fully elucidated. Zuo *et al.* showed that mast cells were activated in one model of partial sciatic nerve injury[33]. The strategy of mast cell stabilization reduced the recruitment of neutrophils and monocytes into the injured nerve, probably by reducing the release of chemokines and other mediators (see above). The potential contribution of neutrophils was studied further by Perkins and Tracey[34], who showed a substantial invasion of endoneurial neutrophils into the damaged nerve — a process that peaked 24 h after injury. The greatest extent of invasion was seen at the site of injury, where any factors that had been released might act on both damaged and intact axons passing through the injury site (FIG. 3b). Relatively few neutrophils were observed either in the distal nerve or in peripheral tissues innervated by the damaged nerve, although this contradicts the findings of other studies[35,36]. Depletion of circulating neutrophils at the time of nerve injury reduced the development of neuropathic pain symptoms but did not reverse established symptoms[34]. Therefore, neutrophils could have an important role in the early stage of neuropathic pain development.

Box 2 | Inflammatory leukocytes and the paradigm of recruitment

Circulating leukocytes respond to injury and/or xenobiotic attack. They leave the bloodstream and reach the site of inflammation — the latter event can occur in the nervous system (see main text) — and we refer to them as inflammatory cells. How do circulating leukocytes sense the opportunity of moving into the nervous system and which subtypes of cell are recruited? These issues have been studied in detail during the past 10–15 years[142], although rarely in relation to the nervous system.

In general terms, classes of adhesion molecules and leukocyte activators act in concert to slow down the fast-moving leukocytes and allow them to interact with the wall of blood vessels. Selectins and their counter-ligands operate in the initial stages of inflammation and promote the phenomenon of cell rolling — the slow-motion movement of leukocytes on the vessel wall, which resembles the motion of a flat tyre. Integrins (expressed by the leukocytes) and their endothelial counter-ligands (immunoglobulin superfamily) are responsible for promoting firm adhesion of the rolling leukocytes.

Between these two events, activation of leukocytes by specific activators that bind to the endothelial surface favours the switch from cell rolling to firm adhesion, the latter signifying a major commitment to recruitment. The expression of a particular receptor by leukocytes, which is coupled to the expression of its agonist (activator) on the vessel wall, explains the selectivity of the recruitment process. Only in recent years has it been realized that chemokines complement the action of classical leukocyte activators (such as leukotriene B_4 and complement factors) — a discovery that has stirred much ongoing research activity. The chemokine family comprises at least 40 ligands and 18 receptors[143], and, so far, pain-related effects have been elaborated for only a few members (see main text).

In damaged peripheral nerves, as in other tissues, macrophages are recruited by chemotactic molecules in the microenvironment. Several lines of evidence indicate that macrophages are important in neuropathic pain models[37–41]. Most of these studies have shown a temporal correlation between the invasion of blood-borne macrophages and the development of allodynia or hyperalgesia. Furthermore, a lack of thermal hyperalgesia in a neuropathic model in the WLD mouse, which shows delayed recruitment of non-resident macrophages, has been reported[38,39]. Depletion of circulating monocytes/macrophages by intravenous administration of liposome-encapsulated clodronate reduces the number of macrophages in the injured nerve and alleviates thermal hyperalgesia[40]. However, Rutkowski *et al.* failed to relieve mechanical allodynia after clodronate administration. They also reported that perineural administration of activated macrophages did not evoke mechanical allodynia[42]. It is possible that during the process of recruitment to a specific tissue, macrophages are 'programmed' for subsequent functions, so direct injection of cells might not mimic their normal role. As the mechanisms of thermal and mechanical hyperalgesia are different, it could be that macrophages are more important for the former than the latter.

During Wallerian degeneration, the Schwann cells that envelop degenerating axons undergo remarkable reactive changes and begin to synthesize a range of potent molecules, including NGF and TNFα. The spared axons in partial nerve lesions run between these Schwann cells. The upregulation of NGF by Schwann cells seems to be driven largely by IL-1β, which is released from macrophages in the damaged nerve[41] (FIG. 3b). It is still unclear which factors regulate the release of other Schwann cell-derived molecules.

Along with or after macrophage recruitment, T cells are infiltrated into damaged nerves, but their involvement in neuropathic pain has been poorly studied. Moalem *et al.* characterized the T-cell infiltration into injured (but not uninjured) sciatic nerves in one neuropathic model[43]. They also showed that the later phase of thermal and mechanical allodynia was weakly reduced in athymic nude rats. Finally, passive transfer of type 1 T cells (which produce pro-inflammatory cytokines) into nude rats increased their neuropathic pain scores to the level of heterozygous littermates. Therefore, the contribution of T cells in neuropathic pain is still unclear, and its role at the periphery could be very limited. Indeed, Tsai *et al.* did not detect a difference in splenocyte proliferation and natural killer cell activity between sham-operated rats and those with a chronic constriction injury of the sciatic nerve (a standard neuropathic model)[44,45].

There is also evidence for recruitment of macrophages and T cells into the DRG, which contains the cell bodies of sensory neurons. Healthy DRG, which lacks a blood–nerve barrier, contains a number of cells that express macrophage markers (such as MCH II)[46] and a small population of T cells that have an immune surveillance function, as in other tissues[46]. Several authors have reported an increase in MCH II staining after peripheral nerve injury[46–48], but the source of this staining is unclear — it might be recruited haematogenous immune cells or activated satellite cells. In any case, there is no direct evidence that these cells are important for neuropathic pain.

Increased expression of INTEGRINS in neuropathic pain models has been reported[49–51]. Intrathecal injection of integrin antisense oligonucleotides or inhibitors blocked the induction of hyperalgesia in such models[49,50]. However, it is not clear whether these treatments simply prevent the normal inflammatory cascade or whether they reflect a specific role of integrins in the development of neuropathy. In addition to promoting inflammatory cell infiltration, chemokines might mediate pain by acting directly on nociceptors[29,52].

Which immune-derived factors produce pain and how? In the family of pro-inflammatory cytokines, TNFα is considered to be the prototype, initiating a cascade of activation of cytokines and growth factors. There is considerable evidence for its involvement in neuropathic pain. Several studies have shown a correlation between the level of TNFα expression and the development of allodynia or hyperalgesia in neuropathic pain models[53–56]. The development of allodynia or hyperalgesia can be increased by the endoneurial administration of TNFα, whereas antagonism of TNFα has the opposite effect[54,57–61]. Interestingly, nerve injury also leads to increased expression of TNFα receptors 1 and 2 by both damaged and spared sensory neurons, and increased sensitivity of both groups of neurons to exogenous TNFα[54,62,63]. Finally, pre-emptive (but not delayed) treatment with etanercept (Enbrel; Amgen/Wyeth) (a TNFα-sequestering drug) can inhibit

INTEGRINS
Dimeric membrane proteins that are involved in several aspects of cell–cell interaction.

Box 3 | **The attraction of anti-inflammation**

The inhibitory role of glycine and $GABA_B$ (γ-aminobutyric acid type B)-receptor activation in spinal neuronal circuits has long been established. The same applies to the negative effect of morphine-like molecules, which have an analgesic action. However, the recent discovery of the importance of microglia in pain sensation opens new avenues for investigating the effects of endogenous inhibitory mediators and pathways. In fact, in other contexts, the concept of anti-inflammation has been suggested to be an important determinant of an inflammatory pathology: malfunction or absence of one or more endogenous anti-inflammatory pathways and mediators could lead to persistent pain and inflammation, and prolong disease[144]. Several mediators fall into this category and, in some cases, new findings about their mechanisms of action and receptor targets have emerged, favouring their exploitation for innovative anti-inflammatory and analgesic drug discovery. It is foreseeable that the same approach could be taken with respect to pathologies of the nervous system and their symptoms, including pain.

Adenosine and its receptors have the potential to reduce both pain sensitivity and microglia activation[85]. The recently discovered neuroprotectins could also fall into this group of counter-regulator mediators, which includes anti-inflammatory cytokines, decoy receptors, melanocortins, haem oxygenase 1, lipoxins, carbon monoxide, glial cell line-derived neurotrophic factor (GDNF) and resolvins[145–147]. The anti-inflammatory effect of GDNF is a good example of a glia-derived mediator that affects the inflammatory response both inside and outside the CNS.

mechanical allodynia in neuropathic models, which indicates that TNFα is particularly important in the initiation of neuropathic pain[54,59]. TNFα might act either directly, on nociceptors, or indirectly, through the prostanoid pathways (see below).

IL-1β is a potent pro-inflammatory cytokine that might be involved in neuropathic pain[61,64]. The expression of IL-1β is upregulated after nerve injury, and neutralizing antibodies to IL-1β receptors reduce pain-associated behaviours in mouse models of neuropathy[61,64]. However, the mechanism of action of IL-1β in the periphery is unclear. Several studies indicate that it might be involved in a complex signalling cascade that leads to the production of pronociceptive compounds (such as nitric oxide, NGF and prostaglandins) from immune cells or Schwann cells. Some of these compounds can lead to changes in gene expression and neuronal excitability (including the development of spontaneous activity) in intact nociceptors (for a review, see REF. 16). IL-1β might also directly excite nociceptive fibres[65] or increase their responses to heat stimuli through an IL-1β receptor type I–PKC pathway[66].

Another cytokine that has been implicated in neuropathic pain is IL-6 (for a review, see REF. 67). Mechanical allodynia has been correlated with levels of IL-6 immunoreactivity or mRNA in the sciatic nerve and DRG, respectively, after nerve constriction injury[68]. Moreover, in this model of neuropathic pain, IL-6-knockout mice show less thermal hyperalgesia and mechanical allodynia compared with wild-type mice. However, one study[69] reported a decrease in mechanoallodynia, but not thermal allodynia, in IL-6-knockout mice, at least for the first 10 days after injury. The mechanism of action of IL-6 is not well established, but might be related to sympathetic sprouting in the DRG[69].

MICROGLIA
A non-neuronal cell type present in the spinal cord and brain (the resident CNS macrophage) that is characterized by its ramified morphology.

Chemokines might have algogenic as well as chemotactic effects[52]. Several chemokine receptors (such as CCR2/3/6/7/8 and CXCR1/2/3/5) are expressed by DRG neurons. Ligands for these receptors can induce calcium transients in dissociated, capsaicin-sensitive DRG nociceptive neurons[29]. Only CCL2 and fractalkine have been studied in neuropathic pain states. It has been shown that CCL2 expression is increased in damaged sensory neurons in a model of neuropathic pain and that direct intrathecal administration of this chemokine induces mechanical allodynia[70]. Mice that lack the CCL2 receptor, CCR2, fail to develop symptoms of neuropathic pain[71]. Some studies have shown an increase in the immunoreactivity of fractalkine (CX_3CL1) and its receptor (CX_3CR1) in the DRG and the spinal cord after nerve injury, and neutralizing antibodies against CX_3CR1 have been shown to delay the development of mechanical and/or thermal hyperalgesia[72,73]. CXCL8 can produce dose-dependent hyperalgesia after intraplantar injection[74], although its role in neuropathic pain is speculative.

A common peripheral mechanism of action of cytokines and chemokines in neuropathic pain might be the induction of COX2, which leads to prostaglandin synthesis in damaged nerves[75]. Ma and Eisenach reported an increased expression of COX2 and prostanoid receptors EP1–4 (EP1 and EP4 were co-expressed with ED1, a marker of infiltrating macrophages) in damaged nerves[76–78]. Interestingly, these studies also showed that perineural or intraplantar injection of ketorolac, a cyclooxygenase inhibitor, reduced pain behaviours and the immunoreactivity of spinal substance P, calcitonin gene-related peptide-α (CGRP) and cAMP-responsive element-binding protein (CREB). The limited effects of cyclooxygenase inhibitors in the clinical treatment of neuropathic pain indicate that this mechanism might be of little importance or be confined to the onset of neuropathic pain.

Central roles of immune cells in pain

Pain associated with peripheral neuropathy. The peripheral nerve injuries that lead to neuropathic pain states cause not only pathology in the damaged peripheral nerve and DRG (see above), but also a series of changes in the central processing of sensory information, which are best characterized at the spinal level. These changes are indirect, as the CNS itself is not damaged. It is remarkable that these central changes include changes in immune cell function.

Two types of immune cell have been studied in this context — haematogenous leukocytes and resident MICROGLIA. Although there have been many studies of immune cell extravasation in the periphery, few have focused on the infiltration of immune cells into the spinal cord after peripheral nerve injury. Extravasation of leukocytes (macrophages and/or T cells) occurs in the lumbar spinal cord 3–14 days after peripheral L5 nerve transaction[51,79]. The specific role of these infiltrating cells is not clear — they could even have neuroprotective or anti-hyperalgesic functions[80].

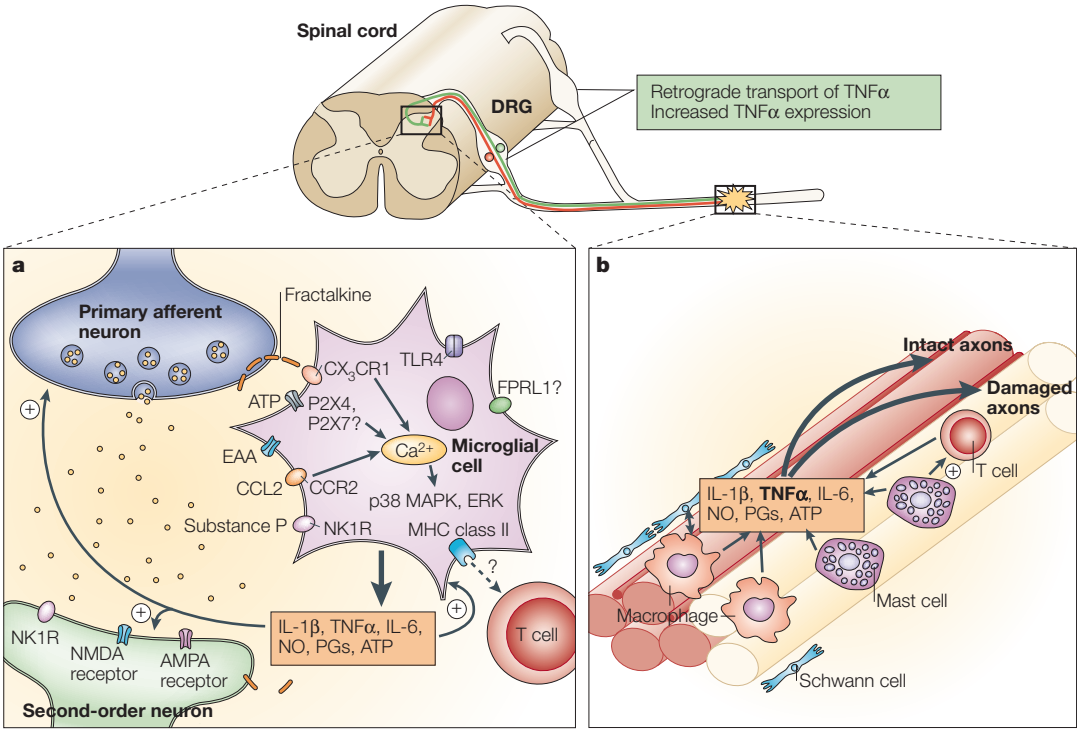

Figure 3 | **Neuropathic pain. a** | Central events that occur after peripheral nerve injury. A primary afferent neuron terminal is flanked by microglial cells that maintain and survey the environment in the spinal cord. In neuropathic pain states, the microglia are activated, probably by the release of transmitters or modulators from primary afferents. The activated microglia release several pro-inflammatory cytokines, chemokines and other agents that modulate pain processing by affecting either presynaptic release of neurotransmitters and/or postsynaptic excitability. The release of inflammatory mediators (such as tumour necrosis factor-α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), nitric oxide (NO), ATP and prostaglandins (PGs)) initiates a self-propagating mechanism of enhanced cytokine expression by microglial cells. This leads to an increase in intracellular calcium, and activation of the p38 and MAPK/ERK pathway. **b** | Changes that occur in sensory neurons after peripheral nerve injury. Damaged and spared fibres are shown juxtaposed after partial injury to a peripheral nerve. The site of injury is typified by the recruitment and proliferation of non-neuronal elements (such as Schwann cells, mast cells, macrophages and T cells), which release factors (such as TNFα, IL-1β, IL-6, chemokine (C–C motif) ligand 2 (CCL2), PGs and nerve growth factor (NGF)) that initiate and maintain sensory abnormalities after injury. These factors might either induce activity in the axons they act on or be transported retrogradely to cell bodies in the dorsal root ganglion (DRG), where they alter the gene expression of neurons. Macrophages and mast cells may recruit T cells, which reinforce and maintain inflammatory reactions. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CCR2, CCL2 receptor; CX_3CR1, fractalkine receptor; EAA, excitatory amino acids; ERK, extracellular signal-regulated kinase; FPRL1, formyl peptide receptor-like 1; MHC, major histocompatibility complex; NGF, nerve growth factor; NK1R, neurokinin-1 receptor; NMDA, *N*-methyl-D-aspartate; P2X4, P2X7, ionotropic purinoceptors; p38 MAPK, p38 mitogen-activated protein kinase; TLR4, Toll-like receptor 4.

Microglia, oligodendrocytes and astrocytes form a large group of CNS glial cells. Microglia express the same surface markers as macrophages/monocytes, which are derived from the periphery (BOX 1). Microglia are activated by events such as CNS injury, microbial invasion and some pain states, which leads to an increase in the production of various inflammatory cytokines, chemokines and other potentially pain-producing substances (see below). Although microglial activation has been found to be beneficial in some circumstances[81], substantial evidence, both direct and indirect, indicates that it can contribute to neuropathic pain after peripheral nerve injury.

Several studies have shown that specific microglial inhibitors and/or modulators can block and/or reverse neuropathic states[82–87]. The most commonly used compounds are fluorocitrate and minocycline. Interestingly, pre-emptive and curative fluorocitrate treatment — which selectively blocks astrocyte and microglia metabolism[88] — inhibits neuropathic pain[82–85], whereas minocycline (a specific microglial inhibitor[89]) blocks the development of neuropathic pain states but does not reduce pain that is already established[86]. The latter study indicates that microglia might be more important in the initial phases of neuropathic pain. Recently, Watkins *et al.* reported that intrathecal minocycline was much more effective in delaying the induction of allodynia than in reversing it in models of sciatic inflammatory neuropathy and acute spinal immune activation with intrathecal HIV-1 gp120 (REF. 87). Moreover, the spinal implantation of microglia that had been activated *in vitro* simulated signs of neuropathic pain (mechanical allodynia)[90].

One way of studying microglia is to monitor their activation status. However, no definitive markers have been identified[91], and most studies have relied on the

Box 4 | **Annexin 1 and the FPRL1 receptor**

The glucocorticoid-regulated protein ANNEXIN 1, an endogenous anti-inflammatory mediator, inhibits inflammatory pain[148,149] and several forms of local and systemic inflammation[149]. So far, its putative role in models of neuropathic pain has not been assessed, although peripheral nerve lesions cause overexpression of annexin 1 in microglia[150], which might indicate the activation of a counter-regulatory neuroprotective phenomenon.

Annexin 1 and its bioactive peptides activate a specific G-protein-coupled receptor, FPRL1, which is expressed by monocytes and granulocytes. Interestingly, this receptor is also present in microglia and its expression is augmented in Alzheimer's disease[151]. FPRL1 is activated by several ligands besides annexin 1, including lipoxins and serum amyloid protein A. It can be activated by the amyloid-β active peptide to promote cell locomotion and oxidant stress[151]. The 24-amino-acid neuroprotective peptide humanin, which was recently discovered through functional expression screening for its ability to suppress the neuronal cell death that is associated with familial Alzheimer's genes, also interacts with FPRL1 (REF. 152). So, FPRL1 is an example of a receptor that transduces both the pro-inflammatory neurotoxic effects of amyloid-β42 and those of humanin. It is not clear whether FPRL1 transduces the inhibitory effects of annexin 1 on activated microglia, which include the downregulation of lipopolysaccharide-stimulated cyclooxygenase 2 and inducible nitric oxide synthase expression[153].

immunostaining of OX-42, which labels complement receptor type 3 (CR3). An increase in OX-42 immunoreactivity has been temporally correlated with symptoms of neuropathic pain after peripheral nerve injury (and some CNS injuries, see below) in many studies[86,90,92–94]. However, a dissociation of microglial activation and the development of tactile allodynia and thermal hyperalgesia have been reported in other cases[93–95]. In addition, local anaesthetic blockade of peripheral nerves or systemic treatment with minocycline markedly reduces the immunoreactivity of OX-42 but does not suppress abnormal pain behaviour[86,93]. Some studies have reported comparable OX-42 induction in the dorsal and ventral horn in neuropathic pain models[92–95]. Together, these findings indicate that microglial activation is necessary, but not sufficient, for the emergence of neuropathic pain behaviour. As there may be different types of microglial activation, other activation markers (for example, integrin αM (ITGAM, also known as MAC-1), Toll-like receptor 4 (TLR4), the CD4 antigen and major histocompatibility complex (MHC) class II) might be more closely correlated with abnormal pain sensitivity than OX-42 (REFS 42,51,86,96–98).

Microglial activation might be just the first step in a cascade of immune responses in the CNS. Zhuang *et al.*[99] recently showed sequential activation of the mitogen-activated protein (MAP) kinase ERK (extracellular signal-regulated kinase) in neurons, then microglia, and finally astrocytes in a neuropathic pain model. Therefore, microglia might be responsible for the initiation of neuropathic pain states, and astrocytes may be involved in their maintenance. It is worth noting that there are several apparent anomalies or contradictions in the literature as to the time course of spinal immune cell activation (for a discussion, see REF. 16).

It is not clear which factors activate microglia in the spinal cord in peripheral neuropathic pain states. On the basis of antisense and somewhat circumstantial pharmacological evidence, Tsuda *et al.* showed that the activation of microglia in neuropathy requires P2X4 receptors, which are upregulated and specifically expressed by microglia in neuropathic pain models[90] (FIG. 3a). Other evidence indicates that P2X7 receptors are expressed on microglia and that their activation by ATP can lead to the production and release of inflammatory cytokines[100]. Indeed, mice that lack this receptor show an impaired ability to develop neuropathic pain[101]. The released cytokines (TNFα and IL-6) might themselves be involved in microglial activation[102,103]. Activated microglia express annexin 1, which might reflect a protective or counter-regulatory response (BOX 3). BOX 4 highlights the potential impact of the annexin 1 system on the activation of microglia, both in the spinal cord and in higher centres.

Fractalkine might also contribute to microglial activation. Verge *et al.* reported fractalkine immunoreactivity in neurons, but not in microglia, in the spinal cord and DRG[73]. Interestingly, its receptor, CX_3CR1, is expressed constitutively by microglia and is upregulated in a spatially specific manner in two neuropathic pain models. Intrathecal pre-emptive and, intriguingly, curative treatment with neutralizing antibodies against CX_3CR1 can inhibit and reverse, respectively, symptoms of neuropathic pain[72]. Consistent with the studies on peripheral neuropathic pain, a good a case can be made for another chemokine, CCL2. It is not expressed at high concentrations in normal sensory neurons, but is upregulated after peripheral nerve injury and transported to central terminals, and CCR2-knockout mice do not express neuropathic behaviours[71].

Finally, a recent study[104] showed that TLR4 can contribute to painful neuropathy. This receptor is activated by several exogenous and endogenous ligands (such as LPS, heat-shock proteins, the extradomain A of fibronectin and amyloid-β), but the mediator functioning in these neuropathic pain conditions was not identified. TLR4-knockout and point-mutant mice did not develop thermal and mechanical allodynia after peripheral nerve injury, and showed reduced microglial and astrocyte activation and strongly decreased expression of interferon-γ (IFNγ), IL-1β and TNFα compared with wild-type mice.

Despite some inconsistencies and uncertainties, microglial activation seems to be an important factor in the development of neuropathic pain. However, it is not clear how microglia produce pain and which mediators are involved. The recruitment of microglia is commonly associated with the activation (phosphorylation) of p38 MAP kinase[54,105–107]. Phosphorylation of p38 is probably a key intracellular signal in microglia that regulates pain-related actions. However, the literature is not entirely consistent, most notably with regard to the time course of p38 activation. The most parsimonious explanation of these findings is that peripheral nerve injuries that are associated with neuropathic pain states lead to p38-dependent activation of microglia in

ANNEXIN 1
A member of a superfamily of proteins named after their ability to annex membranes by binding to acidic phospholipids in the presence of cations; an important endogenous counter-regulator of inflammation.

FPRL1
A member of the formyl peptide receptor family of G-protein-coupled receptors that mediates the anti-inflammatory/protective activities of lipoxins and annexin 1, as well as the activating effects of amyloid-β fragments.

the spinal cord, and then to activation of astrocytes in the relevant spinal cord segments. This process seems to be crucial for the full emergence of neuropathic pain behaviour (for reviews, see REFS 16,108,109).

In pathophysiological conditions, microglia can release various mediators, such as IL-1β, TNFα, PGE_2 and nitric oxide. Some evidence indicates that intrathecal administration of IL-1β or TNFα can lead to symptoms of neuropathic pain in healthy rats[110–112]. Furthermore, the expression of both these cytokines is increased in models of neuropathic pain[113], and blockade of this increase reduces pain and hyperalgesia[54,64]. Despite a lack of strong evidence about their source (that is, neurons and/or microglia), these cytokines can modulate spinal pain processing in several ways. Activation of their receptors on spinal neurons can lead to rapid changes in neuronal excitability[66]. IL-1β or TNFα might also act indirectly through the release of nitric oxide[114,115] and PGE_2 (REF. 116). However, spinal cord cytokines alone are probably not sufficient to explain neuropathic pain, and work in concert with other pronociceptive compounds (for example, substance P and glutamate).

There is conflicting evidence about the roles of COX2 and PGE_2 in the spinal cord after nerve injury[117–120]. Further studies will be required to describe their involvement in the pain that is associated with peripheral neuropathy.

Pain associated with central injuries. Injuries and diseases that directly affect the CNS can also induce a strong immune reaction. In particular, spinal cord injury (SCI) and multiple sclerosis (MS) can be associated with abnormal pain sensitivity.

Loss of function is usually the main consequence of SCI. However, pain severely compromises the quality of life in ~70% of patients with SCI[121]. Mechanisms of SCI-associated pain, although poorly understood, are likely to be manifold[121]. Direct injury of the spinal cord leads to local breakdown of the blood–brain barrier, the release of many intracellular constituents, such as ATP, and the production of reactive oxygen species. As in other tissues, injury of the spinal cord leads to local inflammatory responses and the activation of immune cells, which are similar to those described above. Although direct evidence is still lacking, similar factors and mechanisms might contribute to the abnormal pain state that occurs in SCI. In response to injury, neutrophils, monocytes/macrophages and lymphocytes are recruited to the injury site, and microglia are activated at and beyond this site. The invasion of neutrophils and haematogenous macrophages peaks at 12 h and 5–7 days after SCI, respectively (for a review, see REF. 122). There might be two phases of microglial activation[123]. We have recently found that the microglial inhibitor minocycline reduces both mechanical and thermal hyperalgesia that is associated with SCI[124].

There is direct evidence for the involvement of integrins in the early phase of inflammation after SCI. Two studies have reported that early treatment with a monoclonal antibody against the CD11d integrin subunit limits the trafficking of neutrophils and macrophages into the injury site and reduces allodynia in the contusion model of SCI[125,126]. The mechanism of action of the antibody is unclear, but might involve changes in intraspinal serotonergic innervation[126].

Soon after SCI, the TNFα/CD95 (FAS) complex is secreted, and leads to the autoactivation of microglia and macrophages[127,128]. TNFα might have a double role in pain after SCI: a function in the activation of immune cells and a direct effect on pain-processing neurons (see above). A recent study showed that intrathecal administrations of CCL2 and IL-1β induce rapid activation and recruitment of macrophages and microglia in spinal cord white matter that is undergoing Wallerian degeneration[129], which might, in turn, affect pain processing. ATP is released in SCI, and blocking P2X7 receptors appears to confer some protection against injury[130], which seems to be a neuronal, rather than a microglial, effect.

Almost all of the factors that are released by immune cells and have an effect on pain after peripheral nerve injury might also affect pain processing after SCI. So, TNFα might be involved in SCI pain through the mechanism described above. Other pro-inflammatory cytokines (such as IL-1β and IL-6) and prostaglandins that are produced by COX2 and nitric oxide cannot be excluded. IL-1β could activate macrophages and microglia, which could, in turn, release pronociceptive compounds and/or directly influence pain pathways.

Another mechanism might be neuronal apoptosis. IL-1β can trigger inflammatory apoptotic outcomes through nuclear factor-κB (NF-κB) transcriptional activation, which, in turn, increases the expression of caspase 3 (REF. 131). Moreover, it has been shown that after SCI, apoptosis occurs in an important pain pathway, the spinothalamic tract, through a prompt decrease in BLC2-like protein 1 ($BCL\text{-}X_L$) levels, which are also under the control of NF-κB[132]. So, IL-1β could affect pain-signalling function through a pro-apoptotic effect on neurons of pain pathways.

Some studies have found an anti-nociceptive effect of IL-10 through decreased expression of IL-1β and iNOS in a model of excitotoxic SCI[133,134]. Furthermore, the onset of pain response is accelerated in IL-10-knockout mice[134], and IL-10 can reduce SCI-induced TNFα and improve functional recovery[135]. IL-10 belongs to the group of anti-inflammatory mediators that is discussed in BOX 3.

MS is the most common chronic inflammatory disease of the CNS in humans[136,137]. It is classically associated with demyelination and more recently with neuronal degeneration. Almost 50% of patients with MS experience significant pain at some point during the course of the disease. However, MS-associated pain has rarely been studied and our knowledge is rudimentary. Nevertheless, it is plausible that immune cell factors also contribute to MS-associated pain.

More than 90% of patients with MS have high immunoglobulin G (IgG) concentrations in the brain or cerebrospinal fluid (CSF), which is indicative of the presence of an active inflammatory reaction[137–139].

Histological studies of post-mortem material have shown that inflammatory infiltrates are present at the periphery of MS plaques, with features not dissimilar to those that are characteristic of active infection — T and B lymphocytes, mast cells, macrophages, microglia and astrocytes are all recruited and/or activated[140]. The role of these types of immune cell in the genesis of pain in MS remains largely unexplored, but the participation of microglia is an attractive possibility. It is not clear which mediators are released and how they affect the pain-processing pathways in MS. The activated immune cells can release all the pro-inflammatory cytokines that are involved in neuropathic pain of peripheral origin. It is well documented that the concentrations of cytokines are elevated in clinical cases and animals models of MS[138,139], but which of these cytokines are important in the pain caused by MS? Intriguingly, TNFα might have a more neuroprotective role in MS than in peripheral nerve injury[25]. Mahad *et al.*[139] showed that an increased concentration of CXCL10 and a decreased concentration of CCL2 in the CSF was associated with relapses in MS. Therefore, in MS, CCL2 might be neuroprotective rather than acting as a pain mediator, as it does in peripheral neuropathic pain. Recently, an interesting hypothesis has been proposed about the role of PARs in MS[28]. Elevated levels of tryptase mRNA in mast cells have been observed in patients with MS[140], which might indicate a role for PAR2 in MS-associated pain. As there are reasonable animal models of MS and tools with which to measure and block immune cells and mediators, the role of immune cell function in MS-associated pain can be tested.

Conclusions

We have reviewed the different circumstances in which immune mediators modulate pain processing and the ways in which they might do this. An important point to emerge is that immune cells can act at many anatomical levels: in peripheral tissues that are undergoing inflammation, in peripheral nerves and the spinal cord in cases of peripheral neuropathy, and, as indicated by circumstantial evidence, in some forms of SCI. Under these conditions, a wide range of immune mediators are released, some of which can affect pain signalling systems. It is important to note that immune mediators have many functions, which can be both adaptive and maladaptive to the function of the organism. The challenge, from the perspective of pain, is to exploit this knowledge to develop novel analgesic strategies that do not interfere with the other adaptive roles of these mediators. The clinically successful use of the TNFα-neutralizing strategies in the treatment of several painful inflammatory states shows that this approach is feasible.

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Competing interests statement

The authors declare no competing financial interests.

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