Review article

The neuro-immune balance in neuropathic pain: Involvement of inflammatory immune cells, immune-like glial cells and cytokines

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ABSTRACT

In a large proportion of individuals nervous system damage may lead to a debilitating chronic neuropathic pain. Such pain may now be considered a neuro-immune disorder, since recent data indicate a critical involvement of innate and adaptive immune responses following nerve injury. Activation of immune and immune-like glial cells in the injured nerve, dorsal root ganglia and spinal cord results in the release of both pro- and anti-inflammatory cytokines, as well as algesic and analgesic mediators, the balance of which determines whether pain chronicity is established. This review will critically examine the role of the immune system in modulating chronic pain in animal models of nervous system injury, and highlight the possible therapeutic opportunities to intervene in the development and maintenance of neuropathic pain.

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Damage to the peripheral or central nervous system may result from traumatic injury, surgical intervention, disease such as diabetes, or infection, and leads to an acute phase response, which is characterised by 'nociceptive pain,' inflammation, and restriction of normal function. Usually, following this acute phase, there is a recovery period of diminishing inflammation, reduced pain, healing of the injury and a return to normal function. However, in about 7% to 18% of the general population (Bouhassira et al., 2008; Toth et al., 2009) pain persists despite injury healing, resulting in a state of chronic neuropathic pain. Symptoms of neuropathic pain are often severely debilitating, such as allodynia (pain resulting from a normally non-painful stimulus), hyperalgesia (an increased response to painful stimuli), spontaneous pain, as well as behavioural disabilities (e.g., insomnia and depression). In order to study chronic neuropathic pain several animal models mimicking peripheral nerve injury have been developed, the most widely used of which are: the chronic constriction injury (CCI), most commonly, but not exclusively, of the sciatic nerve (Bennett and Xie, 1988), partial ligation of the sciatic nerve (PNL) (Seltzer et al., 1990), ligation of one or more of the spinal nerves (SNL), which contribute to the sciatic (Kim and Chung, 1992), and the spared nerve injury (SNI), where the tibial and peroneal sciatic nerve branches are ligated, but the sural nerve is left intact (Decosterd and Woolf, 2000). Despite widespread investigation, the pathobiological mechanisms of the transition from acute nociception to a state of chronic neuropathic pain are not completely understood. Much of the initial research on neuropathic pain focused on the properties of neurons following peripheral nerve injury, ultimately leading to the proposal of both peripheral and central sensitisation as important disease mechanisms. Abnormal signals from both injured and intact nociceptors result in amplification of responses to noxious and innocuous stimuli. Peripheral and central amplification is mediated by injury-induced (i) altered expression of receptors, ion channels and neurotransmitters, (ii) increased neuronal excitability and ectopic generation of action potentials, (iii) sympathetic sensory neuron coupling, (iv) neuronal cell death, (v) facilitation and disinhibition of synaptic transmission, and (vi) changes in synaptic connectivity and reorganisation of central nociceptive circuitry. It is not the aim of this article to review the mechanisms of neuropathic pain, and we encourage the reader to see the many excellent reviews on the subject (Costigan et al., 2009b; Latremoliere and Woolf, 2009; Woolf, 2004; Woolf and Salter, 2000). Although it is not disputed that neurons play a fundamental role in neuropathic pain, the strongest pain management strategies currently used, which focus on suppressing aberrant neuronal activity, lack suitable efficacy and produce undesirable side-effects (Varrassi et al., 2010).

In the last decade there has been an explosion of studies which have provided compelling evidence that neuropathic pain pathogenesis is not simply confined to changes in the activity of neuronal systems, but involves interactions between neurons, inflammatory immune and immune-like glial cells, as well as a raft of immune cell-derived inflammatory cytokines and chemokines. Indeed, peripheral nerve injury provokes a reaction from the immune system (e.g., infiltration of inflammatory cells, activation of resident immune cells) which has been observed at various anatomical locations including the injured nerve, the dorsal root ganglia (DRG), the spinal cord and supraspinal sites associated with pain pathways. The aim of this review therefore, is to critically evaluate the key findings which support the notion of neuropathic pain as a neuro-immune disorder. It is also our intention to highlight the therapeutic potential of targeting immune and glial cell responses as well as cytokines and chemokines, following neuropathic injury, in order to interrupt the often self-perpetuating neuro-immune processes which occur during the development of chronic neuropathic pain. An important factor in the longevity of pain following nerve injury is of course the balance between the immune system pro-inflammatory and anti-inflammatory mechanisms. Indeed, tipping the balance in favour of the potentially neuroprotective anti-inflammatory processes may provide a novel therapeutic opportunity to disrupt development of chronic neuropathic pain, a possibility that will be discussed in more detail below.

2. Innate immune response

2.1. Complement

One of the initial immune responses to eliminate microbes and prevent infection is brought about by the complement system. The classical, alternative, and lectin pathways can all lead to activation of the complement system, culminating in the production of C3 convertase, which generates its main effector molecules; the opsonin C3b, which drives ingestion of pathogens by phagocytes; the membrane attack complex (MAC) (C5b-9) responsible for bacterial cell lysis; and the anaphylatoxins C3a and C5a, which are leukocyte chemoattractants (Walport, 2001a,b). Complement activation is essential for host defence against pathogens, however its inappropriate activation can cause severe tissue damage, for instance in demyelinating neuropathies such as multiple sclerosis and Guillain-Barré syndrome (Kerns et al., 2002; Pentland and Donald, 1994). Activated components of the complement pathway have been found following post traumatic neuramomas and crush injury in peripheral nerves, indicating a crucial role of complement activation in the process of myelin clearance following injury, a prerequisite for nerve regeneration (de Jonge et al., 2004). In a model of sciatic inflammatory neuropathy, where the immune system was activated using zymosan, the complement system was found to play a role in pain hypersensitivity, since perisomatic application of the soluble complement receptor-1 (sCR-1) reversed allodynia (Twining et al., 2004). In a more recent study by Li et al. (2007), increased C3 deposition indicative of complement activation, was found in the sciatic nerve as little as 6 h following PNL. Furthermore, inhibition of complement activation by sCR1 led to a decrease in C3 deposition, which not only attenuated pain hypersensitivity for up to 4 weeks, but was associated with a decrease in infiltration of macrophages and T cells to the sciatic nerve (Li et al., 2007). Recently, upregulation of C3 expression following peripheral nerve injury has been found to correspond with a decrease in expression of the cell surface inhibitor of C3, decay accelerating factor (DAF), a natural regulator of complement activation in naive animals (Levin et al., 2008).

Complement activation has also been identified in the DRG following peripheral nerve injury (Levin et al., 2008; Vega-Avelaira et al., 2009). Within the ipsilateral DRG increased C3b expression is predominantly
located on satellite glial cells and infiltrating macrophages, whilst there is decreased DAF expression on the surface of neurons, increasing their susceptibility to complement attack (Levin et al., 2008). Spinal cord activation of several complement components has been identified in several neuropathic pain models (Griffin et al., 2007; Levin et al., 2008), the most important of which appears to be C5a, and its receptor C5aR. Indeed, intraplantar injection of C5a elicited both heat and mechanical hyperalgesia and C nociceptors were sensitised to heat following exposure to C5a (Jang et al., 2010). Intrathecal application of C5a induced pain hypersensitivity in naïve animals, whilst rats administered with C5a antagonists and C5a−/− mice had diminished pain following nerve injury (Griffin et al., 2007). Interestingly, no deposition of MAC was detected in rat injured nerves (Li et al., 2007) and C6−/− mice (which cannot form MAC), had no change in neuropathic pain following nerve injury (Griffin et al., 2007), suggesting no role for the MAC in pain hypersensitivity. The anti-GD2 ganglioside antibody is an effective treatment for neuroblastoma, however it is associated with generalised, relatively opiate-resistant, pain. This pain has been linked to activation of the classical complement cascade, resulting in the formation of C3a and C5a, as well as MAC (Sorkin et al., 2010). In contrast to Griffin et al., 2007, in a model of allodynia induced by anti-GD2 ganglioside antibody, C6−/−deficient rats (which cannot form MAC), had a significant reduction in the anti-GD2-induced pain behaviour (Sorkin et al., 2010). This study also demonstrated that pre-treatment with C5a receptor antagonist completely abolished the anti-GD2 induced allodynia (Sorkin et al., 2010), further supporting an important role for C5a. Additionally, intrathecal administration of sCR1 reduced pain hypersensitivity, presumably by inhibiting production of C5a (Twinning et al., 2005). Given anaphylatoxins, such as C5a, are likely to play significant roles in the migration and adhesion of leukocytes to injured nerves, DRGs and the spinal cord, drugs like the soluble human complement receptor type 1, TP10, may be efficacious in clinical neuropathic pain. TP10 has already been tested in phase II clinical trials to treat complications of cardiopulmonary bypass surgery (CPB), which are associated with complement activation, such as chest pain, myocardial infarction and mortality (Lazar et al., 2004). TP10 was well-tolerated by CPB patients, decreasing mortality and morbidity without any adverse effects.

Complement activation has been associated with macrophage and microglial activation, as well as regulation of T cell responses, during both induction and effector phases of the immune response (Dailey et al., 1998; Kemper and Atkinson, 2007). Taken together, the complement system is involved in essential regeneration and remyelination, but also early activation of immune cells, which through the release of inflammatory mediators increase pain sensitivity following peripheral nerve injury. Therefore, interference of the complement cascade may well provide a novel method to attenuate pain hypersensitivity, and drugs such as TP10 could offer relief from clinical neuropathic pain.

2.2. Mast cells

Mast cells are generally considered to be critical effector cells in allergic disorders, however they are also important initiators and effectors of innate immunity, and are resident in many tissues, including nerves (reviewed by Galli et al., 2005; Metcalfe et al., 1997). It has been demonstrated that mast cells are degranulated at the site of a nerve lesion (Olsson, 1967; Zuo et al., 2003), releasing mediators such as histamine, serotonin, proteases, prostaglandins and cytokines (Galli et al., 2005). Exactly how mast cells are activated by nerve injury has not been demonstrated, however it could be through mechanical damage or increased levels of adenosine or bradykinin at the injury site (McLean et al., 2000; Sawynok et al., 2000). Several mast cell mediators have the ability to sensitize nociceptors, including histamine (Herbert et al., 2001; Mizumura et al., 2000), and tumour necrosis factor-α (TNF) (Sorkin et al., 1997), resulting in increased firing rates; whilst serotonin can elicit hyperalgesia by direct activation of nociceptors (Rueff and Dray, 1993; Sommer, 2004). Many mast cell-derived factors also have chemotactic properties, with histamine (Yamaki et al., 1998), leukotrienes (Malaviya and Abraham, 2000), TNF (Biedermann et al., 2000) and transforming growth factor-β (TGF-β) (Wahl et al., 1993), capable of neutrophil recruitment, which on arrival contribute to the release of additional algesic and inflammatory mediators.

Experimentally it has been demonstrated following PNL, ongoing stabilisation of mast cells with cromoglycate protects them against degranulation and completely eliminates the development of hyperalgesia in rats, which was associated with a reduction in numbers of neutrophils and macrophages at the lesion site (Zuo et al., 2003). Although cromoglycate is used to treat asthma and mastocytosis (Tefferi and Pardanani, 2004), it has not been clinically evaluated in neuropathic pain. Local treatment of the nerve-injured rats with H1 and H2 histamine receptor antagonists also alleviated mechanical, and to a greater extent, thermal hyperalgesia (both established and developing), although pain was not completely eliminated, indicating that whilst important, histamine is not the only mast cell mediator involved in neuropathic pain. Antihistamines, such as H1 antagonists, diphenhydramine and hydroxyzine, have long been used in the treatment of allergic rhinitis and pruritus, and widespread clinical trials in the 1980s demonstrated their mild analgesic effects and potential use as adjuvants to treat dysmenorrhea, trigeminal neuralgia, and thalamic pain syndrome (Rumore and Schlichting, 1986). More recently, diphenhydramine in combination with opioids, has been shown to effectively treat cancer pain that was refractory to treatment (Juan et al., 2001). The mechanism of analgesia induced by antihistamines in humans however remains to be elucidated.

Since the cloning of H3 and H4 histamine receptors around 10 years ago, there has been interest in targeting these receptors in neuropathic pain. Following PNL, systemic or local repeated treatment with H3/H4 receptor inverse agonist or H4 antagonist potentiated mechanical hyperalgesia despite increased numbers of intact mast cells (reduced degranulation) in the injured nerve, whilst treatment with H4 agonist abolished mechanical hyperalgesia (Smith et al., 2007). Contrastingly, two more recent studies have found systemic injections of H4 antagonists produced dose-dependent reversal of mechanical allodynia in rats following either SNL or CCI (Cowart et al., 2008; Hsieh et al., 2010), although these effects may be central rather than peripheral. Therefore, further studies are required to elucidate the effects of H4 antagonists, before considering clinical development for neuropathic pain states. The H3 receptor antagonist, GSK189254, reduces mechanical hyperalgesia and allodynia following CCI (Medhurst et al., 2008), although it is hypothesised to be via enhanced release of monoamines in the CNS. The pre-clinical efficacy of GSK189254 has led to phase I clinical trials to assess its effectiveness against electrical hyperalgesia in healthy volunteers (Vohora, 2007).

Another important mediator is the mast cell-specific serine protease, trypsin, which is released by degranulation and has been shown to trigger inflammatory hyperalgesia and nociceptive behaviour in rats via activation of protease-activated receptor-2 (PAR-2) (Kawabata et al., 2001, 2002; Vergnolle et al., 2001).

As resident immune cells in peripheral nerves, mast cells have a role in the initial stages of inflammation and establishment of pain hypersensitivity accompanying peripheral nerve injury. Despite this, the long-term effectiveness in treating neuropathic pain by reducing mast cell degranulation is open to speculation; on the one hand it may be limited given the involvement of many other immune cell types; on the other, halting activation of one of the first pro-inflammatory immune cell types may stem ongoing immune cell recruitment and subsequent release of pro-inflammatory cytokines.

2.3. Neutrophils

Although not found in healthy nerves, neutrophils (or polymorphonuclear leukocytes) are found in high numbers close (<2 mm) to...
the site of injury and participate in the very early stages following peripheral nerve injury, peaking at 24 h (Clatworthy et al., 1995; Perkins and Tracey, 2000; Zuo et al., 2003). Neutrophil recruitment is mediated by the release of chemotactic agents, such as nerve growth factor-β (NGF-β), CXCL1, monocyte chemoattractant protein-1 (MCP-1) and lektotirenne-B4 (Johnston et al., 1999; Scholz and Woolf, 2007).

Following spinal cord injury (SCI) in rats, early treatment (only during the first 48 h) with a neutralising antibody against the integrin, α4β1, significantly decreases infiltration of neutrophils to the site of injury, as well as attenuating mechanical allodynia (Fleming et al., 2009). In addition, depletion of circulating neutrophils at the time of peripheral nerve injury significantly attenuated the induction of neuropathic hyperalgesia (Perkins and Tracey, 2000). However, following peripheral nerve injury the role of neutrophils in the DRG is uncertain; on the one hand, Morin et al. (2007) have reported a three-fold increase in numbers of neutrophils in DRGs 7 days after SCI in rats, which corresponded with an increase in mRNA for the chemotractant MCP-1. On the other hand, Hu et al. (2007) saw no neutrophil infiltration into DRGs a week following SCI and questioned the exact anatomical locations of supposed DRG invading neutrophils, suggesting that the neutrophil specific immunoreactivity of Morin et al. was limited to the surroundings of the DRGs, particularly the meninges and perineural membranes (McLachlan et al., 2007). Despite controversy over the importance of neutrophil invasion of DRGs in neuropathic pain, co-culture of resting neutrophils and dissociated DRGs, leads to rapid conversion (within 10 min) of neutrophils to an active state undergoing internal oxidative burst, as well as increasing the excitability and firing frequency of the neurons, resulting, within 24 h, in increased numbers of injured or apoptotic neurons (Shaw et al., 2008).

Neutrophils contribute to the inflammatory response by the release of superoxide and other reactive oxygen species (Nathan, 2006). Furthermore, blood-derived neutrophils obtained from nerve-injured rats displaying hyperalgesia appear to be primed, having increased superoxide burst (Morin et al., 2007). Activated neutrophils also release cytokines such as TNF, IL-1β, IL-2 and IL-6, capable of cellular toxicity and directly exciting neurons (see Section 5.1 for more details, Murata et al., 2006; Schafer et al., 2003a). Despite decreasing numbers of neutrophils following their initial peak (Zuo et al., 2003), the inflammatory process is perpetuated by the release from neutrophils of mediators chemotactic for macrophages including macrophage inflammatory protein-1α and -1β (MIP-1α and MIP-1β), IL-1β and defensins (Scapini et al., 2000; Welling et al., 1998; Witko-Sarsat et al., 2000). Interestingly, under inflammatory conditions, neutrophils have been shown to secrete opioid peptides, which bind to opioid receptors on peripheral sensory neurons and mediate antinoceception (Brack et al., 2004; Rittner et al., 2006). However, it seems clear that in neuropathic pain the pro-inflammatory effects of neutrophils, at least in the initial stages out-weigh any possible benefits, and interfering with their infiltration may diminish pain and ongoing inflammation. Despite promising pre-clinical findings, there have been no clinical attempts to block the neutrophil response in neuropathic pain, possibly due to a lack of specific pharmacological agents.

2.4. Macrophages

Macrophages are large phagocytic leukocytes found within tissues, they are produced by the differentiation of monocytes, and generally phagocytose foreign particles (i.e. microbes). In the context of peripheral or central nerve injury macrophages play a central role in Wallerian degeneration, phagocytosing the dead or dying remnants of injured Schwann cells and axonomised axons (Bruck, 1997). Following nerve injury, the resident macrophages (which themselves do not proliferate significantly), are assisted with clearance of cellular debris by a large influx of hematogenously derived macrophages (Bendszus and Stoll, 2003; Hu and McLachlan, 2003). Wallerian degeneration can be interrupted by preventing macrophage infiltration through application of neutralising antibodies for the chemokines MCP-1, MIP-1α and IL-1β (Perrin et al., 2005). The invasion process, which is considered essential for regeneration of peripheral nerves, begins slowly usually around 24 h (as it is probably dependent on neutrophil-derived chemokines), continuing for up to 3 days, and spreading to dense zones further from the site of injury (Zuo et al., 2003).

Besides a role in phagocytosis and regeneration of damaged nerves, macrophages are also implicated in pain-related behaviour. For instance, macrophage depletion by pharmacological means, such as Liposome-encapsulated clodronate, or a genetically impaired macrophage response (i.e. WLD mice), led to reduced pain hypersensitivity in several models of neuropathy (Barclay et al., 2007; Liu et al., 2000b; Mert et al., 2009). However, there are some conflicting reports where macrophage depletion did not relieve mechanical allodynia following neuropathy (Barclay et al., 2007; Rutkowski et al., 2000). At later time-points blood-derived macrophages also infiltrate the DRGs, with their numbers increasing three-fold 1 week after SCI in rats (Hu et al., 2007). Interestingly 7 days following SNI, pain-related behaviour was absent in young (P10) rats compared to adults, with only adult rats displaying ‘ring-like’ macrophages around, mostly-axotomised, A large-neurons, but not small C-neurons of injury associated DRGs (Vega-Avelaira et al., 2009). Microarray analysis of the DRGs, revealed an adult specific increase in transcription of cytokines and chemokines, which selectively attract or activate macrophages, such as IL-6, colony stimulating factor-1 (CSF-1) and MCP-1 (Vega-Avelaira et al., 2008). Following SCI, blood-derived macrophages also invade the spinal cord, which can be attenuated by application of an α4β1-intergrin neutralising antibody, a treatment associated with a long-term decrease in mechanical hyperalgesia and an increase in myelin-containing white matter (Fleming et al., 2009).

Macrophages secrete many cytokines which are potential mediators of hyperalgesia, such as TNF, IL-1β and IL-6 (Sommer and Kress, 2004). A recent study has demonstrated that perineural injection of nicotine (a suppressor of macrophage activation) in PNL-injured mice, prevented upregulation of IL-1β in the sciatic nerve, as well as development of pain hypersensitivity (Kiguchi et al., 2010). Other macrophage derived mediators implicated in pain are reactive oxygen species and prostaglands (PGs, such as PGE2 and PGL2), which sensitise primary afferents directly (Nathan, 1987). Indeed, cyclooxygenase-2, the inducible enzyme which produces PGs, is upregulated in macrophages located at injured nerves (Ma and Eisenach, 2003). Recently, the cysteine protease, cathepsin S (CatS), which can be secreted by activated macrophages (Liuuzzo et al., 1999), has been demonstrated to be upregulated in DRGs and injured nerves of both SCI and PNL-injured rats (Barclay et al., 2007). Furthermore, inhibition of CatS could reverse mechanical hyperalgesia in these animals, whilst intraplantar injection of activated CatS induced mechanical hyperalgesia (Barclay et al., 2007), however the algesic mechanism of CatS remains to be elucidated. On balance, these data point to an important role for macrophages in the genesis of neuropathic pain, through the release of both algesic and chemotactic mediators. Furthermore, macrophages appear to act later than both mast cells and neutrophils, being involved in the fundamental process of phagocytosis and Wallerian degeneration, a prerequisite for regeneration. Targeting macrophages clinically has not been attempted, probably due to the lack of specific drugs that can selectively block their pro-inflammatory and algesic action, while leaving their phagocytic function intact.

2.5. Toll-like receptors

Toll-like receptors (TLRs) are a family of pathogen recognition receptors of the innate immune system, which are expressed by multiple immune and glial cells. Recently, TLRs have been implicated
in Wallerian degeneration and nerve regeneration by macrophages and Schwann cells, as well as in mediating harmful neuroinflammatory responses, through activation of mast cells and microglia (reviewed by Aravalli et al., 2007; Kim et al., 2009a; Lehnardt, 2010).

Initially thought to be activated by pathogens alone, TLRs are now known to be activated by necrotic cells, injured axons (expressing HSP60 and HSP70), and components of the extracellular matrix (Takeda et al., 2003). Furthermore, TLR2, TLR3 and TLR4 are known to be expressed by both macrophages and Schwann cells (Kim et al., 2009a; Lee et al., 2006), which, as has been mentioned above, are critical in the process of Wallerian degeneration. In TLR2−/− and TLR4−/−, as well as mice deficient for the downstream adaptor protein MyD88, there was a reduction in IL-1β and MCP-1 expression, associated with a reduction in macrophage recruitment to the sciatic nerve distal stump following microcrush injury (Bovin et al., 2007). Furthermore, these TLR-deficient mice had compromised Wallerian degeneration, axonal regeneration, and impaired recovery of locomotor function. In contrast, animals that received a single microinjection of TLR2 and TLR4 ligands at the injury site had faster clearance of the degenerating myelin and accelerated motor recovery (Bovin et al., 2007). There is also evidence of a pro-inflammatory response mediated through TLRs, given that cultured rat Schwann cells increase glial activation in the spinal cord, as well as inducing pain hyperalgesia following injection of TLR2 and TLR4 ligands at the injury site had faster clearance of the degenerating myelin and accelerated motor recovery (Bovin et al., 2007).

In a seminal study by Tanga et al. (2005), TLR4, expressed by microglia and normally involved in the recognition of bacterial endotoxin and lipopolysaccharide (LPS), was demonstrated as having a causative role in neuropathic pain. Indeed, interruption of TLR4 signalling in a knockout-mouse or antisense-knockdown rat, resulted in attenuation of pain hypersensitivity in both the development and maintenance phases, following spinal nerve transaction (Tanga et al., 2005). These improvements in pain behaviour corresponded to decreased expression of spinal microglial markers and pro-inflammatory cytokines. More recently, it has been reported that repeated administration of a potent TLR4 antagonist, FP-1, following CCI in mice, resulted in relief of thermal hyperalgesia and mechanical allodynia (Bettoni et al., 2008). In support of a role of the TLRs in spinal neuroinflammation, two further knockout studies in SNL-injured mice, have demonstrated that both TLR2 and TLR3 mediate glial activation in the spinal cord, as well as inducing pain hypersensitivity (Kim et al., 2007; Obata et al., 2008).

The mechanism of microglial-TLR4 activation in neuropathies has recently been elaborated to include the accessory protein, CD14, which is a pathogen recognition molecule expressed by microglia, serving as a carrier linking LPS to cell surface TLR4 (Miyake, 2004). Indeed, CD14−/− mice, displayed significantly reduced mechanical allodynia and thermal hyperalgesia following spinal nerve transaction (Cao et al., 2009b). These investigators also demonstrated that there was increased surface expression of CD14 by lumbar spinal cord microglia (Cao et al., 2009b). Similar accessory proteins may well be associated with signalling through TLR2 and TLR3, although to date none have been investigated.

The widespread role of TLRs in pain pathobiology and the positive outcomes of pre-clinical studies with FP-1, combined with the fact ES564 (another TLR4 antagonist) is well-tolerated in humans (Tidswell et al., 2010), has led to the emergence of TLRs as potential drug targets to treat clinical neuropathic pain. However, alleviation of neuropathic pain by TLRs may well require careful consideration of both temporal and spatial dimensions. For example, initial stimulation of TLRs peripherally to promote Wallerian degeneration and nerve regeneration by invading macrophages and Schwann cells may be desirable; whilst ongoing inhibition of spinal cord TLR signalling may be beneficial to reduce activation of microglia and astrocytes, and thus prevent their trademark release of algesic pro-inflammatory cytokines. In order to specifically target TLRs expressed by different immune or glial cell types it will be necessary to elucidate any specific differences in their signalling.

3. Adaptive immune response

3.1. T lymphocytes

T lymphocytes, which play a central role in cell-mediated immunity and B lymphocytes, primarily responsible for antibody production, are the major cellular components of the adaptive immune response. T lymphocytes themselves are a very heterogeneous group, divided into helper T (Th) cells, cytotoxic T (Tc) cells, and regulatory T cells (Tregs), with several subpopulations of each type. Initially T+ cells were identified at the injury site in three models of neuropathic pain (Cui et al., 2000). T lymphocyte infiltration into injured nerves was then confirmed to occur by 3 days, peaking at day 21, and decreasing thereafter (Moalem et al., 2004). Moalem et al. established a role for T+ cells in neuropathic pain, demonstrating that athymic nude rats that lack functional T+ cells develop significantly less mechanical allodynia and thermal hyperalgesia following CCI, compared to their heterozygous littermates (Moalem et al., 2004). These data have since been confirmed in both nude and Rag1−/− mice, which, unlike wild types, displayed a significant reduction in mechanical hyperalgesia after SNL or SNI (Cao and DeLeo, 2008; Costigan et al., 2009a). The co-inhibitory molecule B7-H1, which binds the inhibitory programmed death receptor-1 (PD-1) on T cells, has recently been identified as an essential anti-inflammatory mediator, with an increase in T cell and macrophage infiltration into lesioned sciatic nerves in B7-H1−/− mice (Uceyler et al., 2010). Following sciatic nerve transection or ligation, T cell infiltration into DRGs as well as the spinal cord has also been reported (Cao and DeLeo, 2008; Hu and McLachlan, 2002; Sweitzer et al., 2002). Spinal infiltration of T cells peaked after 7 days (Cao and DeLeo, 2008), potentially a key time-point in the development of pain chronicity, with T cells interacting with activated glia to exacerbate inflammation and sensitisation of neurons.

The functions of the Th cell subpopulations Th1, Th2, and Th17 are based on their distinctive cytokine profiles (reviewed by Palmer and Weaver, 2010). Th1 cells produce predominantly pro-inflammatory cytokines, such as IFN-γ and TNF, which support cellular immunity, whilst Th2 cells produce anti-inflammatory cytokines, such as IL-4, IL-5, IL-10 and IL-13, which mediate humoral immunity (Mosmann and Sad, 1996). Hence, Th1 and Th2 cytokines can be considered mutually inhibitory for the functions of the reciprocal phenotype. Following CCI, adoptive transfer of Th1 cells into nude rats increased their pain sensitivity to a level comparable with that of heterozygous rats, conversely, passive transfer of Th2 cells into heterozygous rats significantly reduced their pain sensitivity (Moalem et al., 2004). Furthermore, CD4+ T cell (presumably predominantly Th1) adoptive transfer into CD4−/− mice abrogated the reduction in mechanical hyperalgesia following spinal nerve transection, returning their pain hypersensitivity to the level of wild types (Cao and DeLeo, 2008). Following SNL, the absence of spinal infiltration of T cells and mechanical hyperalgesia has been demonstrated in neonatal rats (less than P21) compared to adults, this coincided with expression of IFN-γ, a critical pro-inflammatory mediator released by Th1 cells (Costigan et al., 2009a). Interestingly, in neonates, although T helper populations are limited, they have a larger Th2 population (Morein et al., 2007), which may account for the discrepancy of pain responses compared to adults. Recently, a new helper T cell population, Th17 cells, which produce the potent pro-inflammatory cytokine IL-17, have been implicated in pain. In CCI-mice, T cells at the injury site
were found to express IL-17, with IL-17 expression peaking at 7 days, whilst Rag1 mice failed to express IL-17 in the injured nerve (Kleinschnitz et al., 2006). It seems likely then, that Th1 cytokines, IFN-γ and TNF, and Th17-derived IL-17, are central to the increased pain sensitivity in neuropathic pain, whilst Th2-derived cytokines, IL-4 and IL-10, maybe protective.

The above findings are in line with reports of pain hypersensitivity in animal models of T cell-mediated autoimmune diseases of the peripheral and central nervous systems. For example, rats with experimental autoimmune neuritis (EAN), an inflammatory demyelinating disease of the peripheral nervous system, developed significant thermal hyperalgesia and mechanical allodynia during the course of the disease (Moalem-Taylor et al., 2007). In experimental models of multiple sclerosis, mice with experimental autoimmune encephalomyelitis (EAE) developed thermal hyperalgesia during the chronic phase of the disease (Aicher et al., 2004) and both tactile and cold allodynia, which emerged early in the disease process, often before any signs of neurological deficits (Olechowski et al., 2009). These neuropathic pain symptoms coincided with a significant influx of T cells into the peripheral nerves in EAN (Moalem-Taylor et al., 2007) and the dorsal horn of the spinal cord in EAE (Olechowski et al., 2009). However, the role of T cells in mediating behavioural hypersensitivities observed in these autoimmune diseases remains unclear.

Although most studies highlight a role for T cells in enhancing neuropathic pain, a recent study highlights the analgesic effect of β-endorphin-containing T cells following nerve injury (Labuz et al., 2010). The authors demonstrate that T cells expressing β-endorphin and receptors for corticotrophin-releasing factor (CRF) are present in wild type but not severe combined immunodeficiency (SCID) mice at the damaged nerve. SCID mice had substantially reduced CRF-mediated anti-nociception that was fully restored by transfer of wild type mice-derived T cells (Labuz et al., 2010). Thus, it appears that T cells can also play a beneficial role in painful peripheral nerve injury by releasing endogenous opioids, although it is yet to be established which T cell subpopulation produces β-endorphin.

In summary, these findings indicate that T cells have an important role in neuropathic pain at various levels of the nervous system, particularly in the establishment of pain chronicity (days 3–21), and therefore may make a good target in the management of clinical neuropathic pain. Clearly, the balance between the production of pro-inflammatory cytokines by Th1 and Th17 cells, anti-inflammatory cytokines by Th2, and endogenous opioids, is a perilous one, determining how the adaptive immune system will influence pain sensitivity. As such, a novel approach to tip the balance in favour of the nervous system, being central to many metabolic and immune functions, as well as modulating neuronal activity, through uptake and release of transmitter substances. In the peripheral nervous system, glia are represented by Schwann and satellite cells, while in the CNS, three types of glial cell exist; astrocytes, oligodendrocytes (macroglia) and microglia. Initial evidence that glia contributed to neuropathic pain emerged in the mid 1990s, however the correlation between their activation and pain behaviour was not always robust (Colburn et al., 1997, 1999).

4.1. Schwann cells

Schwann cells are the glia of the peripheral nerves, under physiological conditions they provide trophic support, maintain the local environment, as well as providing myelin sheath to large myelinated axons and enveloping small bundles of unmyelinated C-fibre axons (Remak bundles). In injured nerves however, Schwann cells undergo a phenotypic switch, regaining the ability to proliferate, migrate and secrete numerous mediators that contribute to Wallerian degeneration and nerve regeneration (reviewed by Campana, 2007). In fact during the initial 24 h following injury, before the arrival of blood-derived macrophages, Schwann cells control demyelination by degrading myelin basic protein, through a matrix metalloproteinase (MMP)-dependent pathway, which contributes to early pain hypersensitivity. Indeed, following L5 spinal nerve crush in MMP−/− mice or in rats treated with the MMP inhibitor GM6001, there was a significant reduction in mechanical allodynia (Kobayashi et al., 2008a). Interestingly, MMP9 also promotes macrophage recruitment (Shubayev et al., 2006), possibly to assist in the demyelinating process (Rosenberg, 2002). As a result of compromised myelin integrity of Aδ afferents, these normally mechano-sensitive fibres promote mechanical nociception (Devor, 2006).

Additionally within hours of the peripheral nerve injury activated Schwann cells begin to secrete other mediators including; pro-inflammatory cytokines, TNF (Murwani et al., 1996; Wagner and Myers, 1996), IL-1β (Bergsteinsdottir et al., 1991; Shamash et al., 2002) and IL-6 (Bolin et al., 1995); PGE2 (Muja and DeVries, 2004); ATP (Liu et al., 2005); and chemokines, such as leukemia inhibitory factor (ILF) and MCP-1, which promote continued neuroinflammation through recruitment of macrophages (Tofaris et al., 2002). TNF and IL-1β can directly stimulate neuronal hyperexcitability of A- and C-fibres (see Section 5.1 for more details, Schafer and Sorkin, 2008), whilst ATP can sensitize nociceptors and increase axonal excitability in peripheral nerves (Moalem et al., 2005).

Whilst trophic factors are released by Schwann cells under normal circumstances, following nerve damage they upregulate release of nerve growth factor-β (NGF-β, Lindholm et al., 1987; Matsuoka et al., 1991), glial cell line-derived neurotrophic factor (GDNF) (Hammberg et al., 1996), and BDNF (Tonra et al., 1998). NGF-β, a survival and differentiating factor for neural crest-derived sensory neurons and sympathetic neurons, has been shown to modulate expression of a multitude of pain-related transmitters, receptors, and ion channels (i.e. substance P, (Gilchrist et al., 1991; Lindsay and Harmar, 1989), vanilloid receptor, TRPV1 (Winston et al., 2001) and sodium channels (Friedel et al., 1997)). Furthermore, it has long been known that administration of NGF-β can induce hyperalgesia and allodynia, in both rats and humans (Lewin and Mendell, 1994; Petty et al., 1994). NGF-β antagonism has been shown to prevent hyperalgesia and allodynia from developing when given prior to nerve injury in rats (Ramer and Bisby, 1999; Ro et al., 1999). A more recent study has demonstrated anti-NGF antibody treatment 1 to 2 weeks after nerve injury could completely reverse established tactile allodynia and thermal hyperalgesia following CC1 in mice, as well as in SNL-injured rats (Wild et al., 2007). Following these encouraging results from animal models an anti-NGF antibody, Tanezumab (RN624), is in phase II clinical trials for several pain entities, including osteoarthritis knee...
pain, chronic lower back pain, and diabetic neuropathy. Early indication from one study suggests a single intravenous infusion of Tanezumab results in improved analgesic efficacy in patients with chronic lower back pain compared to the non-steroidal anti-inflammatory, naproxen (Katz et al., 2009). Recently an erythropoietin (Epo)-mediated neuroprotective role has been proposed for Schwann cells. Indeed, following sciatic nerve CCI in rats, systemic Epo treatment limited production of Schwann cell-derived TNF, and was associated with reduced axonal degeneration and facilitated recovery (Campana et al., 2006).

In summary, Schwann cells appear to contribute to neuropathic pain by sensitising or activating the axons of nociceptors, through inflammatory cytokines and NGF-β. Despite this however, the contribution of Schwann cells may not be wholly negative, with potential antinociceptive and protective influences such as release of Epo and GDNF (refer to Section 4.4 for more details on GDNF). Therapeutic agents like the anti-NGF antibody, Tanezumab, and the MMP inhibitor, GM6001, which interfere with Schwann cell nociceptive responses, may prove effective in treatment of neuropathic pain in humans.

4.2. Satellite glial cells

Satellite glial cells (SGCs) are peripheral glia which ensheath neuronal cell bodies within ganglia (i.e., those found in the DRG). They support normal sensory transmission and nociception by maintaining metabolic and ionic homeostasis. In models of neuropathy and peripheral inflammation, SGCs become activated and proliferate (Lu and Richardson, 1991), resulting in increased expression of GFAP and release of pro-inflammatory cytokines, such as TNF and IL-1β (Dubovy et al., 2006; Takeda et al., 2007). Following nerve injury, ATP released from damaged neurons is believed to be one of the critical mediators involved in activation of SGCs through stimulation of purinergic receptors (reviewed by Ohara et al., 2009). In SNI-injured rats, GFAP expression by SGCs peaked after 1 day and was still present on day 10, furthermore DRG nerve blockade with sodium channel inhibitors attenuated activation of SGCs (Xie et al., 2009), indicating their activation is driven by changes in neuronal activity.

Following peripheral nerve injury, levels of NGF-β in the DRG decrease owing to its lack of transport from damaged axons (Lee et al., 1998; Xie et al., 2009). In response to this, several days following injury, SGCs begin to synthesize NGF-β, which is maintained for up to 2 months (Lee et al., 1998; Zhou et al., 1999). As previously mentioned, NGF-β promotes survival and differentiation of neurons, and enhances painful responses (refer to Section 4.1). In a model of inflammatory pain, IL-1β released by activated SGCs has been demonstrated to increase ectopic firing of Aβ fibres of trigeminal ganglion (TRG) neurons, resulting in mechanical hyperalgesia (Takeda et al., 2008). A recent study has demonstrated that autologous application of nucleus pulposus (normally associated with lower back pain and sciatica) to the DRG of naïve rats induces mechanical hyperalgesia which lasted for 4 weeks, a time-course which paralleled activation of SGCs (Otoshi et al., 2010). These activated SGCs displayed an initial increase in TNF expression, coinciding with the development and maintenance of pain hypersensitivity. Additionally, there was a prolonged increase in GDNF expression by SGCs, which may be associated with the recovery of neuronal function and reduction in pain hypersensitivity (Otoshi et al., 2010).

Vit et al. (2008) demonstrated the importance of potassium ion buffering by SGCs, given that RNA silencing of the glia-specific inward rectifying K+ channel (Kir4.1) in the TRG led to spontaneous and evoked facial pain in naïve rats. Moreover, in a model of facial neuropathic pain, expression of Kir4.1 was reduced in the TRG (Vit et al., 2008). Suppression or ablation of Kir4.1 not only leads to dysfunction in potassium homeostasis, but also reduces glutamate uptake by glial cells (Djukic et al., 2007; Kucheryavyykh et al., 2007). Therefore, neuropathic pain may result from deviations in normal potassium and glutamate buffering by SGCs, both of which likely contribute to neuronal hyperexcitability. Another fundamental change in SGC function, following nerve injury, is the increase of the connexin-43 (Cx43) subunit of gap junctions (Ohara et al., 2008; Vit et al., 2006). Cx43 is believed to enhance the intercellular passage of pain mediators, as well as promoting glial activation. However, RNA silencing of Cx43 in TRGs has led to conflicting results, being analgesic following infraorbital nerve constrictions, but evoking spontaneous pain in naïve animals (Ohara et al., 2008; Vit et al., 2006). In conclusion, activated SGCs have an emerging role in establishment of neuropathic pain, by enhancing neuronal firing through both direct (IL-1β) and indirect (potassium and glutamate) mechanisms. Conversely, SGC release of neurotrophic factors, such as GDNF, may be antinociceptive, contributing to the resolution of persistent pain. Owing to a lack of specific pharmacological tools, preventing activation of SGCs may be difficult, although modulating purinergic receptors may well have widespread benefits on pain, through effects on several glial types.

4.3. Microglia

Microglia are a population of normally quiescent resident macrophages, which perform immune surveillance in the CNS, and constitute only 5–10% of total CNS glia. Once activated they can act as phagocytes (Vilhardt, 2005), present antigen to T lymphocytes, and release a plethora of mediators that includes many pro-inflammatory cytokines (Piehl and Lidman, 2001). Despite being derived from myeloid cells, and therefore forming part of the innate immune system, microglia have been discussed here given that they share similar sites of activation and responses with other glial cell types, particularly astrocytes. Microglia can be activated by a wide variety of mediators, released from both neurons, and other glia, including; cytokines (IL-6 and IFN-γ, refer to Section 5.1), chemokines (refer to Section 6.2), ATP, glutamate, and neuropeptides (substance P, calcitonin gene-related peptide (CGRP)). Microglial activation is characterised by the expression of several markers; type 3 complement receptors CR3 or CD11b (OX42), ionized calcium binding adapter protein (IBA1), cluster determinant 14 (CD14) and TLR4 (McMahon et al., 2005), as well as activation of the p38-mitogen activated protein (MAP) kinase pathway (Terayama et al., 2008).

In the context of neuropathic pain, microglia are one of the first spinal cord cell types to be activated within 4 h of peripheral nerve injury (Tanga et al., 2004), which continues for at least several months in experimental neuropathies (Clark et al., 2007; Coyle, 1998). Indeed, following SNI, Jin et al. (2003) observed a rapid activation of microglia, with p38-inhibition reversing this activation (most effectively during the first 10 days), resulting in attenuation of mechanical allodynia. At around the same time Raghavendra et al. (2003) directly inhibited microglial activation, using minocycline, which effectively abrogated mechanical allodynia and hyperalgesia following SNI, but only when given pre-emptively, having no effect on established pain behaviour. Contrastingly, a more recent study from the same laboratory described a role for microglia in long-term maintenance of allodynia, which could be alleviated pharmacologically with propentofylline 4–6 weeks post-injury (Tawfik et al., 2007). Many other investigators have since blocked microglial activation in various animal models of neuropathy, spinal cord injury and post-operative pain, confirming attenuation of both developing and established pain hypersensitivity (Clark et al., 2007; Ledeboer et al., 2005b; Marchand et al., 2009; Obata et al., 2006, 2007). In addition, mirror image pain involving the contralateral spread of pain, was reversed by intrathecal administration of fluorocitrate, a glial metabolic inhibitor (Milligan et al., 2003) possibly due to decoupling of spinal gap junctions which prevents activation of distant glia and release of pro-inflammatory
cytokines (Spataro et al., 2004). A study by Cao et al. (2009b) has recently reported a morphological change in spinal cord microglia collected 3 days following spinal nerve transection, reporting increased size and granularity, indicative of a switch from a resting state to a reactive phenotype. In an interesting study, Zhang et al. (2007) described the ability of hematogenous macrophages to infiltrate the spinal cord, proliferate, and differentiate into microglia following PNL in mice. Furthermore, the authors went on to demonstrate that there was prevention of bone marrow-derived microglial infiltration into the spinal cord in CCR2−/− mice, or rats treated with an MCP-1 neutralising antibody, both of which were associated with a reduction in pain hypersensitivity (Zhang et al., 2007). Thus, a critical role for microglia in the earliest phase of pain hypersensitivity, as well as ongoing pain has been established.

Microglial activation mediated by extracellular ATP (as well as ADP and UTP) through purinergic P2 receptors is an area of huge interest in current pain research (reviewed by Tsuda et al., 2010). Astrocytes release ATP in response to various stimuli or even spontaneously, allowing communication with microglia and neurons (Abbraccio and Cerutti, 2006; Inoue et al., 2007). Hence, astrocytes are likely source of ATP in the spinal cord following nerve injury, and consequently activation of microglia by the purinergic system may not be at its greatest until several days after injury, when astrocyte activation peaks. Nevertheless, the following receptors have been demonstrated to activate microglia and contribute to pain hypersensitivity in models of neuropathy; the ionotropic receptors, P2X4 (Coull et al., 2005; Tsuda et al., 2003, 2008) and P2X7 (Chessell et al., 2005; Clark et al., 2010); as well as the metabotropic, P2Y12 (Kobayashi et al., 2008b; Tozaki-Saitoh et al., 2008). Additionally, many investigators have been quick to demonstrate the potential of inhibiting purinergic receptors, with P2X7 (A-438079) and P2Y12 (MR2395 and AR-C69931MX) antagonists alleviating pain hypersensitivity in animals (Clark et al., 2010; Kobayashi et al., 2008b; McGaraway et al., 2007; Tozaki-Saitoh et al., 2008). These pre-clinical findings, combined with the fact drugs such as the P2Y12 antagonist, clopidogrel, are already used clinically for prevention of blood clots (Savi and Herbert, 2005), highlight the therapeutic potential of targeting the purinergic system to dampen down microglial pro-inflammatory processes in clinical neuropathic pain.

Cannabis has long been known to have antinociceptive properties, alongside its psychoactive effects. Developments in the endocannabinoid field have uncovered two endocannabinoid receptors, CB1 which is widely expressed throughout the brain, and CB2 which is mainly expressed by immune cells (Galliègue et al., 1995). Over expression of CB2 or stimulation with CB2 agonists, JWH-015 and NESS400, led to the attenuation of pain hypersensitivity in several animal models of neuropathy, in parallel with a decrease in microglial activation and an increase in anti-inflammatory cytokines (Luongo et al., 2010; Racz et al., 2008b; Romero-Sandoval et al., 2008b). CB2 downstream signalling has since been demonstrated to suppress neuropathic pain through interference in IFN-γ-signalling, preventing activation of microglia (Racz et al., 2008a). Therefore, modulation of endocannabinoid system, particularly by CB2 agonists, which may be associated with fewer central side-effects than CB1 agonists, may offer a novel therapeutic system to suppress spinal microglial activation in neuropathic pain patients.

A novel mechanism of microglial activation in pain states has recently been hypothesised, involving the communication of infiltrating T cells and microglia, through a CD153–CD40 interaction. Thus far however, there is only circumstantial evidence for this, with increased CD40 expression in the dorsal horn following spinal nerve transection, and reduced pain hypersensitivity in CD40−/− mice (Cao et al., 2009a).

Activation of microglia, and consequent release of mediators, at the first synapse between the primary afferent fibres and second-order nociceptive neurons in the dorsal horn of the spinal cord, is central to the pain enhancing role of microglia. Microglia release a plethora of pro-inflammatory mediators such as; IL-1β (Chauvet et al., 2001), TNF (Hide et al., 2000), IL-6 (Shigemoto-Mogami et al., 2001), NO (Martucci et al., 2008), and PGE2 (Svensson et al., 2003), many of which enhance activation of spinal neurons (Hanisch, 2002; Watkins et al., 2001). In a recent study by Clark et al. (2010), ATP activation of P2X7 was demonstrated as a requirement for microglial release of IL-1β, being aborted in P2X7−/− mice and by P2X7 antagonists. Interestingly, IL-1β release could only occur if microglia had first been primed by LPS, through a TLR4-dependent pathway (refer to Section 2.5 for more detail).

Coull et al. (2005) were the first to demonstrate the trophic factor, BDNF, released from activated microglia can be pronociceptive, causing a depolarising shift in spinal neurons which inverts, normally inhibitory, currents mediated by GABA. Following SNI, Okubo et al. (2010) have recently described an increased expression of three genes associated with leukotriene (LT) synthesis in microglia, thus implying activation of microglia results in the induction of inflammatory LTs. Furthermore, intrathecal administration of LT signalling blockers (AA-861 and pranlukast) suppressed the mechanical allodynia induced by SNI. Drugs of this kind are already in clinical use for the treatment of asthma and allergic rhinitis (Scow et al., 2007) and may be effective to treat neuropathic pain in humans.

Following peripheral nerve injury, in addition to sites in the spinal cord, microglial activation has also been seen at supraspinal sites. In a model of trigeminal neuropathy, rapid (1 day) activation of microglia was observed in the rostral ventromedial medulla (RVM), although this was transient, disappearing after 2 weeks (Wei et al., 2008). In PNL-injured rats, there was an increase in CD11b+ microglia in the hypothalamus and the midbrain periaqueductal gray (PAG) (Takeda et al., 2009). Furthermore, systemic pre-treatment with the glutamate antagonist, memantine, prevented microglial activation in both the hypothalamus and PAG, as well as attenuating mechanical allodynia (Takeda et al., 2009). Therefore, excessive glutamate release, from ectopic neuronal firing of ascending pathways, may be responsible for microglial activation. At this early stage of investigation the behavioural consequences of microglial activity in the brain is far from clear, however it is worth mentioning that both the PAG and RVM are components of the descending nociceptive system, which under physiological conditions control pain transmission in the spinal cord.

Despite the wealth of evidence supporting a pronociceptive and pro-inflammatory role of activated microglia, some endogenous protective mechanisms have also emerged. For example, in response to the anti-inflammatory cytokine IL-4, microglia can release insulin growth factor-1 (IGF-1) (Butovsky et al., 2005), which is associated with neuronal cell survival and renewal. More interesting still, in certain conditions the Th1 pro-inflammatory cytokine, IFN-γ, can promote glutamate buffering by microglia (Shaked et al., 2005), which is protective against glutamate-mediated excitotoxicity. In response to ischemia, microglia have been reported to release the protective neurotrophic factor, GDNF, providing microglia-derived protection to co-cultured astrocytes (Lee et al., 2004). In contrast to this, a recent study suggests GDNF release becomes suppressed when microglia are activated by LPS (Matsushita et al., 2008), indicating some activated microglial phenotypes may lose their protective functionality. However, to date there are no reports of protective actions of microglia in neuropathic pain states.

In summary, a combination of microglial-derived mediators initially contribute to a pro-inflammatory environment at the first pain synapse in the spinal cord, which then persists beyond the original nerve injury, spreading to remote sites, resulting in the development of pain hypersensitivity, as well as long-term maintenance of pain. There are however some exciting possibilities which can specifically suppress microglial activation, pushing them into a quiescent anti-inflammatory state. In particular, modulation of the
purinergic and endocannabinoid systems may have clinical efficacy in chronic neuropathic pain patients, as they do in animal models of neuropathy.

4.4. Astrocytes

Astrocytes represent the largest cell population in the CNS. Through the expression of numerous transporter proteins, astrocytes are able to maintain homeostasis by regulating extracellular ions, protons and neurotransmitter concentrations in their microenvironment. Astrocyte activation is morphologically characterised by hypertrophy and increased production of intermediate filaments, glial fibrillary acidic protein (GFAP), vimentin and/or nestin, and functionally by increased production of a variety of pro-inflammatory substances (Watkins and Maier, 2003). In models of acute pain, spinal astrocyte activation (like microglia), occurs immediately following paw incision, and dissipates within 7 days in parallel with allodynia (Romero-Sandoval et al., 2008a). A recent immunohistochemical study comparing the absolute numbers of activated astrocytes and microglia in the dorsal horn of the spinal cord, found that 1 week following CCI, the number of astrocytes increased modestly (8%), whilst the increase in microglia was more pronounced (20%) (Mika et al., 2009). Following peripheral neuropathy, astrocytic activation occurs later than microglial, around 4 days post-injury, suggesting its role in the maintenance of neuropathic pain (Colburn et al., 1999; Chilardi et al., 2004). A role in pain maintenance for activated astrocytes was recently confirmed, since GFAP−/− mice responded to peripheral nerve injury with normal development of pain hypersensitivity, however the duration was shortened. Furthermore, GFAP antisense treatment in neuropathic-rats reversed established pain 6 weeks after injury (Kim et al., 2009b).

Three months following peripheral nerve injury activated astrocytes are still present in the spinal cord (Coyle, 1998). A recent study has shown that the number of activated astrocytes at 3 months post-injury is inversely correlated with pain hypersensitivity (Deumens et al., 2009). The authors explain this unusual finding through the possibility that astrocytes switch from a pronociceptive to an antinociceptive phenotype as chronicity develops, however this finding should be treated with caution as the study was relatively small in animal number. Indeed, the protective role of glia was also the subject of a recent review (Milligan and Watkins, 2009). However, evidence for an antinociceptive and/or anti-inflammatory role for astrocytes at the cellular level is currently lacking, although an improvement in pain hypersensitivity may relate to the return to normal function of astrocytes in removing excess glutamate from the synapse.

A potential antinociceptive role for astrocytes (and other glia) may be via production of GDNF. Although some studies have suggested a pronociceptive role for GDNF in the periphery (Malin et al., 2006), it has been proposed to have a predominantly antinociceptive role centrally. Spinal application of GDNF by direct injection or production by an injected lentiviral vector both attenuated mechanical and thermal hyperalgesia in rats following nerve ligation (Boucher et al., 2000; Pezet et al., 2006). A more recent study has confirmed intrathecal GDNF treatment was associated with the reversal of allodynia in SNI-rats, but went a step further in identifying that there was an increased expression of somatostatin, a neuropeptide with potential analgesic properties (Adler et al., 2009). Moreover, GDNF applied to dorsal root sensory neurons in vitro increases cell content of somatostatin, leading to an activity-dependent increase in somatostatin secretion, which the authors suggest as the anti-allodynic mechanism (Zong et al., 2009). Although GDNF has not been tested clinically for the treatment of neuropathic pain, clinical trials are under way for the treatment of several neurodegenerative diseases (Hong et al., 2008).

It has recently been demonstrated that astrocyte activation can also occur supraspinally following peripheral nerve injury, resulting presumably from ectopic firing and reduced thresholds of ascending fibres. A study by Wei et al. (2008) demonstrated that following CCI of the infraorbital nerve, there was a prolonged activation of astrocytes, including release of TNF and IL-1β, in the RVM. Moreover, RVM administration of astrocytic inhibitors could attenuate pain hypersensitivity, presumably by restoring control of descending pain transmission. A recent study where sciatic nerve CCI was performed in rats, resulted in increased GFAP and vimentin immunoreactivity, indicative of astrocyte activation, in the PAG (Mor et al., 2010), a region which receives direct spinotectal projections and contributes to descending pain inhibition. Moreover, the level of astrocyte activation correlated, not with the degree of pain hypersensitivity per se, but the level of disability in social interactions. Similar findings have been reported following SNL, with increased GFAP expression in the PAG, which was potentiated further by acute stress, with the level of GFAP correlating with depressive-like behaviour (Norman et al., 2010). The implication of these two studies is that astrocytic activation in the PAG may modulate affective-motivational aspects of pain, which are of course a major concern in clinical presentation of chronic pain, and have often been overlooked in animal models of neuropathy.

Once activated, astrocytes release a plethora of mediators including nitric oxide (NO) (Liu et al., 2000a), PGs (Dirig and Yaksh, 1999; Chilardi et al., 2004), excitatory amino acids (Duan et al., 2003) and ATP (Queiroz et al., 1997), which either lower the activation threshold or, directly excite neurons. Moreover, when spinal astrocytes are activated by local application of HIV coat protein gp120, the resultant increase in GFAP is associated with increased IL-1β, TNF and IL-6 (Milligan et al., 2001; Schoeniger-Skinner et al., 2007). Additionally, these cytokines have been demonstrated to mediate pain hypersensitivity, since intrathecal administration of IL-1β and TNF receptor antagonists, as well as an IL-6 neutralising antibody attenuate pain behaviour in models of neuropathy (Milligan et al., 2001; Schoeniger-Skinner et al., 2007). In summary, peripheral nerve injury leads to central activation of astrocytes, resulting in subsequent release of pro-inflammatory mediators in the spinal cord and the brain, which have been linked to the maintenance of pain hypersensitivity, and affective-motivational aspects of chronic neuropathic pain, respectively. From a therapeutic perspective there are limited pharmacological agents available to target astrocytic activation, although the general glial inhibitor, fluorocitrate, alleviates neuropathic pain in animal models. The most effective method to target the effects of activated astrocytes may therefore be to block pro-inflammatory cytokines once they have been released, a possibility which will be discussed below.

The complex spatial and temporal activation of immune and glial cells throughout the nervous system following peripheral nerve injury has been summarised in Fig. 1, allowing the reader to integrate the seemingly disparate elements of the immune system described in Sections 2–4. In addition, Table 1 summarises the therapeutic potential of modulating immune responses in neuropathic pain.

5. Cytokines

Cytokines are small proteins constitutively expressed on the cell surface in precursor form, which can be cleaved to allow rapid release, whereupon they can diffuse over a relatively short distance to act on another cell. The original name, interleukin (IL), derives from the fact many are produced by leukocytes and act on leukocytes, although in actual fact they are produced by most cell types. Nowadays it is clear that cytokines come in two opposing phenotypes; the pro-
inflammatory, IL-1β, TNF, IL-6, IL-15, IL-17, IL-18, and IFN-γ; and anti-inflammatory, IL-4, IL-10 and TGF-β. Furthermore, it is also apparent that IL-1β, TNF and IL-6 induce the production of each other through positive feedback, acting synergistically to amplify the inflammatory signals (Watkins et al., 1999), which can of course lead to a vicious circle of chronic inflammation if not adequately suppressed. The algesic effects of pro-inflammatory cytokines are often indirect, through the expression of further mediators, such as NO and PGE2, which sensitise nociceptors. A good example of this is the potentiation of heat-evoked CGRP release in a skin preparation by topical application of TNF and IL-1β, which enhances nociception by promoting neurogenic inflammation (Opree and Kress, 2000). However, there is also strong evidence for direct receptor-mediated effects of some cytokines on nociceptors (Sommer and Kress, 2004).

Endogenously, it is anti-inflammatory cytokines which serve as negative-feedback regulators, to maintain a balanced immune response. For example, the most powerful member, IL-10, suppresses genes that encode for pro-inflammatory cytokines, prevents their translation, and down-regulates their receptors (Strle et al., 2001). Therefore, the overall outcome of human chronic pain states may correlate strongly with the balance between pro- and anti-inflammatory cytokines. In support of this notion, patients with complex regional pain syndrome, painful neuropathy and SCI, have systemic increases in levels of pro-inflammatory cytokines TNF, IL-2 and IL-6 whilst anti-inflammatory cytokines, IL-10 and IL-4 are decreased (Davies et al., 2007; Uceyler et al., 2007a,b). This is in complete contrast to patients with painless neuropathy who have elevated anti-inflammatory cytokines (Uceyler et al., 2007b). Thus, modulation of cytokine signalling by blocking certain pro-inflammatory cytokines and/or enhancing anti-inflammatory cytokines may be considered as treatment strategies for chronic neuropathic pain (see Table 2).

5.1. Pro-inflammatory cytokines

5.1.1. TNF

TNF, previously TNFα, is known as a major pro-inflammatory cytokine, with the ability to induce a cascade of additional cytokine production, and has been associated with both the immediate and ongoing stages of chronic neuropathic pain. In naïve animals, topical application of TNF to the sciatic nerve and DRGs leads to changes in the properties of neurons, such as ectopic firing in Aδ-, Aβ- and C-fibres (Sorkin et al., 1997; Zhang et al., 2002), and lowering of mechanical thresholds required to activate C-fibres (Sommer and Sorkin, 2000; Ozaktay et al., 2006). Additionally, subcutaneous application of TNF increases vascular permeability in glabrous skin, increasing the infiltration of leukocytes (Sorkin et al., 1997; Zhang et al., 2002), and lowering of mechanical thresholds required to activate C-fibres (Sommer and Sorkin, 2000; Ozaktay et al., 2006). Behaviourally, electrophysiological changes resulting from TNF application are associated with induction of thermal hyperalgesia and mechanical allodynia (Homma et al., 2002; Murata et al., 2006; Zelenka et al., 2005). Furthermore, following peripheral nerve injury there is increased sensitivity of DRG neurons to exogenous TNF, given that a previously sub-threshold concentration becomes capable of eliciting pain hypersensitivity, with even more rapid onset, and the presence of previously absent spontaneous pain (Schafers et al., 2003a). It is also well-characterised that damage to peripheral nerves is associated with a rapid immune response characterised by endogenous TNF release from Schwann cells, resident macrophages and mast cells (George et al., 1999; Sacerdote et al., 2008; Shamash et al., 2002).
Subsequently, infiltrating blood-derived cells (neutrophils and macrophages), also release additional TNF resulting in a second peak around 3–5 days (Shubayev and Myers, 2000). Moreover, both peaks in TNF protein have been reported to correspond with peaks in decreased pain thresholds (Shubayev and Myers, 2000). Nerve injury also leads to increased TNF expression in the corresponding DRG by SGCs, neuronal cell bodies and invading macrophages (Dubovy et al., 2006). Indicative of a general neuroinflammatory reaction of the nervous system, an increase in TNF has also been observed in more distant areas, such as contralateral and heteronymous DRGs, the dorsal horn of the spinal cord, the RVM, locus coeruleus and hippocampus, presumably from activated astrocytes and microglia (Ignatowski et al., 1999; Jancašek et al., 2010; Sacerdote et al., 2008; Wei et al., 2008). There are many examples where interference of TNF signalling, through either neutralising antibodies or receptor antagonists, can reverse pain hypersensitivity in models of peripheral nerve injury, even if the treatment is delivered after the pain is established (Cuellar et al., 2004; Homma et al., 2002; Shubayev and Myers, 2000).
Table 2

<table>
<thead>
<tr>
<th>Therapeutic means</th>
<th>Target and mechanisms</th>
<th>Effect in neuropathic pain (pre-clinical unless otherwise stated)</th>
<th>References</th>
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<tr>
<td>TNF blockers (neutralising antibodies; Infliximab)</td>
<td>Inhibit TNF signalling, and reduce neuronal hyperexcitability and neuroinflammatory responses.</td>
<td>Anti-TNF strategies block pain hypersensitivity in many animal models of neuropath. Infliximab or Etanercept are used clinically to treat autoimmune disorders (rheumatoid arthritis, inflammatory bowel disease and psoriasis).</td>
<td>Cailler et al. (2004), Homma et al. (2002), Milligan et al. (2001), Onda et al. (2001), Sommer et al. (1998, 2001), Sifakakis (2010)</td>
</tr>
<tr>
<td>IL-1β blockers (neuralising antibodies) (receptor antagonist, IL-1RA; Anakinra)</td>
<td>Inhibit IL-1β signalling, and reduce neuronal hyperexcitability.</td>
<td>Anti-IL-1β antibodies and IL-1RA reduce pain hypersensitivity in animal models of neuropathy. IL-1RA Anakinra, has been approved for treatment of rheumatoid arthritis.</td>
<td>Milligan et al. (2001), Kiguchi et al. (2010), Nuki et al. (2002)</td>
</tr>
<tr>
<td>IL-6 blockers (neutralising antibodies) (IL-6Ra antibody; tocilizumab)</td>
<td>Inhibit IL-6 signalling, and reduce neuroinflammatory responses.</td>
<td>Intrathecal administration of IL-6 neutralising antibodies reduces pain hypersensitivity following CCI. Tocilizumab has recently been approved for the treatment of rheumatoid arthritis.</td>
<td>Lee et al. (in press), Singh et al. (2010)</td>
</tr>
<tr>
<td>IL-15 blockers (neutralising antibodies) (inhibitors, Neurostatin).</td>
<td>Inhibit IL-15 signalling, and reduce macrophage and T cell numbers in the injured nerve.</td>
<td>Both IL-15 neutralising antibodies and Neurostatin prevent macrophage and T cell infiltration following CCI, but effect on pain has not yet been established.</td>
<td>Gomez-Nicola et al. (2008)</td>
</tr>
<tr>
<td>IL-4</td>
<td>Suppresses pro-inflammatory cytokine expression, as well as macrophage/microglial activation.</td>
<td>Pre-treatment with IL-4 gene therapy delays pain hypersensitivity onset following SNI, whilst post-treatment reverses pre-established pain.</td>
<td>Hao et al. (2006)</td>
</tr>
<tr>
<td>IL-10</td>
<td>Suppresses pro-inflammatory cytokine expression, such as TNF and IL-1β.</td>
<td>Intraneural or intrathecal IL-10 and IL-10 gene therapy result in prevention and reversal of pre-established pain hypersensitivity in several models of neuropathy.</td>
<td>Wagner et al. (1998), Milligan et al. (2005a,b)</td>
</tr>
<tr>
<td>Adenosine 2A receptor (A2AR) agonists (ATL1313 and CGS21680)</td>
<td>Increase IL-10 expression, and decrease activation of spinal glia.</td>
<td>ATL131 and CGS21680 reverse pain hypersensitivity following CCI.</td>
<td>Loram et al. (2009)</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>Pleiotropic cytokine which suppresses glial activation.</td>
<td>Intrathecal TGF-β1 reduces established and developing pain hypersensitivity following PNL.</td>
<td>Echeverry et al. (2009)</td>
</tr>
<tr>
<td>Anti-MIPα antibody</td>
<td>Prevents infiltration of macrophages.</td>
<td>Antibodies against MIPα prevent pain hypersensitivity following PNL.</td>
<td>Kiguchi et al. (2010)</td>
</tr>
<tr>
<td>Fractalkine receptor blockers (CX3CR neutralising antibody)</td>
<td>Inhibit fractalkine signalling, and reduce microglial activation.</td>
<td>Intrathecal administration of CX3CR1 neutralising antibodies reduces established and developing pain hypersensitivity following CCI.</td>
<td>Milligan et al. (2004)</td>
</tr>
<tr>
<td>MCP-1 blockers (neutralising antibodies) (CCR2 antagonists; CCR2 RA isoform-[R]).</td>
<td>Inhibit MCP-1 signalling, and reduce microglial activation.</td>
<td>Intrathecal administration of MCP-1 neutralising antibodies or CCR2 antagonists reduces established and developing pain hypersensitivity in several models of neuropathy.</td>
<td>Bhagoo et al. (2007a), Bhagoo et al. (2009), Gao et al. (2009), Thacker et al. (2009)</td>
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Milligan et al., 2001; Onda et al., 2003; Sommer et al., 1998, 2001).

There are already many drugs on the market which target TNF signalling for the treatment of human autoimmune diseases (rheumatoid arthritis, inflammatory bowel disease and psoriasis), including Infliximab or Etanercept (Sifakakis, 2010), however these have yet to be tested against neuropathic pain.

There has been significant effort to uncover how TNF downstream signalling mechanisms can promote pain, some of which will be outlined briefly here (reviewed by Uceyler et al., 2009). TNF elicits its effects through two distinct receptors, the constitutively expressed TNFR1, which when activated results in receptor internalisation, and the inducibly expressed TNFR2, which on activation results in shedding of the receptor–ligand complex (MacEwan, 2002). In naïve rats spinal activation of TNFR1, but not TNFR2, induced pain hypersensitivity in vivo and ectopic firing of DRG neurons in vitro (Schafer et al., 2008). Contrastingly, in nerve-injured animals both TNFR1 and TNFR2 activation lead to pain hypersensitivity and ectopic firing. This finding may be explained given that nerve injury induces a rapid and prolonged increase in expression of both TNF receptors, by peripheral nerve axons, DRG neurons, SGC and Schwann cells (Dubovy et al., 2006; George et al., 2005). Increased expression of TNF receptors has also been reported on primary afferent fibres in the dorsal horn, as well as spinal glia (Bergsteinsdottir et al., 1992; Ohtori et al., 2004; Schafer et al., 2003b). It is apparent therefore that in the intact nervous system TNFR1 is capable of mediating the excitation of sensory neurons and induction of pain hypersensitivity, whilst following nerve injury there is an induction of TNFR2, which along with continuing presence of TNFR1, contributes to ongoing excitation of sensory neurons associated with neuropathy.

Binding of TNF to its receptors in DRGs and in spinal dorsal horn activates nuclear factor-kappa B (NF-κB), which in turn induces transcription of genes encoding pro-inflammatory cytokines and therefore, plays a role in pain facilitation (Ledeboer et al., 2005a,b; Wei et al., 2007). Additional TNF receptor downstream targets in DRG neurons include activation of kinase pathways, ERK, JNK, p38-MAPK and PI3K (Pollock et al., 2002; Takahashi et al., 2006). TNF activation of the p38-MAPK pathways has been demonstrated to promote sodium influx in DRGs (Czeschik et al., 2008; Jin and Gereau, 2006), which may contribute to decreasing excitatory thresholds, and ectopic firing in painful neuropathy. In hippocampal neurons TNF, via a TNFR1-P38K
signalling pathway, has been shown to promote excitability through increases in AMPA receptor expression (Beattie et al., 2002), and decreases in GABA<sub>A</sub> receptors (Stellwagen et al., 2005). In the spinal cord, TNF can promote increased excitatory post-synaptic currents through both AMPA and NMDA receptors (Kawasaki et al., 2008), which may be explained since TNF inhibits the expression and activity of glutamate transporters thereby increasing extracellular glutamate available to stimulate receptors (Korn et al., 2005; Sitcheran et al., 2005). In summary, TNF is released by virtually all immune and immune-like glial cells in close proximity to neurons, and is capable of promoting neuronal hyperexcitability, enhancing excitatory transmission and promoting ongoing inflammation at many levels of the nervous system; making it a central mediator of chronic neuropathic pain and an excellent therapeutic target.

5.1.2. IL-1β

IL-1β is implicated in neuropathic pain since both IL-1α and IL-1β are upregulated within hours following peripheral nerve injury (Gillen et al., 1998; Okamoto et al., 2001; Shamash et al., 2002). The immediate release of IL-1β at the injury site appears to be mediated via the calcium sensitive protein, calpain, which is activated by calcium influx following nerve injury. Indeed, calpain inhibitors completely abrogate local IL-1β release 1 h after CCI (Üeyer et al., 2007c). Whilst early release of IL-1β is not mediated via NMDA receptors, NMDA antagonists can inhibit IL-1β release in the nerve stump 3 days following CCI (Kleinschnitz et al., 2004; Üeyer et al., 2007c). Therefore, different molecular pathways are responsible for IL-1β regulation at different time-points following peripheral nerve injury. Whitehead et al. (2010) were the first investigators to measure spinal cytokines using intrathecal microdialysis, demonstrating that electrically-evoked C-fibre activation results in a 15-fold increase in IL-1β efflux 2 weeks following CCI, compared to sham operated animals. Moreover, treatment with the non-selective glial inhibitor, propentofylline, completely reversed stimulation evoked IL-1β efflux, as well as normalising pain hypersensitivity (Whitehead et al., 2010). An interesting study by Norman et al. (2010) found increased IL-1β expression in the pre-frontal cortex following SNI in mice, an effect that could be potentiated by acute stress. Furthermore, the increased expression of IL-1β induced by SNI and restraint, were paralleled by increased depressive-like behaviour, leading the authors to suggest neuroinflammation following nerve injury can contribute to depression, a common symptom of chronic neuropathic pain (Norman et al., 2010).

Intraplantar injection of IL-1β elicits rapid onset of pain hypersensitivity (Follenfant et al., 1989), associated with transient spontaneous discharges (Fukunoka et al., 1994). DRG neurons are also susceptible to modulation by IL-1β, with brief applications resulting in potentiation of heat-activated inward currents and a shift of activation thresholds towards lower temperatures (Obreja et al., 2002a). The algic properties have also been demonstrated when given intraneurally into rat sciatic nerve (Zelenka et al., 2005) or intrathecally in the rat spinal cord (Sung et al., 2004). Following spinal nerve transection, loss of IL-1 signalling in both IL-1 receptor (IL-1R) knock-outs and transgensics over-expressing the IL-1 receptor antagonist (IL-1RA), leads to a decrease in spontaneous ectopic activity, as well as delayed onset of autotomy and a reduction in its severity (Wolf et al., 2006). Furthermore, pain hypersensitivity is alleviated in animal models of neuropathy by administration of IL-1RA (Millisan et al., 2001), or neutralising antibodies against IL-1β (Kiguchi et al., 2010), and IL-1R (Sommer et al., 1999). The use of the IL-1RA, Anakinra, has been approved clinically for the treatment of rheumatoid arthritis (Nuki et al., 2002), and may offer a potential therapeutic benefit in neuropathic pain.

Like TNF, IL-1β has been demonstrated to increase excitability of neurons. Indeed, prolonged IL-1β exposure has been shown to enhance voltage-dependent sodium currents in TRG neurons, which was dependent on IL-1R, and PK-C signalling (Liu et al., 2006). IL-1β also increases neuronal excitability by enhancing both calcium and non-selective cation currents, as well as inhibiting potassium efflux (Desson and Ferguson, 2003; Shu et al., 2007). IL-1β appears to modulate glutamate receptors in hippocampal neurons, for example, IL-1β reduces AMPA-dependent excitatory post-synaptic currents, but enhances NMDA-mediated calcium influx (Viviani et al., 2003; Yang et al., 2005). More pertinently in spinal cord neurons, IL-1β increases excitatory AMPA and NMDA-induced currents, whilst decreasing spontaneous GABA and glycine inhibitory currents (Kawasaki et al., 2008). It has been suggested IL-1β induction of NMDA-currents may be through PK-C which phosphorylates NMDA subunits, NR1 and NR2B (Brenner et al., 2004; Guo et al., 2002). This idea has been confirmed in a model of orofacial pain, where intrathecal administration of the IL-1RA, attenuated hyperalgesia and reduced NMDA phosphorylation (Guo et al., 2007). Taken together, there is strong evidence for IL-1β in enhancing synaptic transmission and neuronal activity at several locations of the nervous system, and a prominent role in neuropathic pain is likely given its release from numerous immune and immune-like glial cells.

5.1.3. IL-6

IL-6 is a predominantly pro-inflammatory cytokine, released by many cell types, including mast cells, macrophages, lymphocytes, neurons and glial cells, however in certain situations it can modulate anti-inflammatory responses (Jordan et al., 1995; Tilg et al., 1997). Injury of the sciatic nerve induces local upregulation of IL-6 and its receptor, IL-6R, where it appears in Schwann cells (Bolin et al., 1995; Ito et al., 1998; Kurek et al., 1996), macrophages (Ma and Quirion, 2005), as well as neurons (Reichert et al., 1996; Zhong and Heumann, 1995). Induction of IL-6 has also been reported in the DRG of injured nerves (Murphy et al., 1995), whilst a recent study has demonstrated bilateral increases in IL-6 protein in DRGs following unilateral peripheral nerve injury (Brazda et al., 2009). Peripheral nerve injury also increases IL-6 levels in the spinal cord, particularly in the superficial laminae of the dorsal horn (DeLeo et al., 1996; Murphy et al., 1995), in both microglia (Dominguez et al., 2008) and neurons (Yamauchi et al., 2006). The literature predominantly documents the cascade of pro-inflammatory cytokines as beginning with TNF, followed by the stimulation of IL-1β, and subsequent release of IL-6 (McMahon et al., 2005). However this sequence of events has been questioned since following gp120-induced activation of spinal glia, blockade of IL-6 with a neutralising antibody prevented increases of IL-1β and TNF protein in lumbar dorsal spinal cord, and the surrounding lumbosacral cerebrospinal fluid (Schoenig-Skinner et al., 2007). Therefore, in this situation at least, IL-6 appears to have a role in the upregulation of other pro-inflammatory cytokines, and may have a more prominent role in the cytokine cascade than first appreciated.

The role of IL-6 in modulating acute nociception is less clear than TNF and IL-1β, possibly since it can exert opposing inflammatory responses in different conditions. For example, intraplantar injection of IL-6 into naïve animals has led to conflicting results, either inducing mechanical hyperalgesia (Cunha et al., 1992), having no effect on mechanical thresholds (Czlonkowski et al., 1993) or producing thermal hyperalgesia (Flatters et al., 2004). Electrophysiological recordings have also produced conflicting results. Indeed, recordings from dorsal horn neurons in naïve animals, demonstrated peripheral application of IL-6 inhibited the responses to thermal and mechanical stimuli, whilst after nerve injury IL-6 no longer inhibited mechanical responses, but continued to inhibit thermal stimuli (Flatters et al., 2004). In contrast to this Obreja et al. (2002b) found exogenous application of IL-6, in combination with its soluble receptor, increased the heat-evoked release of CGRP from cutaneous nociceptors in vitro, as well as directly potentiating heat-activated inward currents in cultured DRG neurons, resulting in decreased thermal activation
thresholds (Obreja et al., 2005). Unexpectedly IL-6−/− mice have normal nociceptive responses to thermal and mechanical stimuli (Bianchi et al., 1999; Murphy et al., 1999), whilst following spinal nerve lesion they have reduced pain hypersensitivity (Murphy et al., 1999; Ramer et al., 1998), suggesting IL-6 has a role in development of neuropathic pain if not acute nociception. In the spinal cord a clearer role of IL-6 in promoting pain is apparent, since intrathecal administration of IL-6 resulted in development of pain hypersensitivity (DeLeo et al., 1996; Lee et al., 2010), and blocking spinal IL-6 using neutralising antibodies prevents the development of pain hypersensitivity in several models of neuropathy (Dominguez et al., 2008; Lee et al., 2010; Schoeniger-Skinner et al., 2007).

In the context of peripheral nerve injury, IL-6 and IL-6R predominantly signal through Janus kinases (JAK) and signal transducers and activators of transcription (STAT) transcription factor pathways, with differing outcomes in the periphery and the spinal cord. In cultured DRGs IL-6 can enhance neurite outgrowth (Cafferty et al., 2001, 2004), and in vivo nerve regeneration is impaired in IL-6−/− mice (Cafferty et al., 2004; Zhong et al., 1999). These effects of IL-6, and family member LIF, which both signal via the same signal transducing subunit gp130, are mediated by STAT3, demonstrated since blockade of STAT3 signalling in the spinal cord, using a lentiviral vector producing the suppressors of cytokine signalling 3 (SOCS3) following sciatic nerve CCI in rats, led to attenuation of pain hypersensitivity. SOCS3 treatment was associated with the prevention of IL-6, IL-1β, MCP-1, activating transcription factor 3 (ATF3) and GFAP induction, therefore inhibiting downstream mechanisms by which IL-6-mediated JAK/STAT3 signalling promotes spreading of neuroinflammation in the spinal cord. Microglial induction of the chemokine receptor, CX3CR1, via a p38-MAPK pathway has recently been identified as a novel downstream target of IL-6 signalling following CCI in rats. Indeed, intrathecal administration of an IL-6 neutralising antibody, or recombinant IL-6, suppressed or enhanced both CX3CR1 expression and pain hypersensitivity, respectively (Lee et al., 2010). Taken together, IL-6 is predominantly pro-inflammatory in neuropathic pain, promoting exacerbation of inflammation through activation of spinal cord microglia, however at the site of injury it may be antiinociceptive, as well as promoting peripheral axonal regeneration. Although, a humanised monoclonal antibody against IL-6, Tocilizumab, has recently been approved for the treatment of rheumatoid arthritis (Singh et al., 2010), the diverse effects of IL-6 make it a less attractive therapeutic target for the treatment of neuropathic pain, compared to other cytokines.

5.1.4. IFN-γ

IFN-γ is a dimerised soluble cytokine, which is the only member of the type II class of interferons, and has been implicated in many chronic pain states. Following peripheral nerve injury in rats, IFN-γ is released from astrocytes and damaged neurons (Racz et al., 2008a), as well as by spinal cord infiltrating Th1 cells (Costigan et al., 2009a). Furthermore, IFN-γ treatment in cancer patients has long been reported to induce spontaneous pain (Mahmoud et al., 1992; Quesada et al., 1986), whilst intrathecal administration of IFN-γ in naïve but not IFN-γR−/− mice, induced pain hypersensitivity (Robertson et al., 1997).

Application of IFN-γ can cause spontaneous firing of dorsal horn neurons in vivo, as well as increasing wind-up of action potentials in response to repeated electrical stimulation (Vikman et al., 2005). IFN-γ further promotes increased excitability in the dorsal horn by reducing inhibitory tone (Vikman et al., 2007). Thus, IFN-γ appears to induce central sensitisation by several mechanisms. Aside from modulating neurons, IFN-γ is heavily implicated in activation of microglia, and ablation of the IFN-γ signalling in IFN-γ−/− or IFN-γR−/− mice severely impairs both activation of microglia and development of pain hypersensitivity following peripheral nerve injury (Costigan et al., 2009a; Tsuda et al., 2009). IFN-γ-signalling in microglia causes upregulation of several proteins, notably iNOS (responsible for NO production), the purinergic receptor, P2X4, and chemokine receptor, CCR2 (Racz et al., 2008a; Tsuda et al., 2009), all of which contribute to the development of neuropathic pain states. The analgesic properties of endocannabinoids downstream of CB2R relate in part to the reversal of induction of iNOS and CCR2 by IFN-γ (Racz et al., 2008a). In summary, IFN-γ is a potent pro-inflammatory cytokine implicated in the pathogenesis of neuropathic pain, mainly through its actions in the spinal cord on both neurons and microglia, and may offer a good therapeutic target, particularly indirectly by modulating the endocannabinoid system which opposes the effects of IFN-γ.

5.1.5. IL-15

The cytokine IL-15, like IL-2, is a 4-α-helix bundle cytokine, which mainly modulates T lymphocytes; enhancing their proliferation (Armitage et al., 1995), promoting release of T cell chemokines (Badolato et al., 1997; Chen et al., 2005) and increasing their expression of chemokine receptors (Perera et al., 1999). There is an emerging role for IL-15 in the development of chronic pain states, by promoting infiltration of monocytes and T cells to sites of peripheral nerve injury, and the reciprocal levels of the spinal cord. Indeed, in naïve rats intrathecal infusion of both IL-15 and IL-2 resulted in mechanical and thermal hyperalgesia (Cata et al., 2008), whilst intraneural injection of IL-15 led to macrophage and T cell recruitment (Gomez-Nicola et al., 2008). Following CCI, rapid increases of IL-15 expression were observed in the injured sciatic nerve, expressed by damaged neurons and infiltrating macrophages (Gomez-Nicola et al., 2008; Kleinschmitz et al., 2006), as well as in the spinal cord, expressed by reactive astrocytes and microglia (Gomez-Nicola et al., 2008). Following CCI, blocking IL-15 by intraneural application of a neutralising antibody or a physiological inhibitor, Neurostatin, both significantly inhibited macrophage and T cell numbers in the sciatic nerve, as well as promoting a less reactive macrophage phenotype (Gomez-Nicola et al., 2008). In support of this, IL-15−/− and IL-15R−/− mice both have reduced T cell infiltration and microglial activation in the facial motor nucleus, following axotomy of the facial nerve (Huang et al., 2007). Sciatic nerve CCI results in IL-15-dependent increases in expression of iNOS and MHC-II (Gomez-Nicola et al., 2008), molecules which have been linked to widespread inflammation and antigen presentation following peripheral nerve injury (Hu and McLachlan, 2003; Levy et al., 1999). In summary, there is a growing body of evidence of the pro-inflammatory nature of IL-15 following peripheral nerve injury, which warrants further investigation of the effects of blocking IL-15 on pain hypersensitivity in models of neuropathy.

5.1.6. IL-17

IL-17 is a pro-inflammatory cytokine that is produced predominantly by Th17 cells, but also by neutrophils (Ferretti et al., 2003; Kleinschmitz et al., 2006), cytotoxic T cells (Tzartos et al., 2008), as well as glial cells (Kawanokuchi et al., 2008; Tzartos et al., 2008) and plays a central role in nervous system inflammatory disorders such as multiple sclerosis (Tzartos et al., 2008). IL-17 has recently been demonstrated to induce painful behaviour in naïve mice at several levels of the nervous system. Indeed, intraplantar and intraneural...
injection of recombinant IL-17 induced both mechanical allodynia and thermal hyperalgesia, while intrathecal injection produced thermal hyperalgesia (Kim and Moalem-Taylor, 2010). Following CCI, there was increased expression of IL-17 in degenerating nerves, which peaked after 7 days and was co-localised with T cells in the injured nerve (Kleinschnitz et al., 2006). Following sciatric nerve CCI, T cell-deficient Rag1fl/fl mice did not express IL-17 in the injured nerve, as well as displaying reductions in thermal hyperalgesia, macrophage infiltration and MCP-1 expression (Kleinschnitz et al., 2006). A recent study from our laboratory has demonstrated PNL-injured IL-17−/− mice displayed a reduction in mechanical pain hypersensitivity (Kim and Moalem-Taylor, 2010). Furthermore, IL-17−/− also showed a corresponding decrease in infiltration of T cells and macrophages to injured sciatric nerves and DRGs, and decreased activation of microglia and astrocytes in the spinal cord. Taken together, there is growing evidence that IL-17 contributes to immune cell infiltration and activation at the site of injury, and glial activation in the spinal cord following peripheral nerve injury, all of which contribute to the development and maintenance of neuropathic pain. However, additional studies assessing the role of IL-17 in neuropathic pain are required.

5.1.7. IL-18

IL-18 a pro-inflammatory cytokine which is closely related to IL-1β, and acts through IL-18 receptor (IL-18R), however evidence for its role in chronic pain remains limited. In naïve animals, intraplantar injection of IL-18 induced mechanical hyperalgesia (Verri et al., 2007), whilst intrathecal application of IL-18 also induced mechanical hyperalgesia, as well as activation of astrocytes, through an NF-κB dependent pathway (Miyoshi et al., 2008). Following SNI, in the dorsal horn of the spinal cord, there was a striking increase in IL-18 expression by microglia, peaking at 3 days, and IL-18R expression by astrocytes peaking at 7 days (Miyoshi et al., 2008). Increased microglial expression of IL-18 was potentiated by LPS, an activator of TLR4, or attenuated by a TLR4 siRNA, indicating IL-18 induction is downstream of microglial activation through TLRs. However, neutralising antibodies to both IL-18 and IL-18R only partially suppressed injury-induced mechanical hypersensitivity, although phosphorylation of NF-κB and the number of reactive astrocytes were markedly attenuated (Miyoshi et al., 2008). Although there are only a few studies to have investigated the role of IL-18 in neuropathic pain, it appears IL-18 mediates microglia/astrocyte interactions in the spinal cord, which occur several days after nerve injury. Thus, blocking IL-18 signalling may offer a good treatment strategy, though further studies on the involvement of IL-18 in neuropathic pain are necessary.

5.2. Anti-inflammatory cytokines

5.2.1. IL-10

IL-10 is released from activated T cells, B cells, macrophages and mast cells (Uceyler et al., 2009), and is generally considered to be a powerful anti-inflammatory cytokine, inhibiting the release of pro-inflammatory cytokines, IL-1β, IL-6 and TNF (Kanaan et al., 1998; Poole et al., 1995). Cytokine suppression downstream of the IL-10 receptor is believed to be through induction of SOCS3, which inhibits genes normally activated by the JAK-STAT3 pathway (Ogawa et al., 2008; Yoshimura et al., 2003), as well as inhibition of the transcription factor, NF-κB (Driessler et al., 2004). There is evidence that endogenous IL-10 plays a role in normal nociception, since IL-10−/− mice, or mice treated with an IL-10 neutralising antibody had increased thermal pain thresholds (Tu et al., 2003), although application of exogenous IL-10 to naïve rats had no effect on pain sensitivity (Wagner et al., 1998). However, mice pre-treated with IL-10 had decreased pain behaviour following intraperitoneal acetic acid or zymosan, as well as decreased IL-1β and TNF release from peritoneal macrophages (Vale et al., 2003). Using a novel approach, Zhou et al. (2007) demonstrated that subcutaneous inoculation of rats with a HSV–IL-10 vector, 10 days prior to intraplantar formalin, led to a reduction in pain sensitivity and decreased spinal TNF expression. Therefore, the majority of evidence suggests IL-10 can suppress nociceptive pain.

Following peripheral nerve injury, there is an early increase in IL-10 expression, 1 h after injury in the sciatic nerve and 24 h after injury in the DRGs (Jander et al., 1996; Taskinen et al., 2000; Uceyler et al., 2007). However, over a longer time-course there appears to be a gradual increase in expression which peaks after 6 weeks (Okamoto et al., 2001), and may be associated with injury healing. Following CCI in rats, a single intraneural dose of IL-10 significantly attenuated hyperalgesia, macrophage infiltration and TNF expression (Wagner et al., 1998). Furthermore, intrathecal IL-10, or spinal delivery of IL-10 by an adeno-viral vector, both result in prevention and reversal of pre-established pain hypersensitivity in several models of neuropathy, which was associated with decreased IL-1β expression (Milligan et al., 2005a,b). Following CCI, a single intrathecal injection of adenovirus 2A receptor (A2AR) agonist can produce a long acting reversal of pain hypersensitivity, which was associated with decreased markers of activated spinal glia (Loram et al., 2009). Given that A2AR activation can increase IL-10 expression and decrease TNF expression (Coska et al., 2007; Hasko et al., 1996), it was no surprise that intrathecal administration of neutralising antibodies to IL-10 caused the reappearance of pain hypersensitivity eliminated by A2AR agonists (Loram et al., 2009). Taken together, increasing expression of IL-10, by gene therapy or pharmacologically, has substantial inhibitory effects on acute pain and established painful neuropathy in animal models. The mechanism of action of IL-10 is predominantly through suppression of pro-inflammatory cytokines, and the resulting decrease in recruitment and activation of further immune and immune-like glial cells at both the injury site and in the spinal cord. Recombinant human IL-10 is under clinical investigation to treat autoimmune diseases, such as psoriasis and Crohn’s disease (Kimball et al., 2002; Schreiber et al., 2000), and given it may well act as an endogenous suppressor of excessive inflammation after nerve injury, it could be beneficial in the treatment of neuropathic pain.

5.2.2. IL-4

IL-4 is a prototypical anti-inflammatory cytokine, released by activated T cells, mast cells and granulocytes, and is known to inhibit IL-1β, IL-6 and TNF (Cunha et al., 1999; Lord and Lamb, 1996; Tunon de Lara et al., 1994). It has many immune functions including; stimulation of activated B cells; T cell proliferation and differentiation into a Th2 phenotype; and suppression of macrophage activation. There are now several studies which suggest IL-4 is anti-innociceptive in models of acute and chronic pain. For instance, pre-treatment with IL-4 attenuated the pain responses to bradykinin, carrageenan, TNF, zymosan and acetic acid (Cunha et al., 1999; Vale et al., 2003). In each case the antinociceptive effect was associated with decreased production of pro-inflammatory cytokines, TNF and IL-1β, from macrophages. Furthermore, SNL-injured rats pre-treated with subcutaneous IL-4 gene therapy had delayed onset of pain hypersensitivity, whilst post-treatment could reverse pre-established pain (Hao et al., 2006). Decreased pain behaviour was associated with decreased spinal release of IL-1β and PGE2, as well as a reduction in phosphorylated p38-MAPK, indicative of a reduction in numbers of activated microglia. IL-4 can also promote the expression of μ and δ opioid receptors (Borner et al., 2004; Kraus et al., 2001), which may explain some of its analgesic effects. IL-4 antinociceptive effects are largely due to the suppression of pro-inflammatory cytokines and macrophage/microglia activation. Additionally, the ability of IL-4 to promote differentiation of the anti-inflammatory Th2 cell population is well documented; however, whether this plays a role in inhibiting pain behaviour has yet to be addressed and may well uncover an additional analgesic mechanism for IL-4. In summary, despite only a
few studies having investigated the role of IL-4 in pain paradigms, there is already a strong argument for evaluating the potential of recombinant human IL-4 to treat neuropathic pain.

5.2.3. TGF-β

TGF-β family members are pleiotropic cytokines, having a wide variety of functions which are context dependent, for example they may promote cell survival or induce apoptosis, stimulate cell proliferation or induce differentiation (Bottner et al., 2000). Their immune functions however, are mostly anti-inflammatory, with TGF-β1 blocking proliferation and activation of microglia in vitro (Suzumura et al., 1993), as well as inhibiting astrocyte TNF expression induced by IFN-γ and IL-1β (Benveniste et al., 1995). Modest increases in spinal TGF-β1 have been reported following several models of neuropathy (Colosetti et al., 1995; DeLeo et al., 1997), whilst following PNL intrathecal infusion of recombinant TGF-β1 significantly attenuates development of pain hypersensitivity and reverses previously established pain (Echeverry et al., 2009). Improvements in pain behaviour were accompanied by inhibition of microglial proliferation, microglial and astrocytic activation, as well as a decrease in neuronal expression of the stress inducible gene, ATF3, resulting in a decrease in neuronal expression of MCP-1. Recently, TGF-β1 signalling has been studied by deleting Bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI), a naturally occurring negative regulator of TGF-β family members. BAMBI−/− mice have increased levels of TGF-β1 signalling activity, which was associated with a reduction in acute pain behaviours following thermal, mechanical, chemical and inflammatory stimuli, whilst in a model of neuropathy, pain hypersensitivity was also attenuated (Tramullas et al., 2010). The authors suggest TGF-β1 signal suppression involves the opioid system, since mRNA and protein levels of precursor proteins of the endogenous opioid peptides proopiomelanocortin and proenkephalin were increased in the spinal cords of BAMBI−/− mice. In summary, despite only two studies assessing the role of TGF-β1 in models of neuropathy, it appears TGF-β1 signalling can suppress pain development and reverse established pain. TGF-β1 appears to modulate both glia and neurons, suppressing their activation and proliferation, as well as inhibiting pro-inflammatory cytokine release and promoting expression of endogenous opioids. Therefore, even at this early stage of investigation, modulation of TGF-β1 signalling appears to have significant therapeutic potential in chronic of neuropathic pain.

6. Chemokines

Chemokines are small chemotactic cytokines that are important for leukocyte migration and recruitment to sites of nerve injury. Chemokines have been shown to contribute directly to nociception by producing excitatory effects on DRG neurons, induce allodynia after intraplantar injection in rats (Oh et al., 2001), as well as activating spinal glial cells leading to pain hypersensitivity. Due to the large number of studies involving chemokines and neuropathic pain, the section below merely outlines some of the more important findings, however a number of more detailed reviews on chemokines in pain pathobiology are available (refer to Abbadie et al., 2009; Gao and Ji, 2010; Milligan et al., 2008).

6.1. Macrophage modulation by chemokines

Fractalkine is a chemokine which activates the single receptor, CX3CR1, both of which have been implicated in the recruitment and activation of peripheral immune cells following nerve injury. Indeed, macrophages at the site of nerve injury and in DRGs have increased expression of CX3CR1 for up to 1 week following SNL or sciatic nerve transection, whilst the levels of fractalkine itself remain unchanged (Holmes et al., 2008). Moreover, there was a correlation of CX3CR1 expression with the number of CX3CR1-positive macrophages present in the DRG, suggesting fractalkine is a chemoattractant of macrophages. Surprisingly, given the role of macrophages in pain hypersensitivity, intraneural injection of fractalkine into the sciatic nerve significantly delayed the development of allodynia for 3 days in SNI-injured mice (Holmes et al., 2008). Although the mechanism for this anti-allodynic effect is unclear, it has been suggested that it may involve mechanisms other than macrophage recruitment, such as the recruitment of opioid containing immune cells (Holmes et al., 2008), or inhibition of TNF expression (Mizuno et al., 2003; Zujovic et al., 2000). Therefore, in the periphery at least, locally applied fractalkine appears to be antinociceptive.

MCP-1, and one of its receptors, CCR2 have also been implicated in macrophage recruitment to injured nerves. Indeed, in response to SNI, a persistent and marked upregulation of CCR2 mRNA expression, has been demonstrated in the injured nerve and DRGs (Abbadie et al., 2003). Moreover, the development of mechanical allodynia was totally abrogated in CCR2−/− mice, with a corresponding attenuation of macrophage recruitment (Abbadie et al., 2003). MCP-1 is therefore believed to have an important role in the establishment of pain hypersensitivity, and has since been demonstrated to be released by damaged neurons at the injury site and in the DRGs, in several models of neuropathy (Jeon et al., 2009; Jung et al., 2008; Thacker et al., 2009; White et al., 2005). A third macrophage chemokine, MIPx, derived from macrophages, neutrophils and Schwann cells, has also been associated with the development of neuropathic pain, with perineural injection of anti-MIPx, or RNA silencing of its receptors (CCR1 and CCR5) preventing pain hypersensitivity following PNL (Kiguchi et al., 2010). Stromal derived factor-1 (SDF1), through its receptor CXCR4, is another chemokine suggested to be involved in the infiltration of macrophages in neuropathic pain states. Upregulation of mRNA for both SDF-1 and CXCR4 have been demonstrated in DRG neurons in animal models of neuropathy (Bhangoo et al., 2007b; Dubovy et al., 2010; Oh et al., 2001). Furthermore, treatment with a CXCR4 antagonist could attenuate thermal hyperalgesia, in parallel with a reduction in CXCR4 expression in DRGs (Dubovy et al., 2010). In summary, the chemokines, MCP-1, MIPx and SDF-1, appear to facilitate pain hypersensitivity by recruiting peripheral macrophages.

6.2. Spinal cord glia modulation by chemokines

Whilst spinal glial cells are activated by a variety of additional mediators, chemokines are attracting significant attention as candidates for their recruitment and activation. Under physiological conditions fractalkine is expressed in high abundance on the surface of neurons, with the receptor largely confined to microglial cells (Harrison et al., 1998; Lindia et al., 2005; Verge et al., 2004), this has led to the proposal that fractalkine acts as a crucial neuron-to-glial signal. Following peripheral nerve injury, there is increased neuronal activity in the pain-recipient areas of the spinal cord, which leads to both cleavage of fractalkine from neurons, forming a soluble diffusible signal (Chapman et al., 2000), and an upregulation of CX3CR1 by astrocytes. Surprisingly, given the role of macrophages in pain hypersensitivity, intrathecal administration of a neutralising antibody against CX3CR1 attenuates thermal hyperalgesia and mechanical allodynia, whilst exogenous application of fractalkine induces pain hypersensitivity (Milligan et al., 2004). Surprisingly, blockade of CX3CR1 maintained its efficacy in preventing pain-related behaviour even when administered 1 week following SCI, suggesting that endogenous fractalkine also has a role in ongoing pain hypersensitivity (Milligan et al., 2004). It should be noted a nociceptive role for fractalkine in the spinal cord is contrary to its antinociceptive effects in the periphery, however the mechanisms of these spatial differences have yet to be elucidated.

CatS has recently been described as a microglial-derived protease responsible for fractalkine cleavage, released in response to aberrant primary afferent firing in the dorsal horn of the spinal cord (Clark et al.,
Taken together, there is strong evidence that in the spinal cord, glial and neuronal fractalkine, and microglial CatS, are key mediators of microglial activation, resulting in development and maintenance of a vicious circle of pain hypersensitivity and inflammation.

Spinal cord expression of another chemokine, MCP-1, from both neurons and astrocytes, is induced within 24 h following peripheral nerve injury, although microglial expression of its most common receptor, CCR2, remains largely unchanged (Abbadie et al., 2003; Jeon et al., 2009; Thacker et al., 2009; Zhang and De Koninck, 2006). A recent study has shown MCP-1 was primarily induced in spinal cord astrocytes, not neurons, following SNL (Gao et al., 2009). Since areas of microglial activation in the spinal cord match the spatial profile of MCP-1 expression (Thacker et al., 2009; Zhang and De Koninck, 2006), there may either be axonal transport of MCP-1 mRNA from damaged nerves to the spinal cord and production in neuronal terminals or local production from activated astrocytes. Intrathecal administration of an MCP-1 neutralising antibody, 24 h before or 3 days following peripheral nerve injury, both attenuate pain hypersensitivity, in parallel with a reduction in microglial activation (Gao et al., 2009; Thacker et al., 2009). Furthermore, intrathecal administration of CCR2 antagonist reversed tactile allodynia in two models of neuropathy (Bhangoo et al., 2007a; Bhangoo et al., 2009).

It has also been suggested, given CCR2 is expressed by neurons (Gosselin et al., 2005), that astrocytic MCP-1 is an important astrocyte-neuronal signalling molecule. Indeed, MCP-1 is highly inducible in cultured astrocytes, with brief exposure of TNF resulting in >100 fold increase in MCP-1 expression, whilst electrophysiologically, MCP-1 has been demonstrated to directly activate spinal cord neurons (Gao et al., 2009). In summary, MCP-1 may well play an important dual role in central sensitisation of neurons, as well as contributing to microglial activation, associated with the development of pain hypersensitivity and persistent pain. It would seem from pre-clinical studies, blocking chemokines, such as fractalkine and MCP-1, would be extremely advantageous in human neuropathic pain to prevent activation of macrophages and microglia.
7. Conclusions

Nerve injury sometimes leads to chronic neuropathic pain associated with neuro-immune activation and neuroinflammation in both the peripheral and central nervous system. Even though there is growing evidence for specific actions of individual immune and glial cells and their mediators, the complex interactions of these participants remain unclear. It appears that the outcome of nerve injury is likely to rest on the balance of neuroprotective effects by some immune (i.e. Th2) and immune-like glial cells (i.e. Schwann cells), and their anti-inflammatory cytokines, neurotrophic factors, and analogous opioid mediators; and thedestructive effects of other immune cells (macrophages, neutrophils, Th1 cells), and activated glia (astrocytes and microglia), whose release of pro-inflammatory cytokines, complement proteins, peptide transmitters, reactive species, and prostaglandins, promote persistent pain (Fig. 2). Currently, in neuropathic pain states, most evidence suggests that the balance is tipped heavily in favour of pro-inflammatory processes. Therefore the challenge facing clinicians and pain researchers is to uncover novel ways to stimulate endogenous anti-inflammatory machinery, which may be more physiological than completely abrogating pro-inflammatory processes, which are a prerequisite for nerve regeneration. In this respect, induction of anti-inflammatory cytokine (IL-10, IL-4, TGF-β) and growth factor (GNDF) signalling, or activation of natural suppressor immune cells, such as Th2 or Treg populations, may emerge as the leading candidates to successfully manage chronic neuropathic pain, a debilitating condition which is notoriously refractory to current treatment options.

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