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The Role of Neuroimmune Activation in Chronic Neuropathic Pain and New Targets for Therapeutic Intervention

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Therapies for neuropathic pain directed at receptors and channels on neurons have been disappointing, with many suffering patients achieving only partial relief. This chapter will address an alternate and complimentary approach to thinking about and treating neuropathic pain involving the immune system. It will highlight the role of cytokines and neuroimmune activation in neuropathic pain or nerve trauma and offer a list of therapeutic strategies to address these mechanisms. In keeping with the spirit of the conference that inspired this volume, some of the therapeutic avenues will be unconventional.

Neuroinflammation is defined as the infiltration of immune cells into the site of injury in response to damage of the peripheral or central nervous system. Neuroimmune activation involves endothelial cells, microglia, and astrocytes, which when activated produce cytokines and chemokines and induce the expression of surface antigens to enhance the immune cascade independently of immune cell infiltration to the site of injury (DeLeo and Yeziarski 2001). Peripheral nerve stimulation alone is insufficient to induce neuroimmune activation unless the stimulation is sufficient to result in damage to the nerve (Molander et al. 1997). The involvement of cytokines as chemical messengers of tissue trauma or illness is required. Wallerian degeneration can be viewed as the inflammatory response to axonal injury and is primarily attributable to the production of cytokines and chemokines from Schwann cells (Shamash et al. 2002). These cytokines and chemokines regulate macrophage responses and may facilitate myelin breakdown and clearance

(Perrin et al. 2005). Thermal hyperalgesia in mice following a chronic constriction injury (CCI) is temporally related to the onset of Wallerian degeneration, to macrophage infiltration, and to the presence of endoneurial tumor necrosis factor (TNF)- α , whereas mechanical allodynia is temporally related to nerve fiber regeneration. This point is emphasized in a strain of mice demonstrating delayed Wallerian degeneration, which display diffuse alterations in cytokine production and an abnormal onset and duration of neuropathic pain behaviors in response to nerve injury (Sommer and Schafers 1998; Shamash et al. 2002).

Damage to a peripheral nerve can initiate the recruitment of inflammatory cells to the site of injury. Macrophages can remove debris and secrete chemokines to recruit other immune cells and stimulate tissue regeneration (Stoll and Jander 1999). The predominant subtype of T cell defines the type of immune response to injury. Upon activation, CD4⁺ T cells can differentiate into T-helper (Th) cells of two types. Type 1 (Th-1) cells generally produce proinflammatory cytokines, such as interferon (IFN)- γ , interleukin (IL)-1 β , and TNF- α , that can augment the cellular immune response. Type 2 (Th-2) T-helper cells produce anti-inflammatory cytokines such as IL-4 and IL-10 that mediate humoral immunity, suppress macrophage function, and inhibit the production of pro-inflammatory cytokines. Following injury, T cells can infiltrate the injured sciatic nerve and contribute to thermal hyperalgesia and mechanical allodynia. Experiments utilizing passive transfer of Th-1 cells markedly enhanced pain hypersensitivity, while passive transfer of Th-2 cells had a modest inhibitory effect on pain hypersensitivity. The long-term presence of T cells in the injured nerve has been found to coincide with neuropathic pain behavior (Moalem et al. 2004). Th-1 and Th-2 subsets differentially affect sensitivity to neuropathic pain, which suggests that manipulation of the T-cell subsets and their response to injury could be a target for pain therapy.

Cytokines are not typically stored in cells but are regulated by gene transcription following a stimulus. In addition, the messages for most cytokines are unstable, and cytokine synthesis is therefore transient. Cytokines are pleiotropic in multiple ways. One cytokine can act on different cell types, limiting the utility of therapeutic administration of cytokines due to the development of many unwanted effects. Cytokines also are redundant in that multiple cytokines can exhibit the same functional effects. Antagonists to a single cytokine or alteration of a cytokine gene may have limited effects because other cytokines may be capable of compensating for the resulting loss of function. Cytokine production can promote the synthesis of other cytokines in a cascade mechanism. This secondary cytokine production can antagonize the first cytokine or act in synergy to enhance the effect. Often

the outcome will depend upon the milieu surrounding the site of injury or trauma. Cytokines can exert their actions in a local autocrine or paracrine manner or act systemically in an endocrine fashion. Cytokines typically act by binding to ultra-high-affinity receptors on the cell surface such that only a few molecules are needed to effect a response. The concentration of cytokines in a tissue or in the plasma is often low, and thus the inability to detect a particular cytokine does not mean that it is not acting in a given situation. With the exception of TNF- α and chemokines (molecules that stimulate the migration of other immune cells such as macrophages), cytokines exert their actions by causing changes in gene expression, by triggering production of new molecules, or by altering the function of the target cell (Abbas and Lichtman 2003).

INTERLEUKIN-1

IL-1 β levels increase early in both inflammatory and nerve injury animal models of pain (Murphy et al. 1995; Lee et al. 2004). Injection of exogenous IL-1 β can induce thermal hyperalgesia and mechanical allodynia in the rat paw (Wagner and Myers 1996a; Sorkin et al. 1997; Sachs et al. 2002). However, direct nerve injury to the spinal root induces glial activation and enhances expression of IL-1 β bilaterally in the dorsal and ventral horns of the spinal cord. This finding suggests a central neuroimmune reaction (Hashizume et al. 2000a) that is not confined to the innervation pattern of the nerve root. IL-1 β is found in dorsal root ganglion (DRG) neurons and in Schwann cells. Intraneural injections of IL-1 β can produce thermal hyperalgesia and mechanical allodynia, exhibiting a bell-shaped curve with dose-dependent activation at physiological concentrations and reduced firing rates at higher concentrations (Zelenka et al. 2005).

The type-I IL-1 β receptor is highly expressed in intrinsic neurons of the dorsal horn in superficial laminae (Samad et al. 2001). Following peripheral inflammation, central injection of the naturally occurring IL-1 β -receptor antagonist (IL-1RA) or an inhibitor of the IL-1 β -converting enzyme blocked the induction of cyclooxygenase (COX)-2 protein and prevented mechanical hyperalgesia. IL-1 β produced in peripheral tissues can enter the central nervous system (CNS) through a specific, saturable transport system in the blood-brain barrier (Pan and Kastin 2004) and induce central COX-2 isoenzyme expression. Indirect inhibition of COX-2 expression through disruption of IL-1 β production or action may lead to safer anti-inflammatory approaches to pain than those which directly target the COX-2 isoenzyme.

IL-1 β can attenuate the analgesic effects of morphine and may be to morphine tolerance mechanisms. Mice rendered genetically nonresponsive to IL-1 β (through overexpression of IL-1RA or loss of the IL-1 β receptor) have enhanced analgesia from morphine (Shavit et al. 2005). This finding is consistent with reports of IL-1 β elevation in the cerebrospinal fluid of animals following chronic morphine administration. Shavit and colleagues (2005) suggest that the analgesic effect of chronic morphine administration could be enhanced through inhibition of IL-1b function within the spinal cord. Dynorphin-induced hyperalgesia and allodynia involve the action of IL-1b and activation of nuclear factor (NF)-kB and are reversed by IL-10 and IL-1RA (Laughlin et al. 2000).

TUMOR NECROSIS FACTOR ALPHA

TNF- α activity depends on its expression levels, on its activation from an inactive precursor, and on the availability of its receptors. The zinc-dependent protease family of matrix metalloproteinases (MMPs) releases TNF- α from its precursor and facilitates TNF receptor sequestration (Shubayev and Myers 2000). Within this group of proteases are the basal lamina-degrading gelatinases that play a role in Wallerian degeneration by (1) allowing macrophage transit through the Schwann cell basal lamina, the blood vessels, and the blood-nerve barrier; (2) inducing axonal degeneration; and (3) inducing local edema. Following CCI, two peaks of TNF- α are noted corresponding to peaks in pain behavior (Shubayev and Myers 2000). The first peak is derived from Schwann cells, local resident macrophages, and mast cells and corresponds with an increase in MMP-9 activity. The second peak, appearing 5 days after injury, probably represents TNF- α release from hematogenously derived macrophages and corresponds with an increase in MMP-2 activity. Therapeutically, MMP inhibitors and TNF- α protease inhibitor show variable abilities to downregulate TNF- α and TNF-receptor protein levels (Williams et al. 1996; Chandler et al. 1997; Sommer et al. 1997; Glaser et al. 1999).

At high concentrations, TNF- α can trimerize and form a membrane-inserted channel (Baldwin et al. 1996). The importance of this process in vivo has yet to be determined. Specific antibodies to one or more of the TNF- α receptors can block many TNF- α actions, so the mass action membrane insertion appears to be of minor importance.

TNF- α acts predominantly through two main receptors. The type 1 TNF receptor (TNFR1) is constitutively expressed, while the type 2 TNF receptor (TNFR2) can be induced (Vandenabeele et al. 1995). Within a few days

after a crush-type or CCI injury to the murine sciatic nerve, TNFR1 expression modestly increases transiently at the site of injury (George et al. 2005) and mediates thermal hyperalgesia (Sommer et al. 1998b). TNFR2, on the other hand, is markedly increased immediately after injury and remained elevated for at least 28 days. When TNF- α binds to TNFR1 it is preferentially internalized, while interaction with TNFR2 leads to shedding of the complex. Thermal hyperalgesia has been attributed to TNFR1 only (Sommer et al. 1998b). The large increase in TNFR2 and shedding after TNF- α binding suggests that it can act as a TNF- α antagonist because the soluble TNF- α /TNFR2 complex is inactive (George et al. 2005). Antibodies against TNFR1 (but not TNFR2) reduce thermal hyperalgesia and mechanical allodynia in mice that have undergone CCI (George et al. 1999). The predominant subtype of receptor expressed on the target cells, either constitutively or induced by other cytokines, shapes the cellular response to TNF- α (Fontaine et al. 2002; Yang et al. 2002).

The main sources of TNF- α are glial cells and resident macrophages; it is not clear to what extent it is expressed by DRG neurons. TNF- α is constitutively expressed in the sciatic nerve tissue and is induced along with IL-1 β in resident macrophages after injury (Brown et al. 1991; Griffin et al. 1993; George et al. 1999). Peripheral nerve injury or endotoxin challenge induces a large increase in TNF- α in non-neuronal DRG cells (possibly including macrophages, Schwann cells, endoneurial fibroblasts, and mast cells) (Ohtori et al. 2004). TNF- α and TNFR1-expressing microglial cells or astrocytes surround TNFR1-positive neurons (Ohtori et al. 2004). TNFR1 (but not TNFR2) mRNA is constitutively expressed in DRG neurons, in the dorsal root, in afferent fibers, in the dorsal root entry zone, and in laminae I and II of the dorsal horn (Holmes et al. 2004). After endotoxin challenge, TNFR1 was upregulated in neuronal and non-neuronal cells, but TNFR2 is only induced in non-neuronal cells (Li et al. 2004). The near-ubiquitous presence of TNFR1 in sensory neurons may belie their role as immunosensors (Weihe et al. 1991). A linkage to immune-competent cells in the periphery may allow activation during inflammation and immune reactions and may drive illness behavior and fatigue syndromes (Li et al. 2004). TNF- α may act in an autocrine fashion among microglia, inducing its own production and that of microglial IL-10, potentially acting in a negative autocrine feedback loop (Kuno et al. 2005). TNF- α is anterogradely transported in sensory fibers to innervated muscle, consistent with the observation that axotomized and intact muscle afferents develop ongoing activity following a peripheral nerve lesion (Michaelis et al. 2000). While TNF- α may be present in small neurons at baseline, its increase following injury has been observed exclusively in medium to large DRG neurons (Schafers et al. 2002). In this study, DRG

cells were considered the primary source of the anterogradely transported TNF- α . TNF- α may also be taken up through the participation of TNFR1 and TNFR2 receptor systems (Shubayev and Myers 2001). Conflicting data regarding retrograde transport of TNF- α (Shubayev and Myers 2001; Schafers et al. 2002) may be related to the tracer used.

TNF- α immunoreactivity is greater in Schwann cells of patients with painful neuropathy compared to those with nonpainful neuropathy (Empl et al. 2001). Soluble TNFR1 is also elevated in the serum of patients with mechanical allodynia as compared to patients without allodynia (Empl et al. 2001). TNF- α is associated with phagocytosing macrophages, most intensely in acute vasculitis (Oka et al. 1998) and chronic inflammatory demyelinating neuropathies, but not in non-inflammatory neuropathies (Lindenlaub and Sommer 2003). In animal studies, TNF- α applied to the sciatic nerve (Wagner and Myers 1996a) or the dorsal root (Sorkin et al. 1997) increases firing in both C and A δ fibers and reduces the mechanical threshold for paw withdrawal when injected intradermally (Cunha et al. 1992). TNF- α production in Schwann cells after nerve injury (Wagner and Myers 1996b) may mediate injury-associated signals to afferent fibers and could ultimately lead to neurodegeneration. Within hours of nerve injury, resident Schwann cells, fibroblasts, and endothelial cells in the endoneurium upregulate the production of TNF- α (Wagner et al. 1998). Anti-TNF- α antibodies reduce thermal hyperalgesia in both CCI and partial nerve transection mouse models of painful neuropathy, but mechanical allodynia is only reduced by intraoperative administration (Sommer et al. 2001). The TNF- α inhibitor etanercept inhibits both allodynia and p38 phosphorylation only when given before injury and intrathecally (Svensson et al. 2005). Systemic delivery of etanercept is only 50% effective. However, mice deficient in TNFR1 develop mechanical allodynia, but not thermal hyperalgesia, after CCI (Sommer et al. 2001). Enhanced mechanical allodynia is also observed in transgenic mice (with astrocytic targeted chronic expression of TNF- α) following peripheral nerve injury (DeLeo et al. 2000), suggesting a central role of TNF- α in neuropathic pain.

Several animal studies have implicated TNF- α and other cytokines in the etiology of radiculopathic pain and herniated nucleus pulposus-induced nerve damage (Igarashi et al. 2000; Olmarker and Rydevik 2001). Direct application of nucleus pulposus cells or of TNF- α or other cytokines is associated with increased and spontaneous neural activity in spinal root afferent fibers and pain behavior in animals (Olmarker and Larsson 1998). Human herniated disk samples can produce several inflammatory mediators such as TNF- α , IL-1 α , IL-1 β , IL-6, and granulocyte-monocyte colony-stimulating factor (Takahashi et al. 1996). TNF- α , when applied to exposed nerve

roots, has effects that are even more pronounced than those of nucleus pulposus material (Aoki et al. 2002). TNF- α will produce myelin injury (Olmarker et al. 1993, 1994; Kayama et al. 1996), axonal degeneration (Kayama et al. 1996), nerve conduction block (Olmarker and Rydevik 2001), thrombus formation (Olmarker and Rydevik 2001), increased vascular permeability (Olmarker and Rydevik 2001), thermal hyperalgesia (Olmarker et al. 1996; Sorkin et al. 1997), mechanical allodynia, and ectopic discharges when applied in low physiological concentrations to the nerve root (Onda et al. 2002). In studies of nucleus pulposus material applied to exposed nerve roots with extracellular recordings of wide-dynamic-range neurons, TNF- α antibodies markedly reduce after-responses following noxious stimulation, while evoked responses are not inhibited (Onda et al. 2003). Application of TNF- α antibodies to a rat nerve root can partially prevent the nucleus pulposus-induced abnormal nerve discharges (Onda et al. 2003) and nucleus pulposus-induced histological changes (Murata et al. 2004). Nerve roots injured by compression are sensitized to the excitatory effects of TNF- α application (Liu et al. 2002; Schafers et al. 2003).

TNF- α is localized in neurons in several norepinephrine-rich areas of the brain including the locus ceruleus and the hippocampus (Covey et al. 2002). TNF- α in these regions acts in concert with α_2 -adrenergic receptors to inhibit norepinephrine release. During persistent neuropathic pain induced by CCI, TNF- α levels increase in these brain regions, and there is greater α_2 -adrenergic receptor/TNF- α -induced inhibition of norepinephrine release, resulting in decreased norepinephrine function (Covey et al. 2000). Infusion of anti-TNF- α antibodies reverses the thermal hyperalgesia and hyperalgesia. In naive rats, intracerebroventricular infusion of recombinant TNF- α can induce thermal hyperalgesia and mechanical allodynia (Ignatowski et al. 1999). Activation of α_2 -adrenergic receptors increases TNF- α production in primary hippocampal neurons (Renauld and Spengler 2002). Administration of a tricyclic antidepressant, either desipramine or amitriptyline, reduces neuron-localized TNF- α (Ignatowski et al. 1997). After chronic administration of desipramine, zimelidine (Nickola et al. 2000), or amitriptyline (Reynolds et al. 2004a), the TNF- α inhibition of norepinephrine release reverses to facilitation. TNF- α has also been implicated in the antidepressant activity of desipramine in the forced swim model of depression (Reynolds et al. 2004b). These findings suggest that TNF- α is involved in therapeutic actions of tricyclic antidepressants in pain and depression. In the periphery, α_2 -adrenergic receptor stimulation increases the endotoxin-stimulated production and release of TNF- α in macrophages (Spengler et al. 1990), whereas β -adrenergic receptor stimulation decreases TNF- α production in macrophages and microglia (Spengler et al. 1994; Kaneko et al. 2005). Thus, the

sympathetic system may influence the somatosensory system through a cytokine mechanism.

Neuronal apoptosis or cell death has been reported in the dorsal horn of rats after CCI or axotomy (Sugimoto et al. 1990; Whiteside and Munglani 2001). Such apoptosis may occur in a small subpopulation of cells because the total cell count does not significantly decrease (Polgar et al. 2004). Selective dorsal horn neuronal loss may contribute to pain hypersensitivity in these animals through a temporary loss of inhibitory neurons (Ibuki et al. 1997), although this conclusion has recently been challenged (Polgar et al. 2003). An understanding of this apoptotic response and a means to prevent it could have potential value in treating neuropathic pain and its consequences. TNF- α enhances AMPA/kainate-receptor-mediated neuronal injury and plasticity by inducing a rapid upregulation of calcium-permeable AMPA/kainate channels (Ogoshi et al. 2005) and an increase in cell-surface AMPA receptors (Beattie et al. 2002). TNF- α may also be indirectly involved in neurodegeneration and plasticity by inducing the release (Bezzi et al. 2001) and inhibiting the uptake (Fine et al. 1996) of glutamate by astrocytes and microglia. Astrocytes normally take up glutamate to reduce neuronal exposure, but this uptake is inhibited in a dose-dependent manner by TNF- α (Hu et al. 2000; Shaked et al. 2005). Glutamate is also released by activated microglia to act via microglial NMDA receptors in an autocrine fashion to stimulate release of TNF- α (Piani et al. 1992; Bezzi and Volterra 2001). Combined deletion of the genes for TNF- α receptors 1 and 2 almost completely prevents motor neuron cell death after facial axotomy in the adult mouse. These data highlight the role of TNF- α and both subtypes of TNF receptor in the early phase of neuronal cell loss following traumatic neuronal injury (Raivich et al. 2002). The degree of neurodegeneration may be more extensive if the supply of TNF- α is sustained for longer periods.

INTERLEUKIN-6

TNF- α and IL-1 β play important roles in the initiation of persistent neuropathic pain, whereas delayed IL-6 production is a factor in the maintenance of such pain. This pattern is reversed in axotomy models, with IL-1 β and IL-6 peaking earlier than TNF- α (Murphy et al. 1995). Interleukin-6 is one of a family of several similar molecules that act in both paracrine and endocrine fashion. Members of the IL-6 family are grouped according to their three-dimensional shape; they can interact with unique receptors, but they all act through a common mediator, gp130 (Gadient and Otten 1997). When dimerized by an activated receptor, gp130 can activate intracellular

kinases and influence gene transcription. Members of the IL-6 family include leukemia inhibitory factor, ciliary neurotrophic factor, IL-6, and IL-11 (De Jongh et al. 2003). Members of the IL-6 family, like other cytokines, are pluripotent, with broad effects on hematopoiesis, immune response, and inflammation as well as on the induction of acute-phase reactants. Acute-phase reactants are plasma proteins, primarily synthesized in the liver, whose plasma concentrations increase in response to trauma, infection, or direct action of cytokines. These proteins play a role in the innate immune response (Abbas and Lichtman 2003).

IL-6 acts through a specific receptor that is expressed on lymphocytes, macrophages, and other immune cells. Within the nervous system, mRNA for both IL-6 and its receptor are expressed in the hippocampus, neocortex, cerebellum, neurons, and astrocytes (Gadient and Otten 1997). The IL-6 receptor is easily shed by proteolysis; however, the resulting soluble receptor can still complex with many cell types. Thus, the influence of the IL-6 complex is expanded by the immune cell supply of IL-6 receptor. Most soluble receptors act as antagonists, but those for IL-6 function as agonists when coupled with IL-6, a process known as "trans-signaling" (Heinrich et al. 2003). IL-6 mRNA is expressed at low levels in spinal cord neurons in the dorsal horn and is upregulated following peripheral nerve injury, where it may play a role in nociceptive processing at the spinal level (Arruda et al. 1998). IL-6 mRNA is not detected in the normal rat sciatic nerve, but it can be induced in Schwann cells distal to a sciatic nerve crush (Bolin et al. 1995). Upregulation of the IL-6 and IL-6 receptor message is observed in medium to large sensory neurons following nerve injury or axotomy (Bolin et al. 1995; Lee et al. 2004). While gp130 is found in nearly all DRG neurons and is constitutively expressed (Obreja et al. 2002), only 33% of such neurons in one study were able to form functional receptor complexes (Segond et al. 2005). IL-6 is localized in human DRG in more than 75% of cells and co-localized with substance P and CGRP in more than 60% of cells (Nordlind et al. 2000). Membrane depolarization and neuronal activity itself can induce IL-6 in neurons (Sallmann et al. 2000). The depolarization-responsive factor appears to be the glucocorticoid response element-2, possibly accounting for the net inhibitory effect of glucocorticoids on IL-6 transcription (Ray et al. 1990).

IL-6 can exert both neuroprotective and neurodegenerative actions (Gadient and Otten 1997; Okada et al. 2004). IL-6 expression in neurons contributes to activation of glial cells (Klein et al. 1997) and supports the regeneration of peripheral nerves after injury (Zhong et al. 1999); however, chronic exposure can lead to neurodegeneration (Campbell et al. 1993; Qiu et al. 1998). Intrathecal or intraplantar administration of IL-6 in rats with a

CCI mononeuropathy increases cold allodynia (Vissers et al. 2005). Intrathecal injection of IL-6 soluble receptor or IL-6 neutralizing antibody attenuates nerve-injury-induced mechanical allodynia (DeLeo et al. 1996). In other settings, exogenously applied IL-6 reduces neural firing in a model of mechanical allodynia (Flatters et al. 2003). IL-6 does not directly stimulate neurons, but the IL-6 receptor complex can potentiate heat-activated ionic currents in nociceptors through the phosphorylation of TRPV1 molecules (Obreja et al. 2005). Some of the variability of response to IL-6 may stem from the lack of baseline IL-6 receptors in neurons and from the need to stimulate these neurons with a combination of IL-6 and its receptor. In IL-6 knockout animals, mechanical hyperalgesia is attenuated without an effect on thermal hyperalgesia (Ramer et al. 1998). The pleiotropic nature of the IL-6 family is both a challenge and an opportunity. In the same chronic pain condition, several of the family members are present but may act in opposing manners. In certain arthritis models, for example, IL-11 is anti-inflammatory and IL-6 is pro-inflammatory (Gadient and Patterson 1999). The actions of IL-6 may be state dependent and, as Flatters and colleagues (2003, 2004) have proposed, may intriguingly be both pro-inflammatory and antinociceptive under certain conditions. IL-6 (Borner et al. 2004) and other cytokines (Ruzicka et al. 1996; Kraus et al. 2001) can induce the expression of μ -opioid receptors, and morphine can increase IL-6 levels in the plasma (Houghtling et al. 2000) and spinal cord (Raghavendra et al. 2002).

IL-6, whether induced by injury or exogenously applied, forms sympathetic baskets in the DRG (Ramer et al. 1999); IL-6 knockout mice have greatly reduced sympathetic basket formation (Ramer et al. 1998). The invasion of DRG by sympathetic nerves has been linked to mechanical allodynia (Ramer et al. 1999). IL-6 may contribute to human pain states. Herniated lumbar disks produce increased amounts of IL-6 relative to nonherniated disks (Kang et al. 1996) and are associated with mechanical hyperalgesia following surgery. An IL-6 gene variation associated with increased expression and plasma levels of IL-6 has been identified in patients with herniated disks characterized by sciatica (Nojonen-Hietala et al. 2005). Patients with persistent pain 8 weeks after discectomy have a significantly elevated IL-6 level compared to pain-free volunteers (Geiss et al. 1997).

INTERLEUKIN-10

IL-10 is the prototypical anti-inflammatory cytokine. It can act in an antagonistic manner to reverse or oppose many of the actions of pro-inflammatory cytokines. IL-10 can inhibit the production, release, and activity of

TNF- α , IL-1 β , and IL-6; it can inhibit p38 MAP kinase and NF- κ B activation and can downregulate the receptors for pro-inflammatory cytokines (reviewed in Watkins and Maier 2003). IL-10 can be secreted from infiltrating macrophages and lymphocytes to suppress ongoing inflammation. Augmentation of IL-10 appears to be attractive for managing neuropathic pain associated with glial activation because IL-10 inhibits only the pathological functions and increased cytokine activity and does not alter basal activity. Gene therapy methods have been developed to augment IL-10 release, but the delivery system is awkward. IL-10 therapy may be complicated because this cytokine can downregulate the expression of its own receptor in an autocrine or paracrine negative feedback system (Ledeboer et al. 2002). Studies with IL-10 knockout animals or administration of anti-IL-10 antibodies have demonstrated decreased thermal hyperalgesia, suggesting that endogenous IL-10 contributes to nociception (Tu et al. 2003). Clearly, more work is needed to elucidate this area.

FRACTALKINE

Another potential signal for glial activation comes from the neuron itself. The chemokine fractalkine is exclusively expressed on neurons, particularly spinal neurons and sensory afferents, and is clipped off during high levels of neural stimulation to act on fractalkine receptors located exclusively on microglial cells (Verge et al. 2004). Thus, fractalkine represents a true neuron-to-glia messenger (Bacon and Harrison 2000; Hatori et al. 2002). Intrathecal administration of a fractalkine-neutralizing antibody prevents the onset of mechanical allodynia in sciatic inflammatory neuropathy and is even effective when administered 5–7 days after the injury. Intrathecal administration of fractalkine produces dose-dependent mechanical allodynia and thermal hyperalgesia (Milligan et al. 2004).

BEHAVIORAL ISSUES

Cytokine involvement in chronic pain derives from a much broader view of injury-related behavior termed the “sickness response.” This response is composed of a wide range of changes initiated by a peripheral immune or inflammatory challenge (Maier and Watkins 1998). The sickness response includes fever, increased white blood count, activation of the hypothalamic-pituitary-adrenal axis, sympathetic nervous system arousal, decreased social interaction, decreased food and water intake, and increased sensitivity to pain (Wieseler-Frank et al. 2005). Chronic pain is often associated with

behavioral and cognitive alterations. A marked dysregulation (increased levels and shifted circadian cycle) of IL-6 secretion has been reported in patients with major depressive disorder (Alesci et al. 2005). Increased levels of IL-6 following discectomy are associated with depressed mood, increased self-reported stress, and altered morning cortisol secretion (Geiss et al. 1997). This finding is consistent with other reports of behavioral changes associated with prolonged elevated IL-6 levels (Geiss et al. 1997; Papanicolaou et al. 1998). Infusion of IL-1 β or TNF- α for adjuvant cancer chemotherapy is associated with an incidence of nearly 50% of pain syndromes or complaints of pain and tenderness at the injection site (Kemeny et al. 1990; Del Mastro et al. 1995; Elkordy et al. 1997). Immune activation also is associated with a decrease in mood and cognitive function, a common adverse effect of cytokine administration for cancer (Meyers 1999). IL-6 is also associated with inhibition of certain types of learning and memory (Balschun et al. 2004). These findings suggest that some of the behavioral consequences of chronic pain may have an origin in increased IL-6 levels. Conclusions from exogenous cytokine administrations are limited due to the general illness of the patients and the large doses of cytokines that are given, which may contribute to physical illness and could account in part for the behavioral and cognitive changes observed. To address this problem, Reichenberg and colleagues (2001) administered doses of endotoxin to trigger an inflammatory response just short of physical symptoms in normal subjects. After the administration of endotoxin, the subjects reported significantly increased levels of anxiety and depressed mood and decreases in verbal and nonverbal memory. All these behavioral symptoms were significantly correlated with elevated levels of cytokines including TNF- α , IL-1RA, IL-6, and soluble TNF- α receptor (Reichenberg et al. 2001).

GLIA

Glia are recognized as essential participants in the initial and sustained response to injury and in the generation of neuropathic pain (reviewed in Covey et al. 2000; Watkins and Maier 2003; Wieseler-Frank et al. 2004). DeLeo and associates have shown over several studies that microglia are involved in the initial phase of pain hypersensitivity followed by astrocytic involvement in the maintenance phase (Raghavendra et al. 2003; Tanga et al. 2004).

Microglia are the principle sources of the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α as well as the anti-inflammatory cytokines IL-10 and IL-1RA (Aloisi 2001). Microglia, part of the innate immune system, can

initiate the response to injury that can be shaped and controlled by T cells, part of the adaptive immune system. The disruption of this highly controlled and balanced system of interaction between the innate and adaptive immune systems could lead to unregulated inflammation and neurodegeneration and chronic pain. The pluripotentiality of microglia allows these cells to engage in a neuroimmune dialogue (Shaked et al. 2005) that is regulated by the adaptive immune system in physiological conditions but converts to a destructive phenotype in pathological situations. Microglia can therefore be either protective or destructive, depending upon the immune and chemical microenvironment.

What is the link between nerve injury and microglial activation? Partial but significant reduction in hyperalgesia and allodynia behavior can be accomplished by interfering with the function of Toll-like receptor-4 (TLR-4) in microglia (Tanga et al. 2005). TLR-4 functions in the innate immune system as a stable pattern recognition receptor for invariant structures of pathogens. TLR-4 are transmembrane receptors with a cytoplasmic signaling domain; they are similar to the cytoplasmic portion of the IL-1 receptor and have the ability to activate the same pathways as IL-1 β . Activation of TLR-4 is linked to activation of nuclear transcription factor kappa B (NF- κ B) and to induction of COX-2 and other inflammatory mediators. TLR-4 occurs exclusively on microglia in the rat CNS (Lehnardt et al. 2003). TLR-4 can be activated by bacterial wall molecules such as endotoxins or lipopolysaccharide and by endogenous ligands such as heat shock proteins, proteoglycans, and saturated fatty acids released after neural injury and degeneration (Hwang 2001; Lee et al. 2001). In addition, Toll-like receptors control the activation of antigen-specific Th-1 and Th-2 immune responses. Mice lacking TLR4 function either through genetic alteration or through the use of a TLR-4 antisense oligodeoxynucleotide display significantly reduced (but not absent) pain hypersensitivity and reduced expression of mRNA for microglial markers of activation and proinflammatory cytokines (IFN γ , IL-1 β , and TNF- α) following L5 nerve transection (Tanga et al. 2005).

Leukocyte trafficking into the spinal cord increases over time following nerve root injury (Rutkowski et al. 2002). Intercellular cell adhesion molecule 1 (ICAM-1) and platelet endothelial cell adhesion molecule (PECAM) can facilitate hematogenously derived leukocyte infiltration to the CNS. Peripheral inflammation mediated by proinflammatory cytokine activation of the NF- κ B signaling pathway can trigger ICAM and PECAM expression, whereas nerve injury precipitates expression of ICAM and PECAM as well as major histocompatibility complex (MHC) class II, coincident with the development of bilateral mechanical hypersensitivity (Sweitzer et al. 2002). Infiltrating leukocytes can interact with microglia expressing MHC class II

molecules and can act as antigen-presenting cells. Following presentation of antigen in the context of the appropriate MHC molecule, full T-cell activation depends upon the presence of a second positive stimulus or costimulatory factor. The B7 family of costimulators is upregulated in neural tissue damaged through trauma or inflammation. B7.1 and B7.2 are both single-chain glycoproteins, with two extracellular Ig-like domains, a transmembrane segment, and a cytoplasmic tail (Abbas and Lichtman 2003). The B7.2 molecule has been associated with protective immunity, whereas B7.1, another costimulatory molecule, has been associated with destructive immunity such as that observed in multiple sclerosis. Inhibition or elimination of microglial MHC class II expression can reduce pain following nerve transection (Sweitzer et al. 2002). Nociceptive transmitters, substance P, and glutamate can differentially modulate glial MHC expression in a tissue-specific manner (McCluskey and Lampson 2001). The B7.2 molecule is expressed in microglia and not in astrocytes (Menendez et al. 1997); it is strongly upregulated by painful peripheral nerve injury (Rutkowski et al. 2004), axotomy (Bohatschek et al. 2004), endotoxin, IL-1 β , TNF- α , and IFN γ and is inhibited by IL-10, PGE₂, and cyclic AMP-elevating agents (Menendez et al. 1997). Increased B7.2 expression can be prevented by genetic deletion of receptors for IL-1 β or TNF- α (R1 or R2) (Bohatschek et al. 2004). This neuroimmune activation is accompanied by microglial activation and by the release of pro-inflammatory cytokines. The challenge to pain therapeutics is to inhibit the destructive immunity and pro-inflammatory cytokines without compromising the protective immunity elicited by B7.2 and associated molecules. Glutamate can inhibit antigen presentation (Angelini et al. 2002). Consequently, reduction of environmental glutamate by microglia can lead to improved antigen presentation and subsequent activation of T cells, leading to the production of IFN γ and further glutamate uptake (Shaked et al. 2005). Microglia can increase glutamate transporter I expression after nerve axotomy (Lopez-Redondo et al. 2000) and can play an early role in reestablishing glutamate homeostasis. Metabotropic glutamate receptors, present on both microglia and T cells, can sense the environmental levels of glutamate (Heuss et al. 1999; Storto et al. 2000) and synergize with IL-1 β to enhance IL-6 release (Aronica et al. 2005). This regulation occurs at the transcriptional level and provides an additional means of regulation of the inflammatory response (Aronica et al. 2005).

Astrocytes. Activated microglia release proinflammatory substances, including cytokines, and activate astrocytes. Once activated, astrocytes can maintain hyperalgesia and allodynia independent of microglia. The point of conversion to astrocyte-driven sensitization appears to occur within the first 24 hours following injury. Virtually all synapses are enclosed by an astrocyte

casing and allow for a bidirectional excitatory interchange (Araque et al. 1999; Vesce et al. 1999). Astrocytes have a “cellular memory” in that intracellular calcium responses are greatly amplified when astrocytes have previously been repetitively stimulated or exposed to strong synaptic activity (Pasti et al. 1997; Carmignoto 2000). This finding could account for the reports of pain reactivation with new injury, especially in cases of complex regional pain syndrome.

Spinal cord astrocytes exhibit extensive gap junction networks that become more extensive in response to high-intensity or high-frequency neural activity, as seen in chronic pain associated with nerve injury. Microglia do not have these connections at baseline, but they can develop them under pathological conditions (Eugenin et al. 2001). A network of signaling among glia could lead to release of glial-derived substances such as cytokines, glutamate, and adenosine triphosphate at distances far removed from the initial site of excitation and in patterns not predicted by neuroanatomy. In models of inflammatory and traumatic neuropathic pain, low doses of carbenoxolone, a nonspecific, reversible uncoupler of gap junctions (Davidson et al. 1986; Davidson and Baumgarten 1988), dramatically suppresses contralateral or mirror-image allodynia and suppresses IL-1 β and IL-6 production (Spataro et al. 2004). The ipsilateral pain is reduced only at higher carbenoxolone doses. The density of astrocytes and gap junction protein levels is high in the superficial dorsal horn, across the breadth of the spinal cord, and around the circumference of the spinal cord, thus providing a near-continuous signaling path for astrocyte communication and for the potential activation of distant neuronal systems. Astrocyte networks may account for the increased cytokine expression in non-operated nerves found in a transected sciatic nerve model in rats (Ruohonen et al. 2002), casting doubt on studies that have used the nonoperated nerve as an internal experimental control.

MOLECULAR MECHANISMS OF CYTOKINE FUNCTION AND GLIAL ACTIVATION

The link between cytokine binding, glial cell activation, and new cytokine production starts with activation of intracellular protein kinases, especially the mitogen-activated protein kinases (MAPKs) (Watkins and Maier 2003). The MAPK family has at least three groups: extracellular signal-related kinases (ERKs) (Grewal et al. 1999); p38 kinases (Ono and Han 2000); and c-Jun, N-terminal protein kinases (JNKs), also known as stress-activated protein kinases (Gupta et al. 1996). ERKs are primarily activated by neuronal activity, p38 kinases are mainly activated by cytokines, and JNKs are activated

by cytokines and by cellular stress or injury. All may contribute to the various post-injury responses of a nerve.

Activated p38 MAPK is present in spinal microglia and in small TNF- α -positive DRG neurons (Schafers et al. 2003), but not in spinal cord neurons or astrocytes (Tsuda et al. 2004). It is elevated in DRG neurons and in dorsal horn microglia after peripheral inflammation, peripheral nerve injury, and spinal nerve injury (Watkins and Maier 2003). A specific inhibitor of p38 kinase (SB203580), given intrathecally at the time of nerve injury and for 7 to 14 days thereafter, inhibits the development of tactile allodynia (Schafers et al. 2003; Tsuda et al. 2004). CNI-1493, another p38 kinase inhibitor, can reverse established allodynia induced by inflammation of the sciatic nerve (Milligan et al. 2003).

Stimulation of microglia by TLR-4 activation or by TNF- α induces a rapid and lasting activation of the JNK2 isoform. JNK2 may also be responsible for differentiation of T cells into the Th-1 lineage (Yang et al. 1998). Direct inhibition of all JNKs by SP600125, a nonspecific JNK inhibitor, reduces endotoxin induction of activator protein (AP)-1 target genes coding for several inflammatory mediators such as COX-2, TNF- α , and IL-6 (Waetzig et al. 2005).

Transcription factors are the link between receptor-driven cytoplasmic signaling events and changes in gene expression. The transcription factors NF- κ B and AP-1 are essential for the induction of genes involved in inflammation. NF- κ B is an inducible transcription factor that regulates the expression of various genes involved in the inflammatory and immune responses. Many of the pro-inflammatory cytokine genes contain binding sites for NF- κ B (De Bosscher et al. 2003). In the resting state, NF- κ B is inactivated by the endogenous inhibitor I- κ B. Upon cellular stimulation, I- κ B is phosphorylated by I- κ B kinase (IKK) and degrades, freeing NF- κ B to enter the nucleus. Activation of NF- κ B has been described in models of traumatic spinal cord injury (Bethea et al. 1998), nerve injury, and neuropathic pain (Ma and Bisby 1998). Activated NF- κ B is localized to DRG neurons and Schwann cells following partial sciatic nerve injury (Ma and Bisby 1998). Employing an endoneurially applied DNA decoy strategy, Sakue et al. (2001) inhibited NF- κ B activation immediately following L5 spinal nerve ligation and prevented the development of thermal hyperalgesia and the expression of cytokines TNF- α , IL-1 β , IL-6, IFN γ , and of ICAM-1 mRNAs in the injured DRG for up to 2 weeks. Tegeder and colleagues (2004) used a potent inhibitor of IKK (S1627) to block the IL-1 β activation of NF- κ B. S1627 had no effect on baseline nociception or on acute inflammatory hyperalgesia due to formalin, but it did reverse the thermal and mechanical hyperalgesia in inflammatory pain and tactile and cold allodynia in a CCI model of neuropathic

pain. Inhibition of IKK was more effective than nonsteroidal anti-inflammatory drugs and was effective in both reversing and preventing hyperalgesia and allodynia. S1627 did not fully inhibit NF- κ B, which may be advantageous because complete inhibition of NF- κ B might cause too high a rate of adverse effects.

THERAPEUTIC INTERVENTIONS

The systems and mechanisms above represent a departure from the traditional thinking about neuropathic pain. Targeting therapies toward cytokines, glia, or infiltrating immune cells is a new approach for pain therapy, although it has already been employed with some success in oncology and rheumatology. Many neuroimmune mediators participate in a synergy of action and production such that inhibition of one compound will influence the production and action of others. This complex spider web of interactions holds both a promise and challenge for pain therapeutics. In addition, many mediators are involved in activities well beyond pain, including nerve regeneration, control of infection, behavior, and cognitive/memory functions. Therapeutic manipulation of these complex interactions holds promise for addressing the total symptom complex associated with nerve injury and chronic pain. Many of the examples below are from drugs currently on the market but not yet applied to pain conditions. Some may have too narrow a therapeutic index to be routinely used, but in selected patients they may represent an effective strategy. If these targets prove useful, the incentive to develop safer analogues will increase.

Glucocorticoid treatment. The therapeutic value of glucocorticoids has been known for over 60 years, but their utility is tempered by the wide range of adverse events triggered by their prolonged use, including development of tachyphylaxis to steroids, diabetes, impaired wound healing, skin and muscle atrophy, susceptibility to infections, metabolic derangements, and mineral loss from bone (Schacke et al. 2002). Glucocorticoids counteract the production of pro-inflammatory mediators and stimulate the production of anti-inflammatory mediators, including cytokines, through interference in the signaling pathways employing NF- κ B and AP-1. In addition to glucocorticoids, several drugs used to treat chronic inflammatory diseases such as gold salts and high-dose salicylates inhibit NF- κ B activation (Auphan et al. 1995; Yin et al. 1998). When released as part of the stress system, glucocorticoids can serve a negative feedback role to keep in check the immune and inflammatory reactions to trauma.

Glial and immune response inhibitors. Propentofylline, a methylxanthine derivative, was developed to treat Alzheimer's disease but failed to gain approval in Europe. It has a complex set of effects including reduced production of IL-1 β and TNF- α , increased extracellular adenosine, inhibition of glial activation, and release of TNF- α and IL-1 β (Watkins and Maier 2003). When given either systemically or centrally, it both prevents and reverses the development of allodynia in a rat L5 ligation model of neuropathic pain, and it also reduces both microglial and astrocytic activation (Sweitzer et al. 2001). Central administration is more effective, consistent with an effect on spinal glial function.

Intrathecal administration of the tetracycline derivative minocycline can attenuate the induction of mechanical allodynia in the sciatic inflammation model of chronic pain, but it is ineffective if administered after the allodynia is manifest for 7 days (Ledeboer et al. 2005).

The p38 MAPK inhibitor CNI-1493 is structurally similar to an agent clinically available in Japan, gabexate mesylate, a synthetic protease inhibitor that has anticoagulant properties and is used to treat patients with disseminated intravascular coagulation associated with sepsis (Yuksel et al. 2003). Gabexate can also inhibit endotoxin-induced TNF- α production in human monocytes, an effect that is due to an inhibition of both NF- κ B and AP-1 activation (Yuksel et al. 2003). Its utility in chronic pain has yet to be assessed.

In neurodegenerative conditions such as spinal cord injury, the adaptive immune system can be augmented by T-cell-based therapeutic vaccination, literally allowing the body to heal itself (Hauben and Schwartz 2003). Notably, in animal studies the regenerative action of the immune system can be augmented without triggering autoimmune disease (Hauben et al. 2001).

Immunosuppression. Immunosuppressive agents such as methotrexate (Hashizume et al. 2000b) and leflunomide (Sweitzer and DeLeo 2002) attenuate tactile hypersensitivity at doses that inhibit MHC class II expression in rodent radiculopathy and neuropathy models. Leflunomide is approved for clinical use in rheumatoid arthritis and has several anti-inflammatory actions, including inhibition of IL-1 β , TNF- α , TNF- α -activated NF- κ B, and the expression of nitric oxide and COX-2 genes. The drug's potent immunosuppressive effects temper its clinical utility in chronic pain. In a rat mononeuropathy model the active metabolite of leflunomide (A771726), when administered either centrally or peripherally, reduced glial activation and lowered the expression of MHC class II markers at doses that attenuated mechanical allodynia. It did not, however, affect IL-6 levels (Sweitzer and DeLeo 2002). No data were presented for leflunomide's ability to reduce established allodynia.

Methotrexate can inhibit the production of pro-inflammatory cytokines and increase the release of the anti-inflammatory cytokine IL-10. It both prevents and reverses established allodynia and reduces indices of glial activation. One problem is that methotrexate can cause direct astrocytic neurotoxicity. Cyclosporine A, the clinical immunosuppressant, administered at the time of sciatic CCI in the rat, dramatically inhibited the development of heat hyperalgesia (Bennett 2000). Cyclosporine A administration is complicated by weight loss and diarrhea.

Inhibitors of cytokine production and function. Anakinra is a recombinant human IL-1-receptor antagonist approved for use in rheumatoid arthritis (Hallegua and Weisman 2002). No studies have looked at its ability to alter the development or maintenance of other chronic pain states. Successful use of TNF- α monoclonal antibodies or TNF- α -receptor fusion protein has changed the therapy for rheumatoid arthritis and several other chronic inflammatory diseases. The TNF- α monoclonal antibody infliximab has been reported in an open-label study to improve acute sciatica after a single systemic administration, with a reported instant and dramatic reduction in leg pain (Karppinen et al. 2003). Open-label clinical reports have claimed rapid resolution of acute sciatica (involving spinal root irritation) using TNF- α antibodies. One study of 10 patients used intravenous infliximab (Karppinen et al. 2003), and another used subcutaneous etanercept administered once every three days (Genevay et al. 2004) in a different set of 10 patients. All patients had been diagnosed with herniation-induced sciatica. The successful outcomes suggest TNF- α involvement in radiculopathy. In the etanercept study, all outcome variables improved, and the outcome was better than that of a comparison group of 10 patients enrolled in an intravenous steroid treatment study (Genevay et al. 2004).

The presence of IL-6 in several inflammatory conditions has generated much interest in selective inhibition of IL-6 or its receptor. A humanized anti-IL-6 antibody has completed clinical trials in rheumatoid arthritis in Japan and Europe with demonstrated efficacy (Naka et al. 2002; Okada et al. 2004). A fusion protein of soluble IL-6 receptor and IL-6 antagonist (Sporeno et al. 1996) has been employed to bind and inactivate the gp130 molecule (Renne et al. 1998), thereby inhibiting all members of the IL-6 family that use gp130 for signaling. One of the difficulties in using exogenous antibodies or soluble receptors is the need for continuous administration, eventually causing immune-mediated resistance to the treatment. One intriguing way around this problem, at least for IL-6, is to vaccinate an animal against an engineered IL-6 antagonist to induce neutralizing autoantibodies to IL-6 (Ciapponi et al. 1997; De Benedetti et al. 2001). Recently, non-peptide and presumably less antigenic IL-6 antagonists have been reported (Hayashi et

al. 2002). Development of small-molecule antagonists or cytokine mimetics for IL-6 is an ongoing and promising venture because there are few receptor recognition epitopes for the IL-6 family (Bravo and Heath 2000).

Thalidomide was developed as a sedative and anti-nausea drug, but its teratogenic effects and propensity to cause peripheral neuropathy with prolonged use have limited its utility. It is orally active, readily crosses the blood-brain barrier and functions as an immunomodulator by inhibiting the production of a broad range of pro-inflammatory mediators including TNF- α , IL-1 β , IL-6, and IL-8 and by increasing the level of IL-10, IL-2, and IFN γ (Corral et al. 1999). It is also a potent co-stimulator of T-cell function and can mediate a shift to a Th-2 type of immune profile (Haslett et al. 1998). Thalidomide inhibits the activation of NF- κ B (Lokensgard et al. 2000) through the suppression of I- κ B kinase activity (Keifer et al. 2001). It also inhibits the production of TNF- α from human microglial cells (Peterson et al. 1995). Thalidomide can reduce allodynia- and hyperalgesia-related behaviors when given at the time of sciatic nerve injury, but it was found to be ineffective when administered postoperatively (Sommer et al. 1998a). It has poor bioavailability in rats, however, and the dose and duration of therapy may not have been sufficient to reverse allodynia or hyperalgesia. Thalidomide reduces endoneurial TNF- α and increases endoneurial IL-10 and dorsal horn levels of met-enkephalin, but it does not alter IL-1 β or IL-6 levels (George et al. 2000). It can reduce the expression of COX-2 in endotoxin- and cytokine-stimulated peripheral blood monocytes in a partially IL-10 dependent manner (Payvandi et al. 2004). Thalidomide can attenuate the development of vincristine-induced mechanical hyperalgesia in rats (Cata et al. 2004). Clinically, thalidomide has been reported to reduce pain and hyperalgesia in complex regional pain syndrome (CRPS) type I (Schwartzman et al. 2003). More potent, presumably less toxic, analogues, might reduce pain in subjects with CRPS type I as well as other painful conditions (Schwartzman 2005; D.C. Manning, unpublished observations 2005).

Suppression of neuroinflammation has been an active strategy in multiple sclerosis for many years. Much of our knowledge about cytokine and glial therapeutics has derived from treatment of CNS inflammatory immune conditions such as multiple sclerosis. IFN β -1b can downregulate expression of MHC class II (Ransohoff et al. 1991) and adhesion molecules and upregulate IL-10 production (Chabot and Yong 2000). The utility of this and other multiple sclerosis therapies is unexplored in neuropathic pain.

Nutrition and fatty acid therapies. A somewhat unexpected drug class for immunomodulation are the statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors. Following reports that statin treatment produces improvement in a model of multiple sclerosis (Youssef et al.

2002), great interest is now being directed toward this class of drugs (Menge et al. 2005). Treatment with atorvastatin induces the secretion of Th-2 cytokines (IL-4, IL-5, and IL-10) and inhibits the secretion of Th-1 pro-inflammatory cytokines (IL-2, IL-12, IFN γ , and TNF- α). Atorvastatin inhibits MMP-9 (Wong et al. 2001) and reduces CNS penetration of leukocytes and well as reducing the expression of MHC class II molecules (Youssef et al. 2002). This effect on MHC expression is also noted on microglia (Menge et al. 2005). Atorvastatin also reduces the expression of the costimulatory molecules B7.1 and B7.2 (Youssef et al. 2002; Stuve et al. 2003), reduces pro-inflammatory cytokine production, and improves the functional outcome in a rat model of spinal cord injury (Pannu et al. 2005). Another statin, lovastatin, inhibits endotoxin activation of NF- κ B and diminishes the expression of TNF- α , IL-1 β , and IL-6 in rat astrocytes, microglia, and macrophages (Pahan et al. 1997). Statins decrease the migration of leukocytes in the CNS, inhibit MHC class II and costimulatory signals required for activation of pro-inflammatory T cells, induce a Th-2 phenotype in T cells, and decrease the expression of inflammatory mediators in the CNS, including nitric oxide and TNF- α (Stuve et al. 2003). The potential benefit of statins in neuropathic pain is unexplored, but these agents may be effective in preemptive use. How many postoperative or traumatic neuropathic pain states have been avoided by concomitant use of statins?

Shir and colleagues have reported that dietary fat can reduce the neuropathic pain-related behaviors resulting from partial sciatic nerve ligation (Perez et al. 2004). The consumption of unsaturated corn or soy oils suppresses tactile allodynia and heat hyperalgesia, and this effect is accentuated by dietary protein from multiple sources (Perez et al. 2004). Dietary fats can modulate both innate and adaptive immune responses through TLR-4 receptors. Saturated fatty acids activate Toll-like receptors, but omega-3 polyunsaturated fatty acids inhibit agonist-induced TLR activation (Weatherill et al. 2005). Saturated fatty acids increase, and omega-3 polyunsaturated fatty acids decrease, or inhibit endotoxin activation of, B7.1, B7.2, MHC class II, and IL-6 expression in bone-marrow-derived dendritic cells (Weatherill et al. 2005). The effects of these nutritive agents on nerve-injury-induced pain are unexplored. Using nutritional therapies to prevent and possibly treat neuropathic pain raises intriguing therapeutic possibilities.

CONCLUSION

To ease the suffering of our patients, we need to find new approaches to the treatment of chronic neuropathic pain. By changing our perspective and

looking beyond traditional neuroanatomy and neurophysiology to understand the body's response to injury, we may uncover new therapeutic strategies. An appreciation of the role played by the immune system in injury-induced pain states, as summarized in this chapter, represents a new opportunity. Currently available immunomodulatory and immunosuppressive agents need to be cautiously evaluated for their pain-modulating ability. The results of these initial studies will certainly foster more extensive therapeutic development efforts.

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