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Cytokine-mediated neuroinflammation controls the pathogenesis of neuropathic pain

The role of neuroinflammation in neuropathic pain: mechanisms and therapeutic targets

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Neuroinflammation is a proinflammatory cytokine-mediated process that can be provoked by systemic tissue injury but it is most often associated with direct injury to the nervous system. It involves neural-immune interactions that activate immune cells, glial cells and neurons and can lead to the debilitating pain state known as neuropathic pain. It occurs most commonly with injury to peripheral nerves and involves axonal injury with Wallerian degeneration mediated by hematogenous macrophages. Therapy is problematic but new trials with anti-cytokine agents, cytokine receptor antibodies, cytokine-signaling inhibitors, and glial and neuron stabilizers provide hope for future success in treating neuropathic pain.

Inflammation is a pathophysiological state associated with pain. The free nerve endings of peripheral nerve fibers in systemic tissues respond directly to inflammatory factors, such as lowered pH, bradykinin, histamine or prostaglandins, by generating electrical activity that is normally interpreted as painful; this beneficial pain state helps to protect us from adverse environments and can be managed by over-the-counter medications. Neuroinflammation is a more restrictive term referring to inflammatory states within the nervous system (NS) that can give rise to the serious problem of neuropathic pain; the burdensome pain state, for which there is often no effective therapy, that can be debilitating and life-destroying. Stated another way, neuropathic pain is the chronic pain state caused by significant pathological changes in the NS. It can occur secondarily to injury of the central nervous system (CNS) but it occurs most commonly in association with injury to the peripheral nervous system (PNS). These injuries can be caused by tumors compressing peripheral nerves, toxins used as chemotherapy, metabolic or viral diseases, severe ischemic insults and trauma and disc herniation that stretches, compresses or inflames a nerve root. Neuropathic pain is mediated through neuroinflammatory mechanisms affecting NS tissue that is controlled by inflammatory responses to the initial insult. In fact, ingredients of the so-called 'inflammatory soup' that are associated with systemic tissue injury and acute pain states now include proinflammatory cytokines. Proinflammatory cytokines stimulate the production of the traditional chemical constituents of inflammation, such as prostaglandins. When these factors are upregulated within the NS dire consequences can result.

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The key to predicting the development of neuropathic pain is having knowledge of the pathological processes of nerve injury in the context of neuroinflammation. This knowledge provides rationale for new therapies targeted at the predictable temporal events of nerve degeneration and regeneration. Herein we review the temporal course and the consequences of local nerve injury, as well as the cytokine-driven processes of Wallerian degeneration. This understanding explains the link between peripheral nerve injury, abnormal ectopic electrophysiological activity in nociceptive nerve fibers, cellular activation of glia and neurons in dorsal root ganglia (DRG) and the spinal cord, and the delayed development of mirror pain. It provides a neuroimmune explanation of the success of anti-tumor necrosis factor (TNF)-α therapy in the painful human conditions of rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. It explains why, in animal models of painful human disease, experimental interference with mechanisms of cellular activation and retrograde axonal transport can modulate pain states. In total, it provides the basis for a concordant understanding of the histological and molecular states of injured tissue, as well as the behavioral variables of thermal hyperalgesia and mechanical allodynia that are used to define the neuropathic pain state.

Clearly, the pathogenesis of neuropathic pain states is immensely complex, involving structural, physiological and pharmacological changes throughout the neuroaxis (from the site of peripheral nerve injury to the brain and the spinal cord). In this review, we emphasize a TNF-α-driven proinflammatory cytokine mechanism as the basis for understanding the relationship between peripheral nerve injury and neuropathic pain, a process that is controlled by local and distant cellular activation and the development of pathological changes in neural structures. Other proinflammatory cytokines, such as interleukin (IL)-1β and IL-6, are also certainly involved; this has been elegantly demonstrated by Sommer and her colleagues [1–3]. These factors, in association with TNF-α, are complementary and synergistic in complex ways and they can control many other key inflammatory factors, for example inducible nitric oxide synthase (iNOS) mRNA, that also play an important role in neuropathic pain [4,5]. Individual inflammatory factors are differentially expressed with respect to time and cellular elements, further complicating the understanding and therapeutic approaches to neuropathic pain. Our strategy has been to focus on the initial pathological event that gives rise to a progression of chemical and structural changes in the neuroaxis, which define the neuropathic pain state. In this regard, the temporal expression of TNF-α plays a dominant, but certainly not exclusive, role [6], as we will argue later in the text.

Although the cytokine mechanisms of disease are extremely complex and incompletely understood, and cytokine therapies are known to have risks and perhaps unanticipated consequences, the information we present strengthens the rationale for therapies that are specifically targeted at cytokine production and activity, proinflammatory signaling molecules and/or antiglial cellular activators, therein identifying the next generation of pharmaceutical targets for managing herniated disc-related radiculopathies and the many peripheral neuropathies associated with toxic therapies, metabolic disease and trauma.

The sensory neuroaxis

Specialized axon terminals in skin transduce nociceptive and proprioceptive events for processing by first-order sensory neurons in the ipsilateral, segmental DRG. The central axonal extension of the DRG terminates in the dorsal horn (substantia gelatinosa) of the spinal cord. Additional processing occurs in deeper layers of the dorsal horn at the same segmental levels, as well as higher ones. Major somesthetic pathways, including the lateral and ventral spinothalamic tracts and the medial lemniscus, organize sensory fibers in their progression through the spinal cord, brainstem and thalamus before they terminate in the cerebral cortex (Figures 1,2).

The nerve fiber consists of the axon and its Schwann cells (Figure 2). Approximately 90% of all endoneurial cells are Schwann cells; they are the peripheral glia, sharing many molecular characteristics with oligodendrocytes and astrocytes of the CNS. They can be used to model the glial consequences of systemic drugs but they also differ in several ways from oligodendrocytes. Every peripheral nerve axon is sheathed from the root zone to the distal axonal termination site by a continuous basal lamina from sequentially placed Schwann cells (approximately 1 mm apart). A myelin sheath is expressed by Schwann cells on the larger motor fibers and the mid-sized sensory fibers. The smallest polymodal nociceptive fibers are not myelinated. The lipid-rich myelin material enables saltatory conduction of action potentials between nodes of Ranvier – the unmyelinated axon between adjacent myelinated Schwann cells. Each node of Ranvier has an increased density of sodium channels compared with the paranodal area that has a higher concentration of potassium channels (this area is sheltered from the endoneurial environment

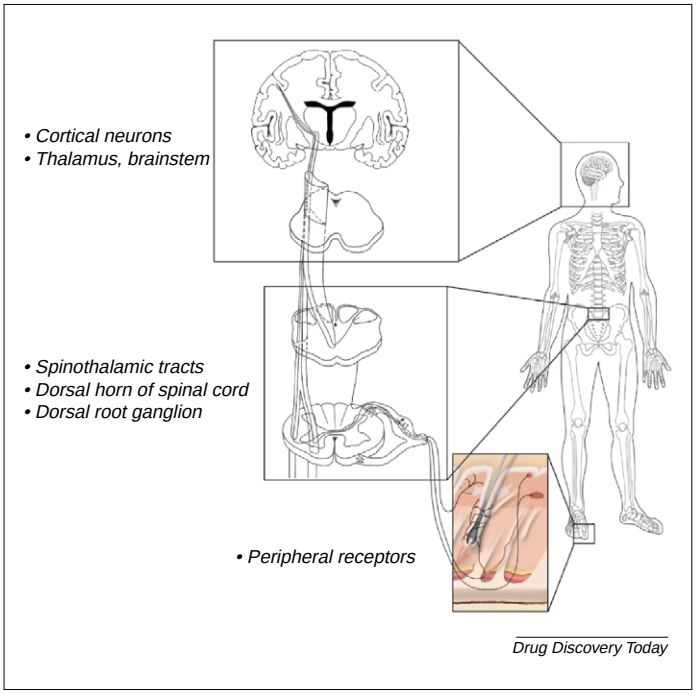

FIGURE 1

Sensory neuroaxis. Specialized axon terminals in the skin transduce nociceptive and proprioceptive events that are processed by first-order sensory neurons in the ipsilateral, segmental dorsal root ganglion. Its central axonal extension terminates in the dorsal horn (substantia gelatinosa) of the spinal cord. The dorsal horn is a primary site for integrating and processing sensory and pain information. Ascending sensory fibers cross through the anterior commissure to the opposite anterior column and form the ventral spinothalamic tract that passes all the way to the thalamus where additional sensory processing occurs. Other major neural structures are involved in arousal and physiological responses to pain but the cerebral cortex integrates and interprets this information in the context of human experience.

by the myelin). The presence of a basal lamina distinguishes Schwann cells from fibroblasts, macrophages and mast cells. The basal lamina is preserved when a Schwann cell degenerates and a duplicate basal lamina is formed by new Schwann cells. The basal lamina has a crucial function in axonal regeneration because it degenerates very slowly after Schwann cell death. Therefore, it serves as a conduit for nerve fiber regrowth in lesions where the axon has not been transected [7]. Schwann cells that are not in contact with axons express nerve growth factor (NGF) and NGF receptor on their surfaces. NGF and NGF receptor also assist in guiding axons down remnant basal lamina tubes. Excessive NGF–TrkA signaling can initiate and sustain neuropathic pain [8].

It should be appreciated that modest injuries to the nerve fiber or injuries targeting Schwann cells (and not the axon) can produce a demyelinating injury, characterized by electrophysiological dysfunction (a conduction block occurs if more than three adjacent Schwann cells are injured), without degenerating the nerve fiber. This can produce paresthesia and loss of function but is generally not associated with debilitating pain states. By contrast, injuries to the axon are much more serious in terms of the prognosis for recovering function and the likelihood of developing neuropathic pain, as discussed in the next section.

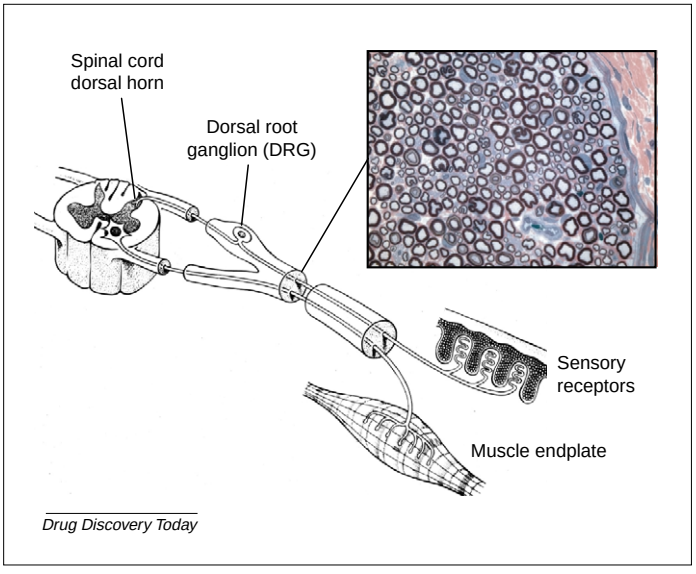

FIGURE 2

The peripheral nervous system. The peripheral nervous system (PNS) includes cranial nerves, spinal nerves, peripheral components of the autonomic nervous system (not shown) and peripheral nerves where the primary sensory neurons are located in the associated dorsal root ganglia (DRG). The DRG is also a part of the PNS. The final motor neuron lies outside the PNS in the spinal cord but motor axons are intermingled with sensory axons in most nerves after their roots join distal neurons to the DRG. Axons are extensions of the cell body and contain a continuous channel of neuronal cytoplasm, innervating sensory endings or muscle fibers. Axons can be myelinated or unmyelinated and are typically organized as a mixed bundle of motor and sensory fibers within fascicles. Many fascicles can comprise a major nerve, such as the sciatic nerve. The micrograph shows a fascicle of a mammalian sciatic nerve with axons encircled by dark-staining myelin (unmyelinated fibers are not observed at the level of resolution provided by light microscopy unless they are stained with an antigen that overextends their size). The perineurium is the fascicular boundary and can be seen as a sheath between the nerve fibers and the pink-staining epineurial collagen matrix. The perineurium and the tight endothelial junctions of vessels within the fascicle comprise a barrier to entry of macromolecules, known as the blood–nerve barrier.

The DRG neurons and their supporting glia (satellite cells) deserve special attention because they are the first-order sensory neurons that respond immediately and directly to axonal injuries [9]. They are also underrepresented as therapeutic targets. DRG neuroprotection strategies could have special importance in limiting the cascade of events that lead on from peripheral nerve injury to neuropathic pain. Two principal mechanisms of communication link peripheral nerve fibers with DRG cells: electrophysiological communication and chemical axonal transport communication. Electrophysiological changes are instantaneous and, in the case of the 'injury barrage', can immediately alter DRG and spinal cord gene expression by upregulating *c-fos* and *c-jun* [10]. Electrical activity after nerve injury could also be part of the initial control mechanism for the activation of nuclear factor (NF)-κB in DRG neurons [11]. The consequences of these changes in immediate early gene expression are incompletely understood and beyond the scope of this manuscript, however reviews of the topic are available [12,13]. Another important subject, the consequences of retrograde axonal communication that occur more slowly and have a protracted effect on the function of central neurons, is presented in detail in this review.

The dorsal horn neuronal complex is recognized as the single most important sensory structure in terms of its role in the maintenance of the neuropathic pain state. However, changes in the excitability of these neurons and their abnormal processing of sensory information are secondary in the cascade of pathophysiological events, initiated by nerve injury. The same neuroinflammatory mechanisms of glial and neuronal cellular activation that are operative in the DRG are probably also operative in the dorsal horn.

Changes in DRG and dorsal horn neuronal activity are further processed in the mid-brain, thalamus and cortical structures of the sensory pathways; they can be modulated significantly by our emotions and previous experiences. Chemical inhibitory influences can modulate dorsal horn neuronal activity [14,15].

Peripheral nerve injury and neuroinflammation

In 1850, Augustus Waller described a pathological process (following nerve transection) that included an initial reaction at the site of injury followed by progressive degeneration and phagocytosis of myelin and axons distal to the injury [16]. This process, now known as Wallerian degeneration, is fundamental to neuropathology and it is tightly correlated with the development of neuropathic pain. The process is complex and will only be briefly reviewed here in the context of neuroinflammation and degeneration. It should be recognized that degenerative events do not stand in isolation, because they are a necessary prerequisite for regeneration of injured peripheral nerves. Depending on the specific experimental conditions, interfering with degenerative events can delay or promote axonal regeneration. Wallerian degeneration occurs after axonal injury of any type, including crush and severe ischemia. Following nerve injury, nonresident hematogenous macrophages invade the injury site, their numbers peaking during the intense period of phagocytic activity. The invasion of macrophages and the process of nerve degeneration are temporally related to the peak periods of hyperalgesia [17]. During the time of gradual axoplasmic disintegration, the axolemma fragments and their contents undergo granular dissolution. Axonal breakdown is mediated by calcium influx and involves activation of axonal proteases [18]. The myelin sheath of the Schwann cell initially remains intact but then it forms

lamellar ovoids surrounded by Schwann cell cytoplasm. Schwann cells can phagocytose myelin debris but hematogenous macrophages reinforce and dominate the process of degeneration and phagocytosis. Therefore, they are effectively required for Wallerian degeneration [19].

The mechanisms by which macrophages potentiate Wallerian degeneration are not entirely known. It is thought, however, that cytokine signaling and secretion of proteases play a central role, as does the expression of adhesion molecules [20]. The purpose of adhesion molecules is to promote transendothelial migration. When leukocytes encounter appropriate activation signals, such as TNF-α, they are stabilized by intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1). These adhesion molecules are upregulated by TNF-α [21]. Further migration through the endothelium follows chemotactic signals that are present in injured nerves. Activated macrophages secrete components of the complement cascade, coagulation factors, proteases, hydrolases, interferons, TNF-α and other cytokines. The activation of macrophages could be related to TNF-α that is expressed by activated Schwann cells, mast cells and endothelial cells. This could be in response to a barrage of substance P (a peptide neurotransmitter expressed in nociceptive sensory neurons) from the injured nerve and the matrix metalloproteinase (MMP)-induced release of soluble TNF-α. Liefner *et al.* [22] have recently used TNF-α –/– mice to demonstrate that the main function of TNF-α during Wallerian degeneration is the induction of macrophage recruitment from the periphery without affecting myelin damage or phagocytosis. Mice (OLA/WLD) with a genetic defect that limits macrophage recruitment to injured nerves demonstrate delayed Wallerian degeneration and reduced pain [23,24].

Role of cytokines in neuropathological processes and pain

Cytokines are small proteins that are released by cells and have specific effects on cell–cell interaction, communication and the behavior of other cells. TNF-α is the prototypical proinflammatory cytokine, although there are other important cytokines in this family, such as IL-1β and IL-6 [1]. This discussion is focused on TNF-α because it is the best understood proinflammatory cytokine and it is upregulated and released immediately after nerve injury by resident Schwann cells, mast cells, endothelial cells and fibroblasts. TNF-α stimulates the upregulation of other proinflammatory cytokines, as well as anti-inflammatory cytokines, and, in so doing, orchestrates the subsequent neuropathological changes in the injured nerve, eventually recruiting immune cells (primarily macrophages) from the circulation to participate in the nerve degeneration process. Blocking the upregulation of TNF-α or the recruitment of macrophages after nerve injury can interfere with the rate and magnitude of Wallerian degeneration, influencing the magnitude and duration of the associated pain state.

The relationship between TNF-α and nerve injury has been of increasing interest because TNF-α has been implicated in the pathogenesis of multiple sclerosis (MS), HIV-associated neurological diseases and peripheral demyelinating neuropathies [25], suggesting that TNF-α is the best biomarker for identifying painful changes in nerves and DRG. Experimental *in vivo* studies in which human recombinant TNF-α was injected into the sciatic nerve demonstrated a transient, dose-dependent, focal endoneurial inflammation followed by primary demyelination and axonal degeneration [26,27]. Low doses of TNF-α injected in rat sciatic nerve resulted in substantial endoneurial edema accumulating throughout the nerve bundle. Higher doses of TNF-α caused extensive splitting of myelin lamellae, forming large vacuoles before demyelination. Myelin sheaths appear as compact spiral lamellae that are alternately light and dark when viewed in osmicated transverse sections by electron microscopy. The light lines, or intraperiod lines, are formed by the approximation of the external membrane surfaces of the Schwann cells. TNF-α causes splitting of the myelin sheath at the intraperiod line giving rise to wide separation of myelin lamellae [26]. Schwann cells are activated after exposure to TNF-α and contain lipid debris, consistent with their phagocytic role. Macrophages invade the tissue after three days to reinforce the phagocytic process and fibroblasts proliferate in the endoneurium after TNF-α exposure and reactive changes in endothelial cells are seen. Occasional axons undergo Wallerian-like degeneration.

Some normal Schwann cells constitutively express TNF-α *in vivo* and there is a significant increase in immunoreactivity during the degenerative phase of the compressive, ischemic, inflammatory neuropathy caused by chronic constriction injury (CCI) [28] to the sciatic nerve of laboratory animals [29]. Using immunohistochemistry staining of TNF-α protein and *in situ* hybridization of TNF-α mRNA sequences, we observed an increase in the number and density of Schwann cell cytoplasmic staining of TNF-α protein and mRNA. The initial increase in TNF-α immunoreactivity (quantified 3–6 h after CCI injury) was doubled during the time of maximum macrophage involvement in the neuropathy. The early increase in Schwann cell TNF-α immunoreactivity following nerve injury has several important functions, including the recruitment of macrophages to the injury site and macrophage activation. This provides the second line of immunological defense and the facilitation of the phagocytic role of Schwann cells that contribute to the initial process of nerve fiber degeneration [30]. This process is then extended and amplified by recruited macrophages.

These combined effects of TNF-α in peripheral nerve cells suggest a new mechanism by which injury to the axon can lead to neuropathic pain states (Figure 3). The local liberation of TNF-α from Schwann cells and other cells at the site of nerve injury causes spontaneous electrophysiological activity in nociceptive and other sensory fibers [31], potentially providing the foundation for ongoing, inappropriate signal amplification by neurons in the dorsal horn. Apart from biologically active TNF-α having the ability to occupy TNF-α receptors in the local axonal membrane, the low pH environment of inflammation enhances the intrinsic ability of TNF-α to interact with membranes by directly binding, inserting and creating ion-channel formation [32], resulting in increased Na^+ influx [33]. In central neurons, TNF-α was recently shown to increase the cell surface expression of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunit GluR1 and to potentiate vulnerability to AMPA receptor-mediated injury in association with an increase in Ca^{2+}-AMPA/Kainite channel numbers [34]. Through these (and possibly other) mechanisms, TNF-α can modulate the number of membrane ion channels, their activity and protein phosphorylation [35], thereby affecting signal transduction pathways that are independent of its receptor-mediated effects.

DRG, central neuron and glial activation can be influenced immediately by massive electrophysiological discharges and, more slowly, by the retrograde axonal transport of cytokines and

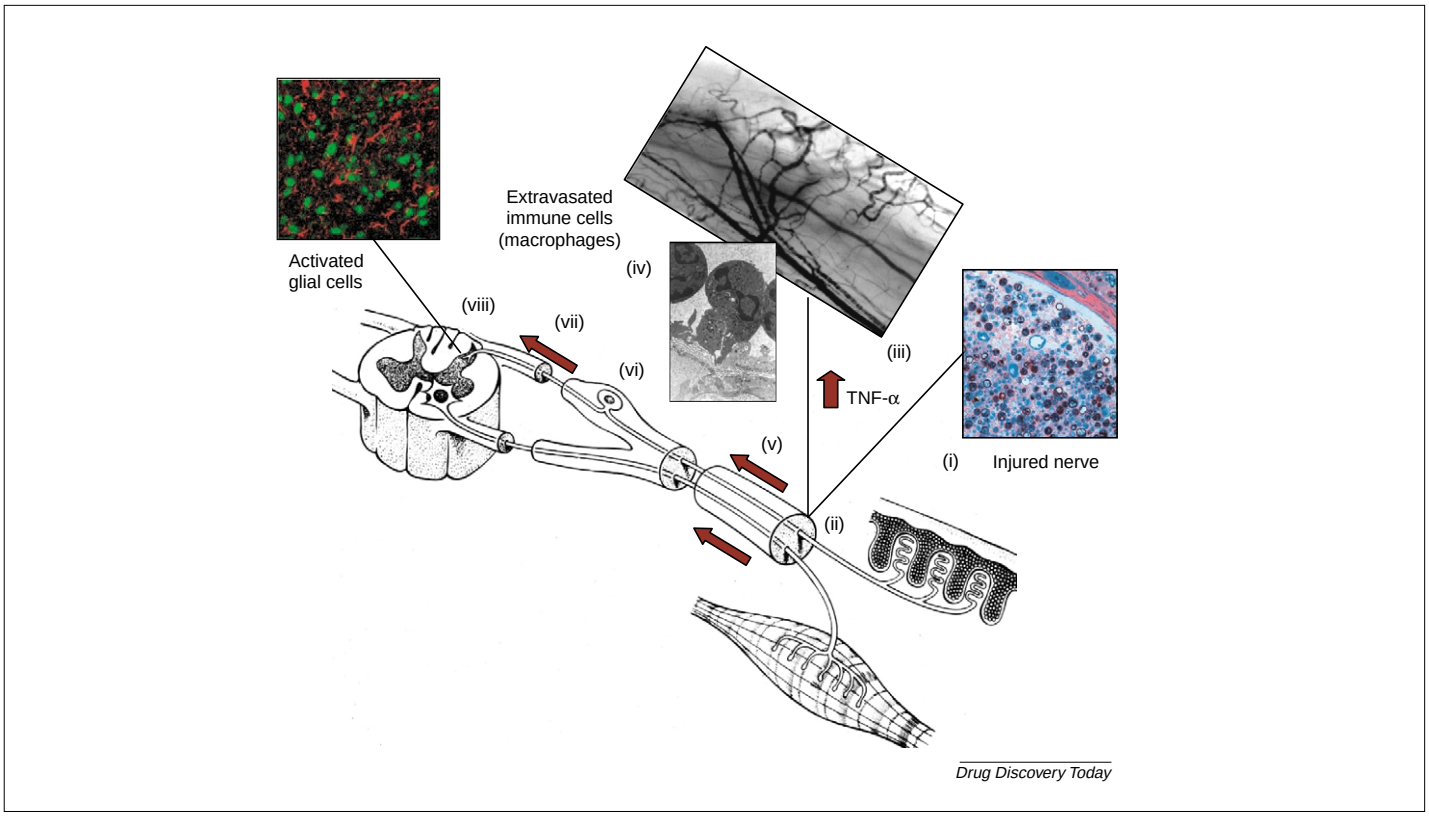

FIGURE 3

Sequence of events that relate peripheral nerve injury to neuroinflammation and neuropathic pain development. (i) Nerve injury causes local upregulation of TNF-α in Schwann cells, mast cells and resident macrophages. Significant injury causes Wallerian degeneration, the process of proximal–distal axonal and Schwann cell degeneration. The photomicrograph shows the pathological consequences at the site of injury, which include edema formation, myelin collapse into dark ovoids, axonal disintegration and macrophage-mediated phagocytosis. **(ii)** During this time local TNF-α causes spontaneous electrophysiological activity in surviving nociceptive nerve fibers. **(iii)** Local TNF-α alters the blood–nerve barrier causing upregulation of vascular adhesion molecules and attraction of hematogenous macrophages that enter the nerve during MMP-related breakdown of the barrier. The micrograph shows a polymorphonuclear leukocyte probing an injured vessel wall on the background of a light micrograph of surface perineurial vessels. **(iv)** Macrophages significantly increase the concentration of proinflammatory cytokines in the injured nerve. **(v)** Local TNF-α and its receptors are retrogradely transported to DRG and ventral spinal cord neurons, which is thought to be a stimulus for **(vi)** upregulation of TNF-α in DRG neurons and glia. **(vii)** Further transport of TNF-α in the anterograde direction occurs from DRG to dorsal horn neurons and glia of the spinal cord, this is thought to be a stimulus for **(viii)** activation of spinal glia and upregulation of TNF-α in glia and CNS neurons. The confocal image shows activated glia (red) in association with dorsal horn neurons (green).

neurotrophic factors. In this review we emphasize the role that cytokines and their receptors could have on DRG gene expression and function via retrograde transport signaling to the cell body. The temporal progression of these retrograde signals is related to activation of central glia, further upregulating proinflammatory cytokines and inflammatory factors to excite spinal neurons.

Schwann cell biology

Schwann cells are extremely sensitive to their environment and to ischemia and, when activated, produce cytokines, such as TNF-α [29,36], as a reaction to these stresses. A similar response is seen in central glia although the response is delayed. Demyelination is primary if the Schwann cell itself is injured; it is secondary, as in the case of Wallerian degeneration, if changes in the integrity of the axon and loss of axon–Schwann-cell contact cause Schwann cell mitosis or apoptosis. TNF-α regulates the integrity of basal lamina via the control of its degrading proteases [37,38]. For reasons that are not yet clear, it is known that Schwann cells produce TNF-α immediately after injury, perhaps as an initial mechanism related to their phagocytic role or to facilitate mitosis or apoptosis.

The relationship between Schwann cells and TNF-α is complex [30]. Our own data clearly confirm that TNF-α induces apoptosis in primary Schwann cell cultures in a dose-dependent manner. Also, inhibition of TNF-α-mediated signaling by blocking p38 activity reduces Schwann cell apoptosis. In some *in vivo* settings it is also clear that Schwann cell apoptosis is mediated by TNF-α. Weishaupt *et al.* [39] reported that administering TNF-α-neutralizing antiserum to animals with experimental allergic neuritis (EAN) reduced the rate of Schwann cell apoptosis, although they concluded that antigen-specific therapy alone could only slightly modulate the rate of Schwann cell apoptosis in this complex disease. Bonetti and co-workers [36] suggest that, although the Schwann cell is a target of TNF-α, TNF-α does not exert cytotoxic effects or apoptosis. Rather, their data suggest that TNF-α influences the fate of Schwann cells by activating transcription pathways, such as NF-κB and c-JUN pathways, and that TNF-α modulates the Schwann cell phenotype by inducing expression of TNF-α receptors and increasing the proportion of non-myelin forming cells that express the p75 NGF receptor. These changes occurred in the absence of apoptosis. The differing conclusions from both groups are partly

based on the use of different *in vivo* models of demyelinating disease [EAN and chronic inflammatory demyelinating polyneuropathy (CIDP), respectively]. CIDP is often a much less severe disease than EAN and it can involve damage to the axon. It might be that this difference partly relates to the expression levels of TNF-α and its receptors and its effect on Schwann cells.

Cytokines and mechanisms of cellular activation

TNF-α receptors and cellular signaling via NF-κB

TNF-α cellular signaling occurs through two distinct cell surface receptors: TNFR1 (p55) and TNFR2 (p75), as shown in Figure 4. Both receptors have significant homology in their extracellular domains but the differences between their cytoplasmic domains lead to the activation of distinct signaling cascades that mediate distinct, often opposing, effects. For example, TNFR1 contains a death domain and is involved in most TNF-α functions, including cell death and proliferation, and TNFR2 preferentially binds to a membrane-bound TNF-α [40,41] that mediates neuroprotection [42]. Transgenic mouse studies have shown that TNFR1 triggers oligodendroglial apoptosis and primary demyelination in the CNS, whereas TNFR2 promotes vascular pathology with no evidence of oligodendroglial apoptosis or primary demyelination [43]. In the PNS, TNFR1 and TNFR2 are induced after injury [37], demonstrating differential regulation during Wallerian degeneration [44] with hyperalgesia and allodynia. This is mediated by TNFR1, not TNFR2 [45]. DRG neurons also show differential expression of TNFR1 and TNFR2 after peripheral nerve injury; TNFR1 functions preferentially on neurons and TNFR2 functions on non-neuronal cells [46]. TNFR1 knockout mice (p55TNFR–/–) that are challenged with myelin immunizing antigen demonstrate normal (wild-type) regression of antimyelin reactivity and tolerance to the immunizing antigen, indicating that the immunosuppressive functions of TNF-α can be sufficiently exerted via TNFR2. However, TNFR2 knockout mice also have some capability of controlling myelin reactivity. Double-deficient TNFR mice are unable to suppress late autoimmune reactivity [40]. In experimental allergic encephalomyelitis (EAE) studies of demyelination, using TNFR1–/–, TNFR2–/– and double-receptor knockout mice, it has been shown that TNFR1 is a crucial mediator of myelin glycoprotein-induced EAE and that TNFR2 signaling has a significant protective role in the clinical progression of the disease [47]. In other studies focused on demyelination and remyelination, the lack of TNF-α led to a significant delay in remyelination [48]; analysis of TNFR1–/– and TNFR2–/– mice in these studies indicated that TNFR2, not TNFR1, was crucial to remyelination. Such studies indicate a dual role for TNF-α and its receptors, causing inflammatory demyelination and cell death on one hand and the promotion of remyelination, by influencing the expression of growth factors, cellular proliferation and neuroprotectionon, on the other hand. This suggests that therapeutically targeting TNF-α can lead to opposing outcomes that depend on the timing and the duration of treatment. It remains to be determined whether TNF-α has different affinities for the receptors.

An important feature of TNFR1 occupation is the crosstalk between the NF-κB and JNK secondary signaling pathways that

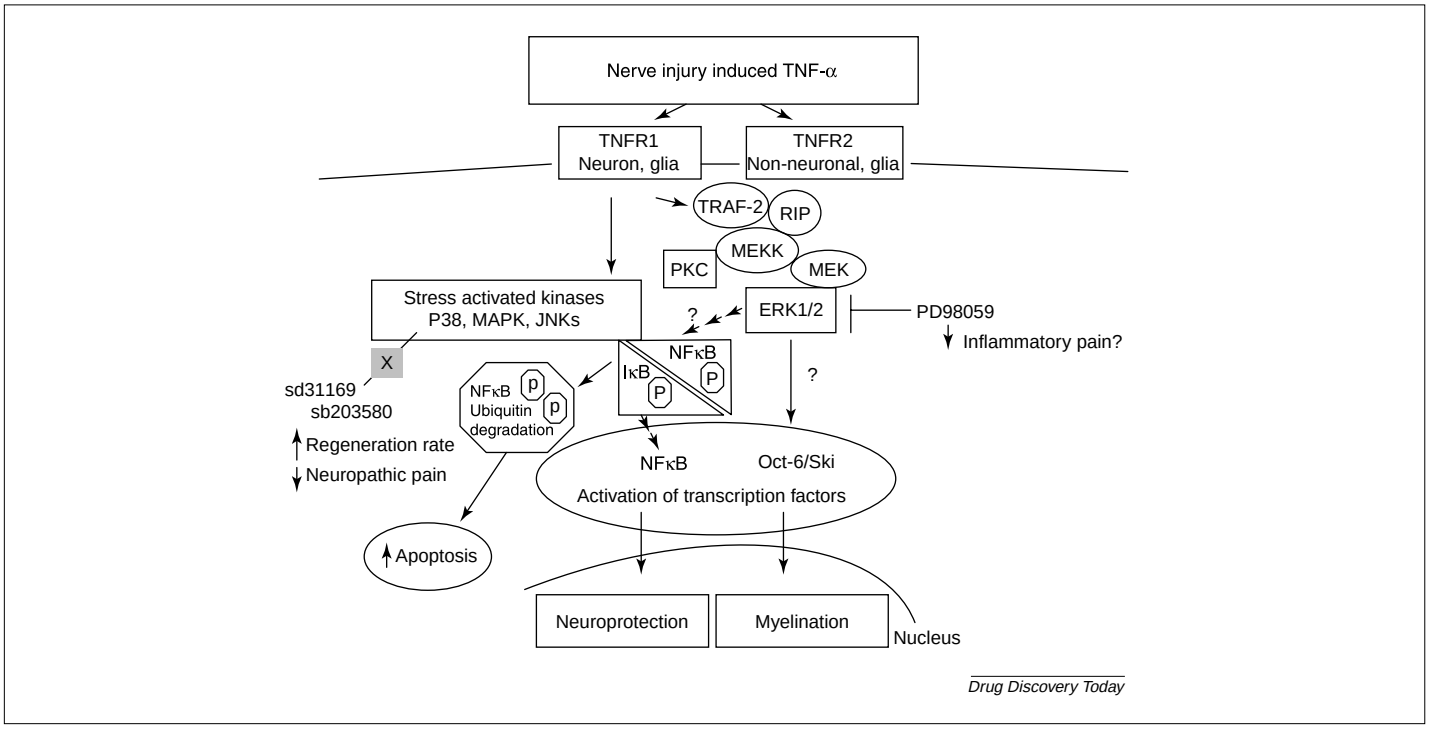

FIGURE 4

TNF-α receptors and signaling molecules. TNF-α is produced after nerve injury and is involved in complex signaling mechanisms. TNF-α binds to both TNFR1 and TNFR2. TNFR1 has intrinsic tyrosine kinase activity, whereas TNFR2 lacks a cytoplasmic domain and initiates ligand-mediated signaling molecules via TRAF-2 and RIP. TNFR1 can induce an apoptosis cascade via stress-activated kinases that can be inhibited by pathways that cross-talk to NFκB and promote anti-apoptotic gene expression. This might be an important pathway in neuropathic pain signaling. TNF-α also activates ERK1/2 in primary Schwann cells, through an unidentified receptor. TNFR2 could also be involved in ERK1/2 pathways. Recently, TNFR2 –/– mice demonstrated an important role for TNFR2 in myelination, however, the signaling pathways have not been well characterized. It is probable that TNFR2-mediated signaling pathways leading to Oct-6 and Ski transcription will be involved.

affect apoptosis. Via the activation of NF-κB, TNFR1 mediates signaling for activation and cell death as a consequence of a complex interaction with the c-JUN transcription factor and the downstream production of caspases and inhibitory factors. In the absence of NF-κB activity, cellular susceptibility to TNF-α-induced apoptosis increases, whereas enforced activation of NF-κB protects against apoptosis [49].

TNFR2 lacks a cytoplasmic death domain and occupation of this receptor results in the binding of TNFR-associated factor (TRAF)1 and TRAF2 to the cytoplasmic portion of TNFR2, as well as the recruitment of protein inhibitors of apoptosis. TRAF2 and receptor interactive protein (RIP) stimulate pathways that activate MAPK and NF-κB, respectively. Studies in mice and humans have shown that NF-κB is a repressor of apoptosis, whereas MAPK can either inhibit or promote apoptosis. As reviewed by Gupta [41], the potential mechanisms of TNFR2 occupation and apoptosis are uncertain. TNFR2 could serve as a high-affinity trap of TNF-α that delivers TNF-α to TNFR1. Others have proposed that the cytoplasmic domain of TNFR2 can potentiate apoptosis or even directly induce apoptotic death (i.e. TNFR2 occupation leads to upregulation of TNF-α production, stimulating TNFR1 and resulting in apoptosis). Therefore, the complex relationship between the biologically active TNF-α protein and TNFR1 and TNFR2 occupation stimulates or inhibits NF-κB and apoptosis via mechanisms that modulate the availability of active TNF-α and TNFR molecules (converted by MMPs and interactions with RIP, MAPK and JNK). Recent work suggests that interference with TNF-α signaling via the p38 MAPK pathway causes compensatory cytokine actions that can mimic or bypass the traditional glial activation pathways. These relationships are obviously complex and incompletely understood. However, they could be the basis for the mechanisms of neurological complications reported in association with anti-TNF-α therapy.

p38 MAPK signaling

In 1994, p38 was first identified as a MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells [50]. At the same time, the cloned target for an anti-inflammatory drug (SB203580) was found to be identical to p38 [51]. It is now known that the regulation of cytokine biosynthesis in many different cell types is mediated through p38 activation [52]. It is also appreciated that various forms of cellular stress activate mammalian p38 and that p38 participates not only in inflammatory responses, but also in stress-induced signaling in cell proliferation and in apoptosis [53]. To date, six distinct isoforms of p38 have been identified and it is believed that functional differences between the isoforms are related, in part, to their differential expression, activation and substrate specificity [54].

The p38 pathway controls the activity of multiple transcription factors and the expression of many genes, some of which have probably not yet been identified. The p38 pathway phosphorylates and enhances the activity of many transcriptional control factors, including activating transcription factor, ELK-1, NF-κB, heat shock transcription factor-1 and SAP-1. In neuronal pathology, the p38 MAPK pathway is believed to mediate apoptosis resulting from acute neuronal injury [55].

Several drug companies have developed p38 inhibitors and their application has a wide appeal in strategies designed to interfere with cytokine expression. SB203580 (GlaxoSmithKline) is a broad-band p38 inhibitor that is being replaced by more-specifically targeted compounds. In HIV-dementia clinical trials, an inhibitor of p38 phosphorylation (CPI-1189, developed by Centaur) was thought to provide protection by inhibiting neuronal apoptosis [56,57]. Another compound (SD31169, developed by Scios) inhibits the activity of phosphorylated p38 MAPK. The compound is particularly effective in promoting axonal regeneration by affecting Schwann cell viability and protecting against TNF-α signaling that causes apoptosis [58]. Intrathecal administration of p38 MAPK inhibitors has been demonstrated to inhibit the development of allodynia and hyperalgesia in several models of pain, presumably by blocking p38 phosphorylation in microglia [59–64]. Although effective in preventing enhanced pain responses, most p38 inhibition has failed to reverse established pain states [60,61]. However, Cytokine Pharmascience's p38 inhibitor (CNI-1493), administered intrathecally, has been reported to prevent and reverse allodynia associated with extraneural inflammation [62].

Relationship between TNF-α and MMPs

MMPs belong to a large family of zinc-dependent endopeptidases that comprises collagenases, gelatinases, stromelysins, membrane-type MMPs and a disintegrin adhesion metalloproteinase (ADAM) including TNF-α converting enzyme (TACE) [65]. Elaborate interactions between TNF-α and MMPs exist at several different levels. First, TNF-α induces gene expression of some MMPs, including gelatinases [66,67]. Second, MMPs, such as gelatinases, matrilysin [68] and TACE [69], control TNF-α release and the availability of its active soluble form by cleaving the extracellular C-terminus from the transmembrane form. Third, MMPs control the processing and inactivation of the TNF-α receptors [70]. Proteolytic shedding of TNF-α cell surface receptors is performed by some MMPs and presents an important balancing mechanism that sets thresholds for TNF-α function [71].

Degenerating diseases of the CNS are largely modulated by MMPs and TNF-α [72]. Direct intracerebral injection or induction of MMP-2, MMP-9 and TNF-α all produce nerve degeneration or primary demyelination [29,73], leukocyte and macrophage recruitment, and breakdown of the blood–brain barrier, producing vasogenic brain edema [27,74]. MMPs contribute to MS and Guillain-Barré syndrome [72]. The use of synthetic hydroxamate-based MMP inhibitors (MMPIs) has been shown to be effective in reversing established EAN [75]. MMPIs have also been shown to be effective in treating localized inflammatory pain, reversing endotoxin-induced hyperalgesia and reducing the painful consequences of peripheral neuropathy [76,77]. A limited amount of detail is known about the role of TACE in the NS but its mRNA is expressed abundantly in the CNS [78]. In the peripheral nerve TACE is upregulated transiently in Schwann cells and macrophages within one week of CCI [37]. All of these MMP-mediated effects are thought to occur in relation to changes in the activity of TNF-α and its receptors.

Anti-cytokine therapy: success, limitations and the future

Pain behavior and experimental anti-TNF-α therapy

Pain in rodents can be measured by the behavioral response to nociceptive stimuli and it is typically characterized in terms of thermal hyperalgesia and mechanical allodynia. These are important endpoints in models of nerve injury that relate directly to TNF-α activity. Several rat and mouse models of neuropathic pain have been used to explore the mechanisms and treatment of neuropathic pain states [79,80]. We prefer the chronic constriction injury model

because it produces robust Wallerian degeneration with additional inflammation (caused by the chronic gut sutures used for nerve constriction). Because some nerve fibers survive the injury, behavioral testing can also be used to assess pain.

Before the commercial development of anti-TNF-α monoclonal antibodies or soluble receptors, thalidomide was tested as a means of reducing the painful and pathological consequences of CCI [81]. Thalidomide reduces the production of TNF-α by activated macrophages, increases the recruitment of IL-10-positive epineurial macrophages [82] and reduces the degree of thermal hyperalgesia during the peak of endoneurial macrophage influx following CCI. Interestingly, thalidomide therapy reduced TNF-α but not IL-1 or IL-6 in the injured nerve and, when treatment ended, hyperalgesia increased. Thalidomide is currently used in the clinic for the treatment of various painful TNF-α-mediated diseases [83].

Upregulation of TNF-α also leads to compensatory increases in anti-inflammatory cytokines, such as IL-10, that downregulate TNF-α production. IL-10 (injected directly into the rat sciatic nerve following CCI) reduced hyperalgesia [84]. Thus, exogenous IL-10 therapy could be a means through which the detrimental effects of TNF-α can be regulated, a strategy now being explored in clinical trials for the management of sepsis and congestive heart failure [85].

The commercial development of specific soluble TNF-α receptors greatly advanced the field of cytokine based therapy. These biologics provide an excess of soluble TNF-α receptors that can occupy the biologically active TNF-α protein before it encounters receptors on cell surfaces. This reduces its biological effect. Of particular interest is Enbrel®. Enbrel® consists of recombinant human TNFR-p75–Fc fusion protein. The product is made by encoding the DNA of the soluble portion of human TNFR-p75 with the Fc portion of IgG. It acts as a competitive inhibitor of TNF-α function by preventing the binding of TNF-α to its membrane receptors. The therapeutic use of the soluble receptor is restricted because the half-life of the molecule is short; therefore combinations of the soluble receptor with immunoglobulin (Ig) or other molecules have been trialed in an attempt to increase the half-life of the drug, enabling therapeutic applications. Enbrel® has a history of success in managing the progression of rheumatoid arthritis and related diseases [86] and it has been used experimentally in painful neuropathy [87] and in uncontrolled human studies to treat low back pain [88,89].

Remicade® is a chimeric monoclonal TNF-α antibody approved for the treatment (in combination with methotrexate) of rheumatoid arthritis and (alone) for the treatment of Crohn's disease, ankylosing spondylitis, psoriatic arthritis and ulcerative colitis. It is currently undergoing formal double-blind, randomized clinical trials for sciatica [90] and has been studied in laboratory animals with spinal disc herniations [91]. The laboratory data suggest it is effective in reducing pain behaviors, although so far the clinical data are equivocal.

Humira® is the newest TNF-α biological response modifier to be approved in the USA. It is a completely human recombinant IgG1 anti-TNF-α monoclonal antibody. It has an adverse effect profile comparable with Enbrel® [92], including autoantibody formation and, on rare occasions, lupus-like syndrome. Several other similar anti-TNF-α biologics are currently under development.

Anti-TNF-α therapy and neuropathy

It is well known that thalidomide is neurotoxic, although it is not clear if it produces a primary neuronopathy or axonal 'dying-back' neuropathy [93]. It is not even agreed as to whether the neurological injury is dose-related. In one series of patients with cutaneous lupus erythematosus who were treated with thalidomide 50% developed sensory axonal peripheral neuropathy. Other reports note the involvement of large and small nerve fibers. The nature of the neurological injury is important because it would appear to be refractory to recovery after drug removal. With better knowledge of its neurotoxic mechanisms and targets, it might be possible to protect against the development or progression of the neuropathy. That is, it would be useful to know to what extent interference with p38 MAPK and/or NF-κB activity is involved in the pathogenesis of the neuropathy. It is known that in addition to dysregulation of TNF-α production, thalidomide inhibits the activation of NF-κB (and NF-κB activation is crucial for NGF-mediated sensory neuron survival) [94]. This effect might be part of the mechanism of either its dorsal ganglionopathy or distal axonopathy. Celgene has undertaken a developmental program to reduce the complications of thalidomide and their thalidomide analogs (CC-1088 and CC-10004) have undergone, or are currently undergoing, human clinical trials [95–96].

Newer forms of anti-TNF-α therapies can cause life-threatening complications through their immunosuppressive action, and demyelinating disease. In June 2004, Scheinfeld [97] reviewed >150 reports of complications arising after the use of the TNF-α blockers: Enbrel®, Remicade® and Humira®. Major complications included congestive heart failure, lymphoma, infection, lupus-like syndrome, induction of autoantibodies and injection-site reactions. These complications are well known to physicians and are fairly well understood in terms of their relationships to TNF-α suppression. The nature of the acute neuropathy that has been reported after TNF-α monoclonal antibody therapy, the exacerbation and frequency of MS attack and the production of MS-like disease in individuals not previously diagnosed with this disorder are all complications that are much less understood [98,99]. The incidence of these demyelinating diseases might increase when TNF-α monoclonal antibody therapy is used to treat painful neuropathy because there is already glial and neural involvement in these diseases and perhaps, therefore, a lower threshold for complications.

Although the mechanisms of the neurological injury caused by anti-TNF-α therapy are not known, failed clinical studies with lenercept in MS patients have provided insight into the role of TNF-α in MS exacerbation that could also apply to other demyelinating diseases. Cytokines are pleiotropic factors that act interdependently in complex ways. Their actions can be both proinflammatory and anti-inflammatory. So, in a paradoxical way, removal of TNF-α could potentiate disease – just like interferon-gamma (IFN-γ) provokes MS attacks. Therefore, TNF-α blockage could contribute to demyelination in subclinical disease states.

Retrograde axonal transport

Several signals from an injured nerve influence the function of cells in the mammalian DRG. These events have been studied in detail with *Aplysia californica* by Ambron and Walters [100] in the context of priming events and retrograde injury signals controlling the cellular and molecular biology of nerve regeneration. They characterize four phases of signals to the nucleus after nerve injury, including early-phase signals (seconds to minutes after injury), intermediate-phase signals (hours to days after injury), late-phase signals (days

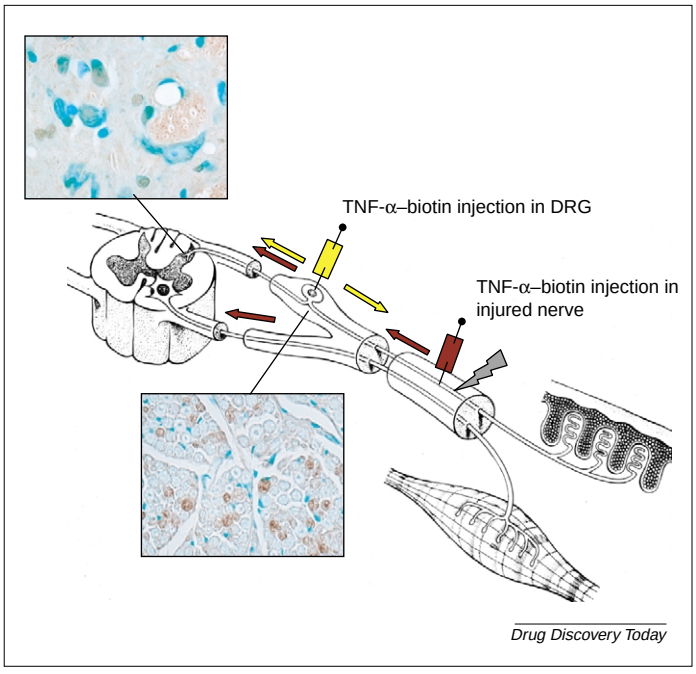

FIGURE 5

Fast axonal bi-directional TNF-α transport in injured neuroaxis. Histochemical tracing of biotinylated TNF-α using avidin-tagged peroxidase after its injection (250 ng in 3 μl volume) into the sciatic nerve following chronic constriction injury (red arrows) showed that TNF-α was transported at a rate of 300 mm/day retrogradely from the injury site to the spinal nerve adjacent to the ipsilateral DRG where it was seen in axons and Schwann cells (brown immunohistochemical stain in tissue micrograph; 200x). From the DRG it was further transported anterogradely to the spinal cord where it localized in axons and non-neuronal cells (top photomicrograph from ipsilateral spinal cord dorsal horn). It was also transported to the ipsilateral motor neurons in the ventral horn. Neurobiotin, an aminoderivative of biotin, was used for axonal tracing as a control to distinguish TNF-α-selective transport from unselective biotin-mediated transport. Biotin was transported intraaxonally and showed no evidence for selective uptake, Schwann cell reactivity or non-neuronal cell reactivity in spinal cord (not shown). When TNF-α was injected in the ipsilateral L5 DRG (yellow arrows) it was transported anterogradely in the sciatic nerve where it localized in injured axons, as well as in neural and glial structures of the dorsal horn of the spinal cord. Sections for photomicroscopy were developed with 3′,3-diaminobenzidine (DAB, brown) and counterstained with methyl green.

to weeks after injury) and completion signals (for the regeneration process). Early- and intermediate-phase signals arising during axon degeneration are linked to the development of neuropathic pain states.

Early-phase signals are primarily electrophysiological and result in the acute release of neurotransmitters onto neurons and the activation of kinases that phosphorylate constitutive transcription factors. This effect can be blocked by preemptive local anesthesia. Intermediate-phase signals are retrograde transported injury signals that include target-derived factors. Ambron *et al.* [101] performed a key experiment by collecting the axoplasm extruded from the injured ends of axons and injected it into the cell bodies of uninjured sensory neurons. One day later the cells showed the same increase in excitability as seen with axonal injury. By contrast, axoplasm from normal nerves had no significant effect when it was injected into the cells. Other studies of nerve injury noted that the hyperexcitability of the cell soma was not seen until 1–3 days after axon injury (depending upon how far the injury site was from the sensory neuron). They also showed that this hyperexcitability was not induced if the nerves were injured and then exposed to colchicine, a drug that blocks retrograde axonal transport [102].

Within a week of mammalian peripheral nerve injury, dynamic reorganization within the damaged sensory nerve fibers causes an increase in retrograde axonal transport of small proteins (including neurotrophins), representing a rapid and transient response to sensory nerve injury and providing a way of introducing regeneration-modulating factors into neuronal cell bodies of DRG. In the injured peripheral nerve many of the genes for neurotrophic factors and cytokines that control cell function and survival are induced at the injury site within hours of injury.

The bidirectional nature of axonal transport is important for the communication between the injured axon and the surviving DRG. With the exception of NGF that transports retrogradely, neurotrophins such as BDNF, neurotrophin (NT)-3 and NT-4 are known to undergo both retrograde and anterograde trafficking. Immunomodulatory signaling molecules, such as p38, JNK and ERK, also transport signals bidirectionally. For example, receptor-mediated NGF transport activates retrograde transport of p-ERK1/2, p-p38 and p-Akt in DRG neurons [103], whereas JNK3 (a JNK that is expressed primarily in the NS) is capable of anterograde and retrograde transport along the sciatic nerve [104]. Our tracing studies consistently show the ability of exogenous TNF-α to transport signals bidirectionally [105,106] (Figure 5).

Mechanisms of trophic currencies in neural networks have been reviewed [107]. Here, we discuss axonal transport of immunomodulatory TNF-α, the only cytokine that is known to undergo axonal transport and activate central neuropathic pain pathways following nerve injury. Retrograde transport of other proinflammatory cytokines has not been studied in the context of neuropathic pain. Endogenous TNF-α transport is induced after chronic constriction injury of the rat sciatic nerve [105,108] and it returns to basal levels by day seven, post-injury [85]. This observation is temporally similar to the increase in axonal transport of NGF, brain-derived growth factor (BDGF), glial-derived neurotrophic factor (GDNF), NT-3 and NT-4 [109–111].

TNF-α tracer injected at the injury site undergoes fast (200–300 mm/day) retrograde transport from the injured sciatic rat nerve to L4 and L5 DRG; this is followed by anterograde transport to the spinal cord from the DRG [105,106]. Neurobiotin is an amino derivative of biotin that can be used to distinguish between TNF-α-specific and biotin-specific axonal transport. Four unique characteristics of TNF-α transport have been identified: first, TNF-α uptake and transport is selective to certain nerve fibers, localizing both intraaxonally and within the associated glia; second, TNF-α transport along sensory afferents is distinctly different between normal and injured nerves (i.e. TNF-α uptake by DRG soma is inhibited after injury [105] but, after injecting TNF-α into DRG, it is transported to the spinal cord via injured axons and not via normal axons [86], suggesting the importance of injury-specific signals and packaging of the TNF-α transporting complex); third, the transporting mechanism (vehicle) for TNF-α has not been specifically identified but it has been suggested that TNF-α receptors facilitate its transport [105]; fourth, in contrast to neurobiotin that is transported within axons, TNF-α tracer conjugated to biotin is seen in both neuronal and non-neuronal (presumably glial) structures (Figure 5). The fourth characteristic further supports the hypothesis that suggests

TNF-α plays a role in the glial activation that is important for the development of neuropathic pain.

Thus, these data suggest that TNF-α and TNFR activation and retrograde transport from the site of nerve injury represent a mechanism for the central cellular activation and TNF-α upregulation in the spinal cord, as previously speculated [112,113]. These data could also suggest possible mechanisms that lead to changes in the levels of TNF-α, TNFR2, p-p38 and p-ERK in apparently uninjured adjacent neurons, as well as helping to explain the role of TNF-α in mirror-image pain [114].

Glial activation and pain

Neuropathic pain following damage to peripheral nerve cells is associated with neuronal plasticity in peripheral and central sensory afferents and the spinal cord [105]. Peripheral nerve injury has been associated with an increase in spinal glial TNF-α for some time and has been implicated in the generation of neuropathic pain [112]. Activation of spinal cord glia, including both microglia and astrocytes, occurs in a temporal pattern that is consistent with retrograde transport of TNF-α. Using specific glial inhibitors, it has been shown that microglial activation is superseded by astrocyte activation. This astrocyte activation dominates the central neuroinflammation environment and the further development of the neuropathic pain state [115–118].

Antiglial activation therapies

A therapeutic strategy has been proposed that targets the activation of CNS glia to reduce the development of neuropathic pain [119]. Existing drugs, such as the antimicrobial agent minocycline, have anti-inflammatory properties and have recently been shown to attenuate mechanical allodynia and proinflammatory cytokine expression in microglia [120]. Administered intrathecally, minocycline selectively inhibits microglial activation [121] and delays the development of allodynia; however, it does not effectively reduce established allodynia [120] and its effect on peripheral glia has not been reported.

It has also recently been shown that a novel NF-κB inhibitor can inhibit inflammatory cytokine secretion from mouse microglial cells in culture [122]. Although further experimental work and clinical trials are required, these reports establish the feasibility of targeting glia to interrupt the cascade of neuroinflammatory events that produce neuropathic pain.

Neuroprotection: erythropoietin

It is worth considering how neuroprotective strategies can eliminate or obtund the mechanisms of cellular activation or death mediated by the upregulation of proinflammatory cytokines. Some agents can be given immediately after injury or, ideally, before surgical iatrogenic injury to protect neurons and glia from injury or death. Although the links between nerve damage, apoptosis and chronic pain are unconfirmed, there are several reports that suggest neuronal death is an important mechanism in neuropathic pain [123–125]. Rationale for this form of therapy derives from the trials of neurotrophic factors, such as NGF-β and NT-4, that possess potent neuroprotective activity [126,127]. Unfortunately, it was discovered that these factors are limited therapeutically because they have a tendency to induce hyperalgesia [128]. Erythropoietin (Epo), by contrast, does not induce hyperalgesia and is a neuroprotective agent for primary sensory neurons, spinal neurons and their supporting glial cells. Experimentally, Epo therapy enhances recovery from neuropathic pain states [125] and protects neurons from death induced by ischemia [128], proinflammatory cytokines [129] and glutamate excitotoxicity [130].

In rats, systemic treatment with recombinant human Epo (rhEpo), administered one day before L5 spinal nerve crush and daily thereafter, significantly reduces the neuropathic pain consequences of injury [125] and corresponds with the prevention of apoptosis of primary sensory neurons and glia. The mechanisms of Epo protection are not completely understood but it is known that Epo regulates TNF-α levels [131]. In peripheral nerve cells, realtime qPCR showed that local TNF-α mRNA upregulation after nerve injury could be reduced by 50% using rhEpo therapy [132]. Thus, it appears that rhEpo regulates TNF-α mRNA levels but it is not known if rhEpo stabilizes TNF-α mRNA or directly regulates transcription. Immunohistochemistry studies demonstrated that EpoR is localized to Schwann cells *in vivo* and *in vitro* [133] and that rhEpo treatment downregulates TNF-α in Schwann cells after nerve injury, an observation that can relate directly to its effects on pain behavior. It has also recently been shown that rhEpo prevents or reverses nerve disorders and some pain manifestations in diabetic rats [134]. Finally, one caveat to Epo therapy is its primary hematopoetic effect, which results in significant elevation of hematocrit.

BOX 1

Although there is no guarantee that interference with any individual pathological event, cytokine or molecule will reliably alter the course of neuropathic pain without adverse or unintended consequence, current research suggests the following targets might be considered to further explore the complex problem of neuroinflammation and neuropathic pain.

Potential therapeutic targets:

At the injury site
- Schwann cell, mast cell, endothelial cell activation
- MMP degradation
- Proinflammatory cytokine upregulation
- Proinflammatory cytokine receptor upregulation
- Anti-inflammatory cytokines (e.g. IL-10)
- Vascular adhesion molecules
- Macrophage function

Retrograde transport
- Inhibition of transport signals
- Inhibition of transport mechanisms

Dorsal root ganglion
- Satellite cell stabilization
- Neuronal stabilization
- Antibodies to proinflammatory cytokines

Dorsal horn
- Glial stabilization
- Neuronal stabilization
- Upregulation of inhibitory neurotransmitters
- Glutamate inhibition

Common chemical mediators
- TNF-α, IL-1β, IL-6
- MMP-2, MMP-9, TACE
- Phosphorylated p38, ERK and JNK
- NF-κB

Therapy with peptide fragments of the Epo molecule might provide neuroprotection without increasing hematocrit [135–136].

Conclusion

The development of neuropathic pain often occurs after an initial injury to the PNS. It is closely associated with the pathological process of Wallerian degeneration, a process that is driven by proinflammatory cytokine upregulation. A cascade of neuropathological events develops, beginning at the site of nerve injury and proceeding rosterally to involve the glia and neurons of the DRG and the dorsal horn of the spinal cord. Local upregulation of cytokines initiates a cycle of neuroinflammation and cellular activation that might be refractive to traditional therapy. Activation is associated with abnormal function of these cells and precipitates the development of neuropathic pain. Potential targets for new therapy (Box 1) could focus on interfering with the local upregulation of proinflammatory cytokines and the activation of central glia and neurons that further upregulate proinflammatory cytokines.

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