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Gene Therapy directed at the neuroimmune component of chronic pain with particular attention to the role of TNFα

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Abstract

Identification that neuroimmune activation in the spinal cord is an important factor in the development of chronic pain has opened the possibility that gene transfer of anti-inflammatory peptides may be used to reduce pain neurotransmission. We review the published evidence regarding gene transfer to meninges to express the anti-inflammatory peptide interleukin 10, and gene transfer to dorsal root ganglia using replication incompetent HSV vectors to express interleukin 4, interleukin 10, or the soluble (p55) tumor necrosis factor receptor (sTNFR). The results of these experiments suggest a novel role for "reverse signaling" through the full length membrane form of TNFα in the modulation of chronic pain.

Keywords

gene therapy; gene transfer; herpes simplex virus; cytokines; tumor necrosis factor; neuropathic pain

There is substantial evidence, detailed in other manuscripts in this volume, to indicate that the development of chronic pain is accompanied by activation of spinal cord astroctyes and microglia ("glia") with the consequent production of pro-inflammatory cytokines that appears to play an important role in the persistence of pain [4,5,40,41]. The role of this neuroinflammatory process in the pathogenesis of chronic pain can be assessed in animal experiments that utilize gene transfer methods to deliver and express anti-inflammatory peptides in the spinal cord; this approach has the potential to be translated into novel treatments of intractable pain in patients. In this chapter, we will review the preclinical studies of gene transfer in the treatment of chronic pain.

In rodent experiments, Watkins and co-workers have demonstrated that plasmids encapsulated in liposomes, or adenovirus- or adenoassociated virus-based vectors constructed to express IL-10 injected intrathecally result in release of IL-10 into the cerebrospinal fluid, and that the anti-inflammatory cytokine prevents the development of pain or reverses established pain in models of neuropathic pain caused by nerve constriction or injection of pain-causing substances into the nerve sheath [19,25,26]. The reduction in pain-related behaviors is usually long-lasting, although for reasons not entirely clear, a pair of injections a few days apart appears to be more effective than a single injection of the gene transfer vector.

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An alternative approach involves the use of herpes simplex virus (HSV)-based vectors to transduce neurons of the dorsal root ganglion (DRG). HSV is a double stranded DNA virus that is spread by contact, infecting and replicating in skin or mucous membrane. Viral particles released in the skin are taken up by sensory nerve terminals and carried by retrograde axonal transport to the neuronal perikaryon in the DRG, where the wild-type virus may either re-enter the lytic cycle, or establish a latent state characterized by persistence as non-replicating intranuclear episomal elements. Vectors constructed from HSV that retain the targeting properties of the parental virus are thus efficiently taken up from skin inoculation and delivered to the DRG (Fig. 1).

HSV vectors engineered to express several different inhibitory neurotransmitters have shown analgesic properties in rodent studies. A replication defective HSV based vector containing the preproenkephalin (PENK) gene and injected subcutaneously into the paw expresses enkephalin in DRG neurons in vivo [1] and reduces hyperalgesic C-fiber responses [42]. In addition, vector-mediated enkephalin expression reduces pain-related behaviors in a rodent model of polyarthritis induced by injection of complete Freund's adjuvant (CFA) [2] and also prevents cartilage and bone destruction in the inflamed joints, an effect that appears to result from release of enkephalin from the peripheral sensory terminals in the joint. We have shown that subcutaneous inoculation of an enkephalin-producing non-replicating HSV vector produces an analgesic effect in the formalin injection model of inflammatory pain [11] and in pain caused by cancer in bone [10]. The analgesic effect of the vectors is continuous over time, additive with morphine, and persists in animals that are rendered tolerant to the analgesic effects of morphine [14]. In the infraorbital nerve constriction model of craniofacial pain, Pohl and colleagues have demonstrated that inoculation of the enkephalin-expressing vector in the face produces a significant reduction in mechanical hypersensitivity on the affected side [24]., Yoshimura *et al*. showed that following injection into the rat bladder, HSV-mediated enkephalin effectively attenuated capsaicin-induced bladder irritation and resultant bladder hyperactivity [9,46], and Westlund and colleagues recently reported that direct injection of the enkephalin-expressing HSV vector into the pancreas attenuated evoked nocifensive behaviors in a rodent model of acute pancreatitis [22]. Other inhibitory neurotransmitters that have been delivered by HSV-mediated gene transfer to reduce pain include gamma amino butyric acid (GABA) produced by a vector expressing glutamic acid decarboxylase [15,20,21] and endomorphin-2 [45].

Based on the extensive evidence of the effectiveness of HSV mediated transfer of inhibitory neurotransmitters we have examined the effects of HSV-based vectors expressing anti-inflammatory peptides in models of both inflammatory and neuropathic pain.

Vector-mediated expression of interleukin (IL)-4 in the DRG after subcutaneous inoculation of an IL-4 expressing HSV vector does not alter sensory thresholds in normal animals, but significantly reduces mechanical allodynia and reverses thermal hyperalgesia in the selective spinal nerve ligation model of neuropathic pain [13] (Fig. 2 a,b). The effect of vector-mediated IL-4 expression correlates with a block in the increase of IL-1β and phosphorylation of p38 MAP kinase in the dorsal horn characteristic of neuroimmune activation in chronic neuropathic pain (Fig. 2 c,d).

Several lines of evidence indicate that tumor necrosis factor-alpha (TNFα) plays an key role in the development of chronic pain. In response to peripheral nerve crush, the amount of TNFα in spinal astrocytes and microglia is increased [29]. In the chronic constriction injury model of peripheral neuropathic pain, neutralizing antibodies directed against TNFα or the p55 TNF receptor (TNFR) reduce thermal hyperalgesia and mechanical allodynia [35], and intrathecal administration of the recombinant p75 soluble TNFR (sTNFR) peptide (etanercept) prior to selective spinal nerve ligation reduces mechanical allodynia [32,37]. TNFα is a member

of the superfamily of type II transmembrane proteins containing an intracellular N-terminus that are biologically active as self-assembling non-covalent bound trimers [16]. The full-length membrane-spanning TNFα (mTNFα) is cleaved by the inducible TNF alpha converting enzyme (TACE) [27] to release a diffusible peptide (soluble TNFα [sTNFα]) [8]. The activity of sTNFα can be terminated in part by binding to cleaved soluble forms of the TNFR (p55 or p75 sTNFR). And recombinant p75 sTNFR has substantial anti-inflammatory properties and is approved for use in the treatment of several inflammatory diseases.

We examined the effect of vector-mediated transfer of the gene coding for the p55 sTNFR to the dorsal root ganglion (DRG). A nonreplicating HSV gene transfer vector (T0TNFSR) constructed to express the p55 sTNFR injected subcutaneously to transduce DRG does not alter sensory thresholds in normal animals, but delays the development of both mechanical allodynia and thermal hyperalgesia after spinal nerve ligation, and inoculation 1 week after spinal nerve ligation results in a statistically significant reduction in pre-existing mechanical allodynia and thermal hyperalgesia (Fig. 3 a,b). The vector-mediated effect on mechanical allodynia peaks 2 weeks after inoculation before waning and is established by reinoculation. Inoculation of the p55 sTNFR-expressing vector prevented the increase in mTNFα in the spinal cord measured by western blot; by immunocytochemical staining mTNFα co-localizes with GFAP-labeled astrocytic processes and in a restricted focal distribution in OX-42-labeled microglia.

We examined other markers of neuroimmune activation including phosphorylation of p38 in microglia of the spinal cord [17,23], and release of IL-1β [38,44]. P-p38 immunoreactivity was localized to OX-42-positive microglial cells in the dorsal horn [13]. Animals with pain caused by spinal nerve ligation and inoculated with a control vector showed a statistically significant increase in both IL-1β and p-p38 in the dorsal horn of spinal cord measured by ELISA. These increases were significantly attenuated in animals inoculated with T0TNFsR (Fig. 3 c,d). P-p38 and mTNFα increases in the lateral hemisection model of below-level central pain of spinal cord injury were also attenuated by the p55 sTNFR-expressing vector. Following lateral hemisection animals develop mechanical allodynia; subcutaneous inoculation of T0TNFSR into the plantar surface of both hind paws 1 week after hemisection resulted in a significant reduction in pain-related response that was present by 1 week after inoculation and peaked 2 to 3 weeks after inoculation.

We tested a nonreplicating HSV vector expressing IL-10 in the delayed phase of the formalin test. TNFα mRNA and protein in the dorsal quadrant of the lumbar spinal cord are both increased 45 minutes after injection of formalin, a time point that corresponds to the peak of nocifensive behavior, and correlate with an increase in p-p38 [47]. Subcutaneous inoculation of the IL-10 expressing HSV vector resulted in a significant reduction in nocifensive behavior, concomitant with blocking the increase in mTNFα expression and the phosphorylation of p38 MAPK [47]. There was no sTNF detected in the spinal cord after formalin injection, and mTNFα immunoreactivity in the dorsal horn was found in restricted zones in activated microglia, identified by OX42 immunostaining [47].

Neuroimmune activation plays an important role in the development of chronic pathological pain after damage to the nervous system [4,41]. The evidence includes observations that: 1) in rodent models of peripheral neuropathic pain, partial peripheral nerve damage results in activation of glia in the spinal cord [43]; 2) treatments that reduce pain also block glial activation; and 3) disruption of spinal glial activation reduces nocifensive behaviors [19,30]. Specific evidence that TNFα plays an important role in this process is provided by studies which have demonstrated that: 1) spinal TNFα is increased after nerve injury [29]; 2) application of TNFα sensitizes DRG neurons to painful stimuli [28,36]; and 3) damaged nerves are hypersensitive to the proalgesic effects of TNFα [3,31]. Intrathecal administration of the

soluble form of p75 TNFR (etanercept) reduces pain-related behaviors and blocks the increase in phosphorylation of p38 in the spinal cord in the spinal nerve ligation model [32]. The antinociceptive effect of p75 TNFR in this model was observed only when the peptide was administered prior to injury. In other studies, intrathecal administration of neutralizing antibodies against the p55 TNFR (but not antibodies against the p75 TNFR) reduce pain in the chronic constriction injury model of peripheral neuropathic pain [34].

An emerging body of evidence in the immunology literature suggests that "reverse" signaling through mTNFα may serve to regulate the immune response [6,7,18,39]. The evidence from our studies suggests sTNFR released from the terminals of transduced DRG neurons binds to mTNFα to activate a reverse signaling mechanism that inhibits the phosphorylation of p38 and reduces expression of TNFα and IL-1β (Fig. 4). TACE is responsible for both cleavage and release of sTNFα from mTNFα as well as for the release of p75 sTNFR from p75 TNFR [33]. Released soluble cleaved receptor binding to sTNFα provides one mechanism that can result in termination of the action of sTNFα. The demonstration in this study that p55 sTNFR can down-regulate TNFα(and IL-1β expression by reverse signaling through mTNFα provides a second mechanism. Whether this process occurs directly in microglia, or through cell-cell signaling mediated by astrocyte-expressed TNFα has not been established. In the immune system, full-length transmembrane mTNFα serves to mediate bidirectional cell-to-cell interactions through binding to the membrane receptor complex (p55 or p75 TNFR) on neighboring cells. Similar to other members of the TNF superfamily, in immune cells the membrane-anchored ligand has been demonstrated to transduce "reverse" signals into the ligand-expressing cell when activated by the cognate receptor(s). The experimental evidence reviewed suggests a "reverse signaling" role for mTNF in the pathogenesis of neuropathic pain.

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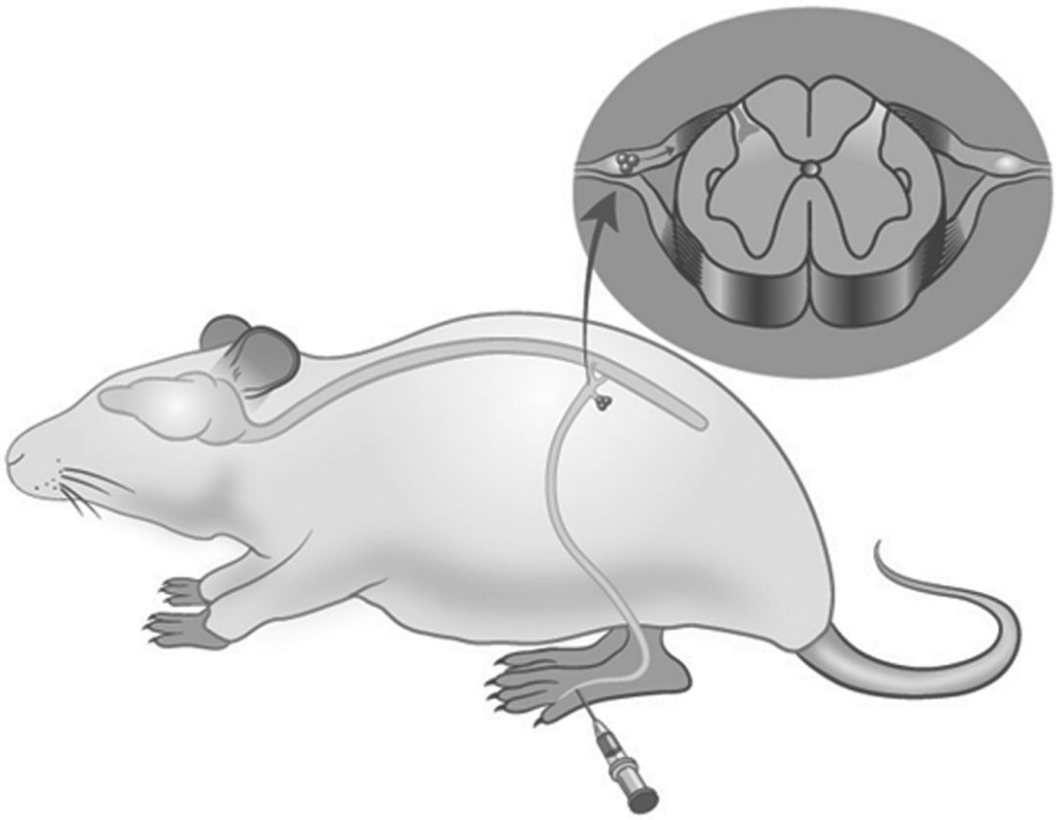

Figure 1.
Non-replicating HSV vectors injected subcutaneously are taken up by nerve terminals in the skin and carried by retrograde axonal transport to the DRG. Transgene products produced from the otherwise latent genomes are carried by anterograde axonal to nerve terminals in the spinal cord.

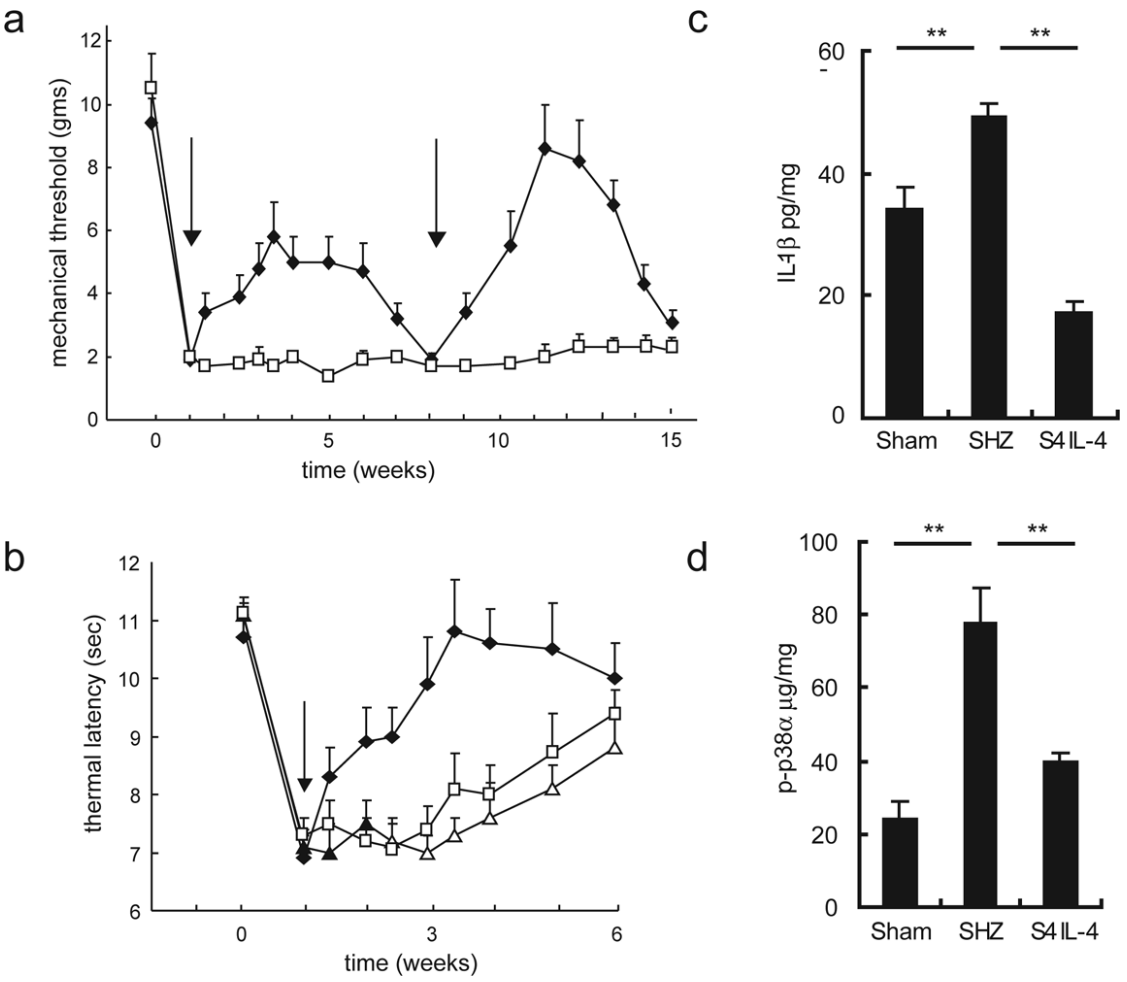

Figure 2.

Antinociceptive effect of S4IL4 in rats with neuropathic pain resulting from SNL. Filled diamonds - S4IL4; open squares - SHZ; open triangles - vehicle control. Mechanical allodynia is identical in vehicle control and SHZ-inoculated animals. Values presented as mean ± SEM, n=6-8 animals per group. Arrows indicate time of vector inoculation. X-axis: weeks after SNL. In each case, $P < 0.01$ comparing S4IL4-inoculated to control, using the general linear model (GLM) for repeated measures.

(a) Mechanical allodynia in animals inoculated with vector 1 week after SNL. (b) Thermal hyperalgesia in animals inoculated with vector 1 week after SNL. (c, d) IL-1β and p-p38αexpression in the spinal cord dorsal horn in neuropathic rats. Rats were given gentle tactile stimulation to left paw for 10 min 3 weeks after SNL or sham surgery. Ipsilateral dorsal horn was harvested 2 h after gentle stimulation. Inoculation of S4IL4 but not control vector significantly suppressed the increase in levels of IL-1β (c) and p-p38α(d) (*P <0.05, **P <0.01, ANOVA, post-hoc Scheffe test).

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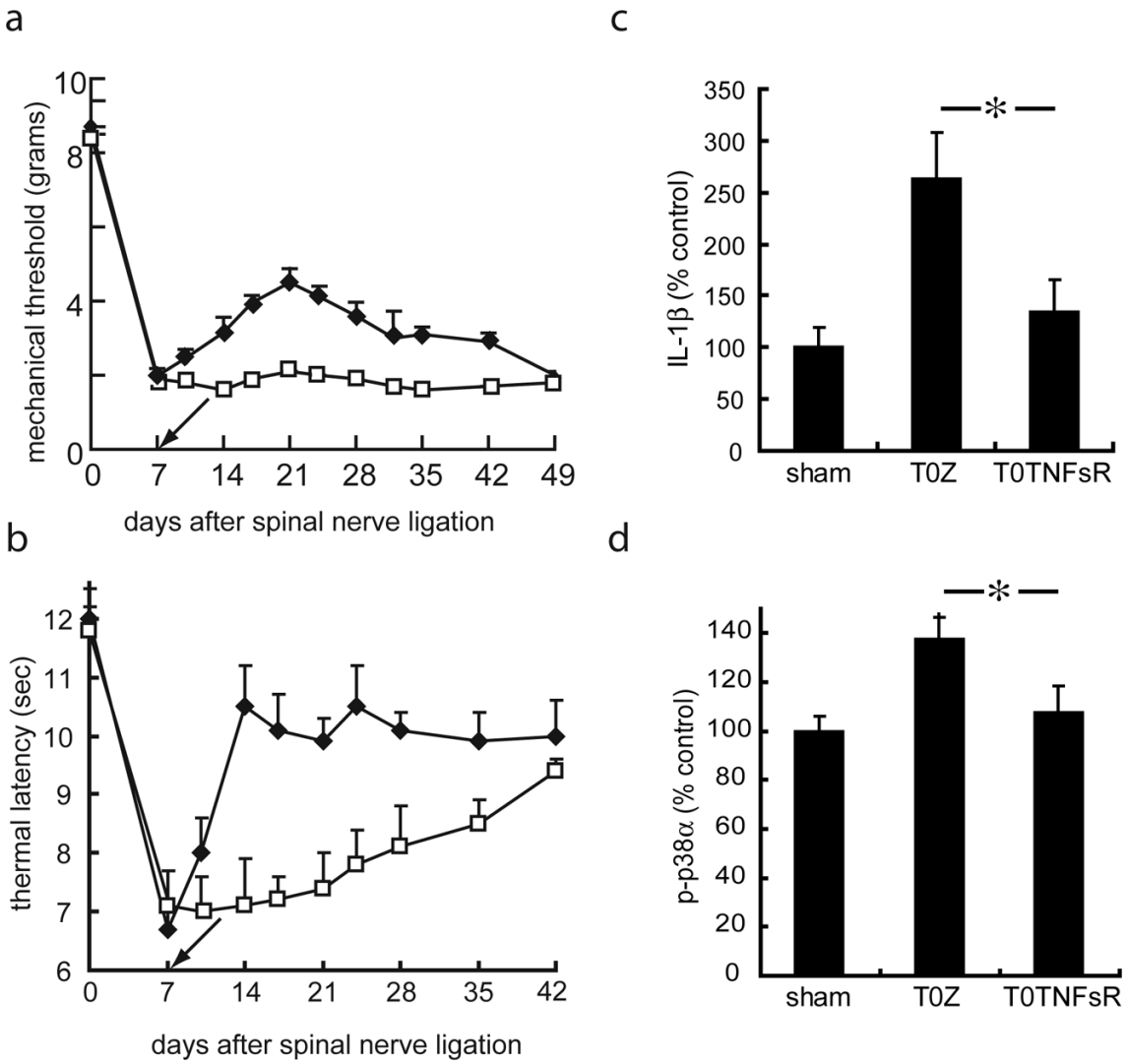

Figure 3.

Mechanical allodynia (a) and thermal hyperalgesia (b) in rats inoculated with vector T0Z.1 (open squares) or T0TNFsR (filled diamonds) 7 days after () selective L5 spinal nerve ligation. The time of vector inoculation is indicated by the arrow in each panel. T0TNFsR reversed mechanical allodynia ($P<0.01$, $n=4$-8) and thermal hyperalgesia ($P<0.01$, n=4-8). The statistical significance of differences between curves was determined using a general linear model with repeated measures analysis (SPSS 13.0).

(c, d) IL-1βand p-p38αexpression in the spinal cord dorsal horn determined by ELISA. Animals inoculated with T0TNFsR showed significant suppression in the increase of IL-1β(c) and p-p38α(d) compared to control vector T0Z.1. *$P<0.05$, ANOVA.

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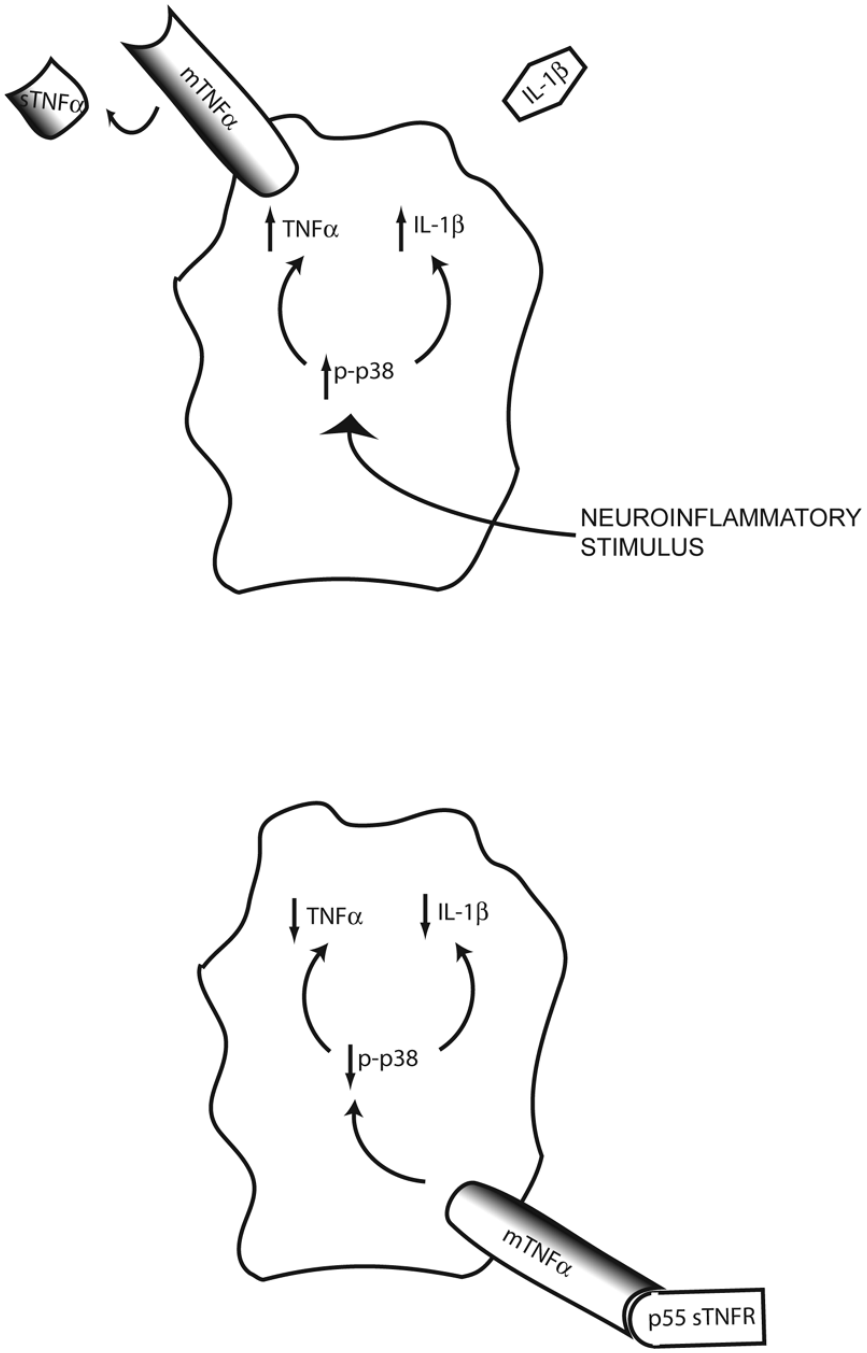

Figure 4.
Schematic diagram of the role of microglial TNFα in neuropathic pain. A neuroinflammatory stimulus caused by injury to peripheral nerve results in increased phosphorylation of p38α and elevations in IL-1β and TNFα in microglia; the latter is cleaved by TACE to release sTNFα (top). In our experiments, vector-produced p55 sTNFR acting through mTNFαeffects a reduction in p-p38, IL-1β, and mTNFα (bottom).