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Microglial modulation as a target for chronic pain: From the bench to the bedside and back

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Abstract

With a widespread Opioid Epidemic and profound biopsychosocial implications, chronic pain is a multi-faceted public health issue requiring urgent attention. The treatment of chronic pain is particularly important to anesthesiologists given our unique role as perioperative physicians and pain medicine specialists. The present review details the recent shift from a neuronal theory of chronic pain to one that includes complex neuron-glia interactions. In particular we highlight microglia, the myeloid-lineage cells of the central nervous system, as initiators of a post-injury neuroimmune response that contributes to the acute to chronic pain transition. We discuss ever-advancing preclinical studies, wherein significant success has been made through pharmacologic and genetic modulation of microglia, and we emphasize where these approaches have made the transition to the clinical realm. Furthermore, we highlight the most current, novel efforts to visualize glial activation *in vivo* using positron emission tomography (PET) and improve the diagnosis of chronic pain through radiotracer binding of specific targets, like the 18 kDa translator protein (TSPO) in microglia and myeloid-lineage cells. Our rapidly advancing knowledge about microglia and their involvement in pain suggests that the era of glial-targeted therapeutics is just beginning so long as we refocus our attention on optimizing preclinical studies using a clinically-informed approach, prior to translation.

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INTRODUCTION

Chronic pain is a significant public health problem of rising prevalence and consequence. With 100 million Americans alone experiencing chronic pain, this is an issue facing a larger population than that affected by cancer, heart disease, and diabetes combined; moreover, it is estimated that pain poses an economic burden of $635 billion each year in medical treatment and lost productivity.[1] Further complicating matters is the widespread Opioid Epidemic, which has consumed more lives with each year in recent history.[2] As Anesthesiologists, we have a crucial role in the prevention and management of pain both in the perioperative and outpatient settings. Our pre-operative assessments and pre-habilitation protocols aim to optimize patients before surgery, while our intra-operative drug administration can alter the likelihood a patient develops chronic post-surgical pain, and our post-operative pain management continues to influence functional recovery.

The interaction between the nervous system and the immune system is particularly important to the development of chronic pain conditions.[3] New technologies using whole system single-cell immune profiling of patients before and after surgery have demonstrated the complexity of the peripheral immune response to surgical injury and how modulation of this response may impact to post-surgical outcomes.[4] Despite these advances, there is little known about the central nervous system (CNS) immune response to injury. The CNS is considered to be “immune privileged,” since under physiologic conditions the entry of peripheral innate and adaptive immune cells is tightly controlled by the blood-brain barrier.[5] However, the recent dramatic increase in knowledge about microglia and astrocytes, the non-neuronal cells in the CNS, has brought fitting attention to their function as resident brain and spinal cord immune cells with key contributions to CNS health and disease.[6]

As pain mechanisms have been further studied, an exclusively neuronal theory fails to explain chronic pain in its entirety; glial cells – particularly microglia – are now widely implicated in the initiation and progression of persistent pain. As a result, the number of articles focused on glia and pain has risen considerably since the 1990s (Figure 1), reflecting this important contribution. In recent years, several comprehensive reviews have effectively detailed the numerous potential molecular mechanisms underlying the connection between glia and pain.[3,7,8] In this narrative review, we aim to provide a more clinically-informed basic science introduction to the physiological roles of glia. We then discuss the emerging importance of glia in pain conditions, reviewing both the extensive basic research and limited clinical studies available on the potential of targeting glia as a therapeutic approach for the prevention and management of chronic pain. Finally, we provide insight into novel imaging techniques to visualize glial cells *in vivo* for early diagnosis and therapeutic decision making.

A PRIMER ON GLIAL CELLS

Glia, the non-neuronal cells of the nervous system, are present both peripherally and centrally in larger quantity than neurons (Figure 2).[9] Three types of glial cells exist in the CNS – microglia, the resident myeloid-lineage cells of the CNS; astrocytes, responsible for modulating neuronal activity; and oligodendrocytes, providing the myelin sheath that

insulates neurons. Beyond their role as CNS support cells, activated glial cells release cytokines/chemokines, and regulate neuronal signaling through a process termed gliotransmission.[10]

Microglia, the myeloid lineage cells of the CNS and the focus of this article, are in fact the only myeloid cells derived exclusively from yolk-sac progenitors.[6] Beginning at embryonic day 8.5, microglia travel to the developing CNS,[11,12] and the blood brain barrier forms at E13.5 to segregate them from the periphery.[13] These cells have immense importance in the developing brain, comprising approximately 10% of the total CNS cell population. Microglia express numerous cell surface markers, many of which are common to other myeloid-lineage cells, including CX_3CR1 and CD11b,[14] and several recently discovered microglia-specific genes including TMEM119 and Sall1.[15,16] Microglia serve multiple important functions during and after development including triggering apoptosis of neurons and engulfing dead cells in the developing brain to control cell number.[17–20] They also facilitate synapse maturation and remodeling in addition to synaptic pruning,[21] though further investigation is necessary to elucidate the precise mechanisms by which microglia mediate such plasticity.[22] After injury, microglia are closely involved in identifying injured tissue, and through the production of complement, can clear away neuronal debris and prevent damage from spreading to nearby intact neurons.[23]

In their resting state, microglia exhibit a ramified (highly branched) morphology and actively survey their surroundings for potential danger.[24] For many years, it was accepted that the presence of injury led to microglial activation and transformation to an amoeboid (less branched) morphology expressing increased levels of markers such as CD11b and Iba1 as well as the release of pro-inflammatory cytokines like IL-1, IL-6 and TNF-α.[25,26] More recently, this view has evolved as a result of data showing a dissociation between morphological microglial activation and expected neuroinflammatory outcomes,[27,28] suggesting that the M1 (pro-inflammatory) versus M2 (anti-inflammatory) nomenclature ascribed to macrophages may not translate directly to microglia.[29] More likely, microglia exist in a range of states that are environment-, location-, age- and even sex-dependent. This has been more clearly delineated using next generation sequencing (NGS) methods to investigate the microglial transcriptome under varying conditions, capitalizing on cell surface markers such as CD11b and the recently identified microglia-specific TMEM119 to distinguish microglia from CD45-containing infiltrating macrophages.[15] For example, Grabert et al.[30] performed RNA transcriptome sequencing on microglia isolated from different areas of the brain (cortex, hippocampus, striatum, cerebellum) and found that the microglial transcriptome is regionally heterogeneous. In addition, they found distinct age-dependent microglial signatures with the cluster of immune regulation genes most sensitive to aging. The spectrum of microglial states has also been highlighted by comparing acutely isolated microglia to those maintained in culture for hours or days. These studies have shown gene expression profiles that differ based on time in culture,[31] resulting in de-differentiation of microglia to more inflammatory states that themselves even exist with slightly different transcriptional programs.[32] Given the multitude of states and functions, microglia have now been implicated as contributors to several neurologic disorders including Alzheimer's disease,[33] multiple sclerosis[34] and autism spectrum disorder,[35] among others. A recent elegant study using RNA sequencing on microglia isolated from resected human brain

tissue found that microglial-specific genes overlapped significantly with genes implicated in a host of neurodegenerative and psychiatric disorders.[31] Such studies reflect the potential for an improved understanding of microglial behavior in disease and thus allow for the development of more targeted strategies to modulate microglia to treat these conditions.

PAIN MECHANISMS AND THE CONTRIBUTION OF GLIA

Broad prevention and management of pain has historically eluded the medical community in part due to the mechanistic complexity and heterogeneity of pain. When injury occurs, an acute pain state is elicited wherein primary afferent fibers, including Aδ and C fibers, signal via their cell bodies within the dorsal root ganglia (DRG) to the dorsal horn of the spinal cord and ultimately to the cortex by way of the brainstem and thalamus.[36–38] The presence of injury lowers the threshold required to activate nociceptors, permitting the healing of damaged tissue; however, in some instances, due to peripheral and/or central plasticity, this sensitization can become persistent, causing spontaneous pain, pain from typically non-noxious stimuli, and/or pain that is exaggerated in severity and duration[37,39]. In such cases, while the initial injury may heal, persistent pain becomes a malignancy in and of itself, with chronic CNS changes contributing to the perpetuation of pain signals.[39] Pain that has reached a chronic stage is more refractory to treatment than acute pain,[40] which is why inhibition of the transition from acute to chronic pain is imperative. Mediators responsible for this pain progression must be identified: microglia may represent such a target (Figure 3).

Several papers in the 1990s first documented glial reactivity in mouse models of pain.[41–43] Specifically, they demonstrated that after peripheral nerve injury microglial (and astrocytic) “activation” markers (CD11b, GFAP) were increased in the spinal cord dorsal horn ipsilateral to the injury and pro-inflammatory cytokines were detected in the same tissue (also see Figure 2).[3] Furthermore, microglia were suggested to be the first responders to injury, becoming activated at 24 hours post-injury, increasing in number for 1 week following injury,[43] and ultimately remaining activated chronically post-injury.[41,44–46] Several studies[47,48] further demonstrated that microglia are important for the initiation but not the maintenance of pain, with astrocytes taking on this latter role.[49] These findings are particularly interesting in the context of elegant work demonstrating that microglia can trigger astrocytic activation and subsequent loss of synaptic support through the release of IL-1α, TNF and C1q.[50] A host of microglia-specific molecules have been shown to be upregulated in nerve injury models including $P2X_4R$, CSF1R and Trem2, among others, comprehensively reviewed recently by Inoue and Tsuda.[8] Current use of NGS technologies have taken a systems-approach to identify a whole cell microglial-specific pain relevant transcriptome. In a study evaluating the RNA signature of microglia after spinal nerve ligation (SNL), transcriptome-wide analyses using RNA-seq revealed 17 genes that were upregulated after SNL compared to sham.[51] Further, chromatin immunoprecipitation (ChIP) sequencing and ChIP-qPCR revealed 16 enhancers (regulatory regions) that were induced for weeks after injury, suggesting a mechanism for long-lasting transcriptional changes that may contribute to pain chronification. Taken together, these studies suggest that modulation of microglial reactivity may prevent the aberrant synaptic plasticity associated with chronic pain.[52]

TARGETING MICROGLIA TO TREAT PAIN: A TRANSLATIONAL PERSPECTIVE

While there are no currently approved drugs that specifically target microglia, some clinically available agents exhibit a degree of microglial modulation and are being explored as potential analgesics (see Table 1). In addition, multiple substances released from injured neurons have been implicated in triggering microglial activation via specific receptors, and these may be viable targets for further drug development.[7,8] As outlined below, this microglial targeting has already had considerable success at attenuating upregulation of inflammatory mediators in the spinal cord and reversing anti-nociceptive behaviors in preclinical rodent models, however, for most, their efficacy in humans is in need of further study.

Microglial "Modulators"

Minocycline.—Minocycline, a tetracycline antibiotic, is one of the most frequently used medications purported to selectively inhibit microglia in mouse models of pain. Minocycline has been shown to prevent injury-induced sensitivity through the inhibition of microglial activation and subsequent TNF-α and IL-1β release when pre-emptively administered intrathecally or intraperitoneally.[25,53–55] While some preclinical studies show minocycline is effective at reversing existing allodynia and hyperalgesia,[45,56] not all studies support these findings.[25,53,54] A double-blind placebo controlled randomized controlled trial (RCT) administering 200 mg minocycline or placebo prior to carpal tunnel or trigger finger release, followed by 100 mg twice daily for 5 days after surgery demonstrated no meaningful reduction in the time to pain resolution between minocycline-treated and placebo-treated patients.[57] Importantly, in one subgroup of patients with elevated post-traumatic stress disorder (PTSD) symptoms, minocycline was actually associated with a longer time to pain resolution. Another multi-center RCT supported these findings, with no significant change in pain scores after pre-operative administration of minocycline in patients undergoing lumbar discectomy.[58] In another study, Sumracki et al.[59] gave unilateral sciatica patients intradermal capsaicin and used their known heightened response as a screen for novel anti-neuropathics. In this small study, patients received a one-time dose of minocycline or pregabalin (the positive control) prior to capsaicin administration and found no significant effect of either treatment compared to placebo, suggesting that this paradigm may not be sufficiently sensitive to detect an effect. In contrast, a small RCT showed that two weeks of daily minocycline improved the numerical rating scale (NRS) of patients with subacute lumbar radiculopathy by 1.47 points, a small but statistically significant effect size.[60] Finally, the efficacy of twice daily minocycline was evaluated in 25 patients with diabetic peripheral neuropathy and significant changes in neuropathic symptoms were found in the minocycline group; however, visual analog scores were significantly different in both the minocycline and placebo groups.[61]

It is possible that the existing limited efficacy of minocycline in the clinical context results from low selectivity of minocycline for the microglial phenotypes present in persistent pain states, or more likely, that this antibiotic has many non-microglial targets which may hinder its analgesic properties. This theory is supported by a preliminary study that concluded

minocycline inhibited expression of the anti-inflammatory cytokine IL-10, limiting its potential benefit.[53] Furthermore, a recent critical review of the preclinical literature on minocycline clearly outlines the diversity and extent of its targets including peripheral immune cells (monocytes, T cells, neutrophils) and even neurons.[62]

Propentofylline.—Another example of a non-specific glial modulator which showed great preclinical promise for preventing and reversing neuropathic pain is the atypical methylxanthine and phosphodiesterase inhibitor, propentofylline.[63,64] Unfortunately, one clinical trial in patients with post-herpetic neuralgia (PHN) failed to show any effect of the drug after 4 weeks, with a species difference in microglial activation between rodents and humans proposed as the explanation.[65] This study was criticized for several methodological flaws including lack of preclinical data in PHN models, unknown bioavailability and low CNS penetration of propentofylline.[66]

Drugs Targeting Specific Microglial Genes

Toll-like Receptor 4.—Toll-like Receptor 4 (TLR4), the receptor for lipopolysaccharide and danger-associated molecular patterns such as high-mobility group box 1 (HMGB1), has been investigated as a possible initiator of microglial activation and presents a promising target for pain reversal and prevention.[67] While all myeloid-lineage cells express TLR4 peripherally and centrally, it may also be expressed by CNS astrocytes[68] and neurons[69], suggesting that TLR4 targeted drugs may have effects beyond glial modulation. Tanga et al. [70] demonstrated that TLR4 knockout (KO) or point mutation significantly abrogated mechanical and thermal sensitivity following peripheral nerve injury, and knockout mice had lower levels of CD11b and CD14, markers of microglial activation, as well as decreased mRNA expression of IFN-γ, IL-1β, and TNF-α at all time points following nerve injury. In other animal models of pain, TLR4 KO prevented the spreading of post-injury pain,[71] a phenomenon that presents great difficulty in a clinical setting. Interestingly, several investigations have highlighted that a direct interaction between TLR4 and the opioid antagonists naloxone and naltrexone may explain their analgesic efficacy.[72–74] These agents were initially used as competitive opioid antagonists for the treatment of opioid addiction and alcoholism. However, at doses approximately 1/10th that used for substance use disorders, “low-dose” naltrexone (LDN) came into clinical use as a theoretical microglial modulator or CNS anti-inflammatory agent, as low doses exhibit minimal opioid receptor binding and instead target TLR4 directly.[75] In one randomized, double-blind, placebo-controlled crossover study, LDN was shown to significantly reduce baseline pain and improve life satisfaction and mood compared to placebo.[76,77] In clinical practice, we (Dr. Tawfik) prescribe LDN 4.5 mg daily for the treatment of fibromyalgia and other refractory pain conditions given its safety profile and relatively low cost.

Purinergic Receptors.—One of the first studies to link microglial activation to persistent pain identified $P2X_4R$ on microglia as an essential mediator of post-injury neuron-microglia signaling through the release of neuronal ATP.[44] Rigorous immunohistochemistry confirmed the specificity of $P2X_4R$ to microglia and treatment with an antisense oligodeoxynucleotide antagonizing $P2X_4R$ offered a partial reversal of anti-nociceptive behaviors.[44] Subsequent work by the same group[78–80] has outlined an intracellular pathway after $P2X_4R$ activation

that leads to microglial p38 MAPK activation and subsequent NFκB-mediated cytokine release. More recently, they have developed $P2X_4R$ antagonists with high specificity and efficacy in preclinical models of neuropathic pain and PHN.[81] Subsequent testing in human trials is reported to be ongoing but limited information is currently available.[8]

Interestingly, another purinergic receptor expressed on microglia, $P2X_7R$, has been evaluated as a potential target to dampen neuroinflammation.[82] One clinical study showed no effect of a $P2X_7R$ antagonist in patients with refractory rheumatoid arthritis,[83] however, the low CNS penetration of the drug may explain these results.[84] The $P2X_7R$-associated nonselective ion channel, pannexin-1 (Panx1), may provide an alternate pathway target as deletion of this gene in microglia blocked the development of allodynia in mice.[85] Further, the widely used anti-gout agent, probenecid, is a Panx1 inhibitor, reversed existing allodynia and decreased spinal IL-1β in a mouse model of joint pain, suggesting a new use for a clinically-available drug with a good safety profile.[85]

p38 Pathway Inhibitors.—The mitogen-activated protein kinase (MAPK) family includes several members crucial for intracellular signaling in neurons and glia. p38 MAPK is a particularly attractive target as multiple receptor pathways converge on p38 including $P2X_4R$, $P2X_7R$, and TLR4, and p38 engages NFκB to produce proinflammatory cytokines.[86] Several studies have found that phosphorylation of p38 MAPK in spinal microglia is required for persistent pain after nerve injury and targeting phospho-p38 with selective inhibitors reverses allodynia in preclinical models,[87] though the effect may be sex-specific in some cases.[88] In a rat model using first-degree burn to induce central sensitization, inhibition of p38 MAPK by preemptive treatment with the ATP-competitive SD-282 resulted in partial reversal of mechanical, but not thermal sensitivity, in a dose-dependent fashion.[89]

Clinical translation of p38 inhibitors has had mixed results. In a multicenter, double-blind, placebo-controlled cross-over trial studying the effects of the p38 MAPK inhibitor, dilmapimod, for patients with nerve injury, radiculopathy, or carpal tunnel syndrome, dilmapimod-treated patients reported a significantly greater reduction in pain intensity compared to the placebo group, in addition to improvements in numerous affective outcome measures.[90] In contrast, an RCT evaluating the analgesic properties of the p38 inhibitor, losmapimod, for patients with lumbosacral radiculopathies demonstrated no significant differences in NRS for losmapimod-treated patients vs. placebo, although the losmapimod group did report clinically meaningful improvements in secondary measures like sleep, general health, and social functioning.[91] Losmapimod's poor analgesic efficacy was supported by an additional study from this group, which found no significant difference in NRS between the losmapimod and placebo groups for patients whose neuropathic pain resulted from traumatic peripheral nerve injury; in this study, however, there was also no significant difference in secondary outcomes between groups.[92]

Cytokine Antagonists.—Glia are the likely source of CNS pro-inflammatory cytokines such as IL-1β and TNF-α.[50,93] Once released into the synapse, these cytokines act on their cognate receptors on pre- and post-synaptic neurons to directly modulate excitatory synaptic transmission.[3,93] Pre-synaptically, activation of IL-1R by glial released IL-1β results in increased glutamate release from primary afferent neurons[94] and measurable increases in the

frequency and amplitude of excitatory post-synaptic currents.[95] Post-synaptically, TNF-α makes a key contribution to homeostatic synaptic scaling, an important type of whole cell activity-dependent modulation, by altering the trafficking and cell surface expression of post-synaptic AMPA receptors.[93,96]

Numerous efforts have been made to understand whether antagonizing pro-inflammatory cytokines can successfully prevent or reverse post-injury pain. One preclinical study using a rat model of complex regional pain syndrome (CRPS) demonstrated TNF-α elevation in ipsilateral hindpaw skin, nerve tissue, and bone following tibial fracture and immobilization[97]. When soluble TNF-R1 was administered to inhibit TNF, allodynia was decreased, but limb edema and temperature remained unchanged, suggesting that TNF contributes to allodynia, but not the additional vascular changes seen in CRPS. Another series of studies using a rodent model of neuropathic pain confirmed the anti-allodynic effect of TNF antagonism using the drug etanercept and further demonstrated that the mechanism of allodynia reversal was dependent on suppression of TNF-induced phospho-p38 in DRG neurons[98] and spinal microglia.[99] Unfortunately, randomized controlled trials of infliximab and etanercept, both TNF antagonists, have failed to alleviate pain in patients with lumbar radiculopathies[100,101] or discogenic back pain,[102] despite promising preclinical studies suggesting the safety of such agents.[103] Taken together, these studies reflect the promise and complexity of cytokine inhibition, and suggest that further studies are warranted to identify which combinations of cytokines may be targeted simultaneously to reverse persistent pain.

Recent attention has also focused on the contribution of IL-1β to pain development and maintenance. Several early studies demonstrated efficacy of the IL-1β receptor antagonist, IL1-ra, at preventing or reversing existing pain in preclinical models.[104,105] In order to hone in specifically on microglial cytokines, Grace et al.[106] used a rigorous method involving Gq- and Gi-coupled Designer Receptor Exclusively Activated by a Designer Drug (DREADD) in order to selectively stimulate (Gq) or inhibit (Gi) microglial activity. The DREADD CD68-hM3Dq was utilized for its ability to increase intracellular Ca^{2+}, because elevated intracellular Ca^{2+} has been shown to activate microglia and initiate the release of inflammatory cytokines.[107] When administered intrathecally, CD68-hM3Dq induced allodynia in mice within four hours. The study subsequently confirmed the dependence of this allodynia on IL-1β by administering IL1-ra, which entirely prevented the development of hypersensitivity following CD68-hM3Dq administration. This strategy represents an exciting breakthrough that should clarify the precise contribution of microglial cytokine release to pain progression and maintenance.

At this time, several other pharmacologic agents, including cannabinoids, which act in part on cannabinoid receptors on microglia,[108] and ibudilast, a phosphodiesterase inhibitor,[109] show promise as analgesics for patients with neuropathic pain, though studies of these agents are in preliminary stages.[75] Numerous clinical trials are in progress to advance our understanding of the analgesic efficacy of glial modulators (Table 1) and with further translational efforts (discussed below) more compounds will enter the pipeline shortly.

CLINICAL TRANSLATION: PITFALLS & PROMISE

Improving Preclinical Studies

Thus far, clinical studies of glial modulators have shown limited efficacy at reversing and preventing persistent pain. We can, however, learn from these failures to better design future studies and drugs. As outlined in several recent reviews, this failure to translate is not specific to glial modulators in pain but has been the case for many promising analgesic drugs.[110,111] Some suggested explanations for these failures include the heterogeneity of clinical pain conditions, the complexity of the underlying mechanisms and the need for more reliable preclinical models and measures of pain.[111] For example, preclinical experiments often administer drugs preemptively or very shortly after induction of the pain model, while in a clinical setting, treatment may not be initiated for up to 5 years after the inciting injury.[112] In addition, the use of strictly evoked or reflexive responses, like paw withdrawal, need to be expanded to include higher order cognitive tasks such as place preference or avoidance that more aptly capture the affective-motivational aspects of pain.[113] This suggests that additional preclinical studies with clinically-informed protocols may be necessary to optimize all potential analgesics including glial modulators prior to translating them.

In the particular case of glial-targeted agents, the low reported clinical efficacy may also be explained by the lack of specificity of our purported glial modulators,[62] oversimplification in our understanding of microglia reactivity,[29] incomplete knowledge of sex-specific effects,[55,114] or lack of understanding of the time-dependence of microglial contributions to persistent pain. It is encouraging that our knowledge in these areas is rapidly expanding and actively being pursued through clinically-informed basic science experiments in many laboratories, including our own. Also promising is that NGS studies have provided a deeper understanding of the microglial transcriptome and perhaps more importantly, how closely human and mouse microglia compare. For example, Gosselin et al.[31] evaluated the microglia transcriptome from human and mouse cortex and found that the overall pattern of gene expression was similar, with the majority of gene pairs expressed within a fourfold range. Although clear differences were also found, knowledge of these also improves our ability to design human microglia-targeted drugs.

Imaging Microglia to Improve Targeted Treatment

As described previously, microglia are likely the initiators of a cascade leading to astrocyte dysregulation of neuronal crosstalk. The timing of microglial-directed treatments must therefore consider how involved microglia are, at that particular moment, in the overall maintenance of pain. One major difficulty with clinical studies is that the glial activation status of patients is unknown, and likely variable over the course of disease, but developments in clinical imaging may provide an invaluable technique to guide and monitor glial-targeted interventions in real-time.

Positron emission tomography (PET) is a non-invasive imaging technique that enables visualization of cellular and molecular processes in living intact subjects and can be used clinically for diagnosis and disease management, or preclinically in animal models.[115] PET shows great promise as a method to visualize neuroinflammation, and specifically glial

activation, in a variety of neurologic disorders through the use of radiotracers;[116,117] small molecules, peptides, particles, or engineered protein fragments that are specific for a molecular process/target of interest and labeled with a positron-emitting radionuclide (e.g., 18F, 11C, 64Cu). The most widely used class of PET tracer for imaging glial activation are those that bind the translocator protein 18 kDa (TSPO) – a mitochondrial membrane receptor upregulated in activated glia and myeloid lineage cells (monocytes/macrophages). The exact function of TSPO is not yet clear, however, a series of studies suggested that it may contribute to recovery from injury as TSPO agonists decreased allodynia and thermal hyperalgesia in a model of neuropathic pain.[118,119] One preclinical study, using a first generation TSPO tracer, 11C-PK11195, found a significant increase in binding in the lumbar spinal cord of rats after partial sciatic nerve ligation, correlating with elevated glial activation.[120] In the clinical setting, Loggia and colleagues showed an elevated TSPO-PET signal in the brain of patients suffering from chronic low back pain (LBP), using 11C-PBR28, a promising second generation TSPO tracer.[121] Specifically, there was a significantly higher standardized uptake value ratio (SUVR) in the thalamus (normalized to whole brain uptake) for LBP patients, in addition to increased SUVRs in the pre- and postcentral gyri and paracentral lobule – although the differences in the thalamus were the most dramatic and consistent. Likewise, in a study of eleven CRPS patients versus twelve controls, 11C-PK11195 was shown to detect glial activation in the thalamus, caudate nucleus, putamen, and nucleus accumbens of CRPS subjects as per the significantly elevated distribution volume ratio.[122] In an exciting attempt to pair such imaging with microglial modulation, a current clinical trial (ClinicalTrials.gov identifier:) is recruiting patients with low back pain to undergo TSPO-PET scans before and after a 2-week trial of minocycline, placebo or no treatment. Such PET imaging studies of the molecular underpinnings of pain will likely provide critical insights into the *in vivo* systemic spatiotemporal dynamics of neuroimmune interactions involved in the development of chronic pain, and could also be advantageous in early diagnosis, therapeutic decision making, and ultimately the prevention of many pain syndromes.

CONCLUDING REMARKS

We have presented in this review significant evidence supporting a glial contribution to persistent pain, with microglia acting as the first responders to initiate post-injury neuroinflammation. Through glia-neuron crosstalk, sensitization occurs to propagate a centralized persistent pain cycle that poses a significant challenge to both patients and clinicians. Glial modulators represent a class of medications with proven efficacy in preclinical studies that warrant further investigation for the treatment of chronic pain. Moving forward, improving the extent to which preclinical studies are clinically-informed – for example, optimizing the dose and timing of drug administration relative to the injury, using non-reflexive tests of sensitivity and considering variables such as type of pain, sex and age – all increase the likelihood that we make meaningful translational advancements. Moreover, the use of non-invasive imaging techniques will likely help with patient selection and disease monitoring and could serve as an important endpoint in clinical trials of new glial modulators. In addition, our rapidly expanding knowledge about glia in animals and humans, through advanced transcriptomics, suggests that glial “activation” is not an all-or-

none phenomena, and strategies that more subtly shift glia back to their homeostatic functions may have greater success in treating high-impact pain.

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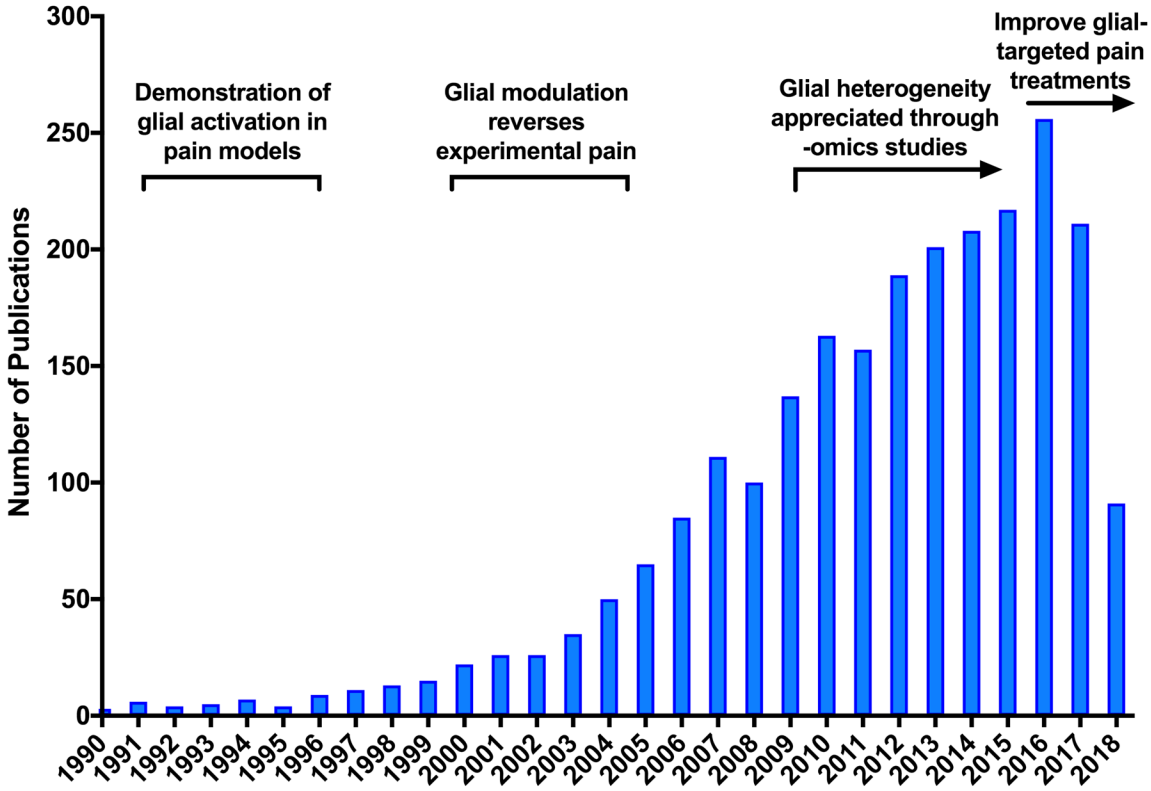

Figure 1.

Exponential increase in the number of published papers on glia and pain since the 1990s. PubMed search hits for “glia and pain” were tabulated from 1990 through 2018 to track growing interest in glial biology as it pertains to pain. Major milestones from this research are also noted, including the need to use the newest knowledge on glia to improve glial-targeted treatments.

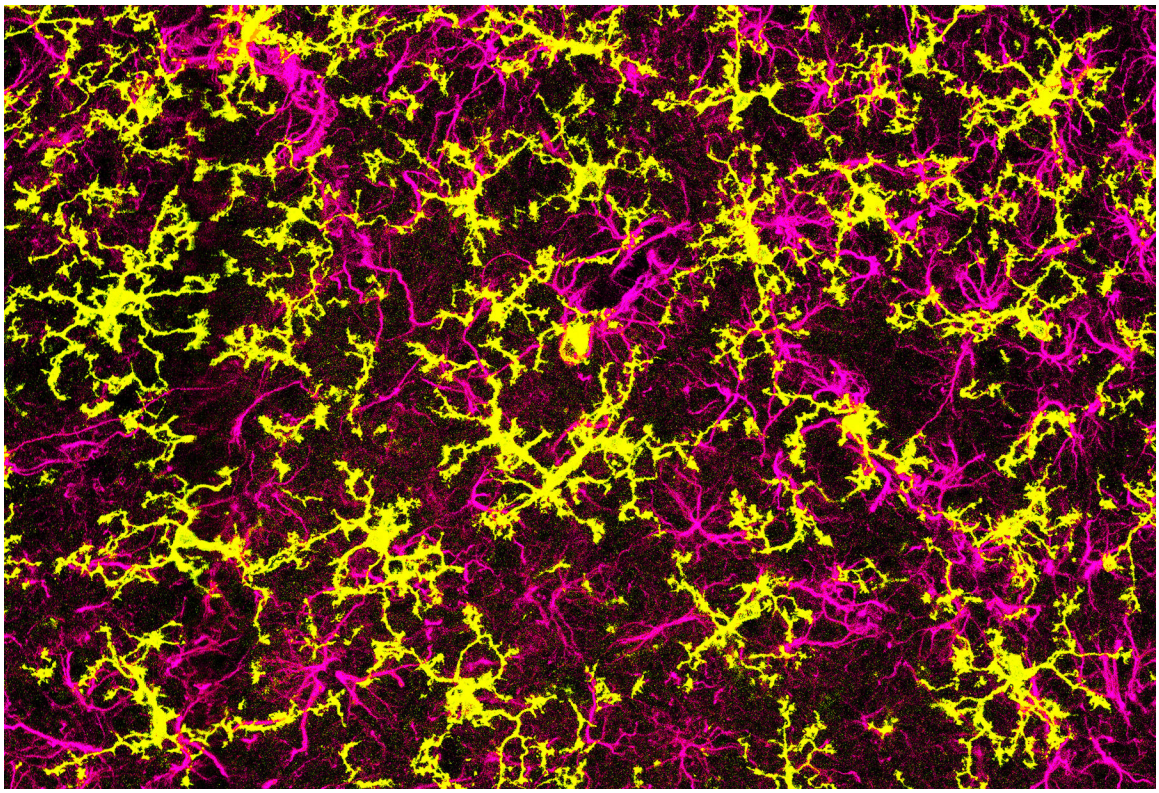

Figure 2.

Microglia and astrocytes comprise the majority of glial cells in the CNS. Microglia are labeled with CD11b (yellow), a marker of microglial activation, and astrocytes are labeled with the astrocytic marker GFAP (pink) in the dorsal horn of the spinal cord of mice that underwent tibial fracture and casting as a model of complex regional pain syndrome.

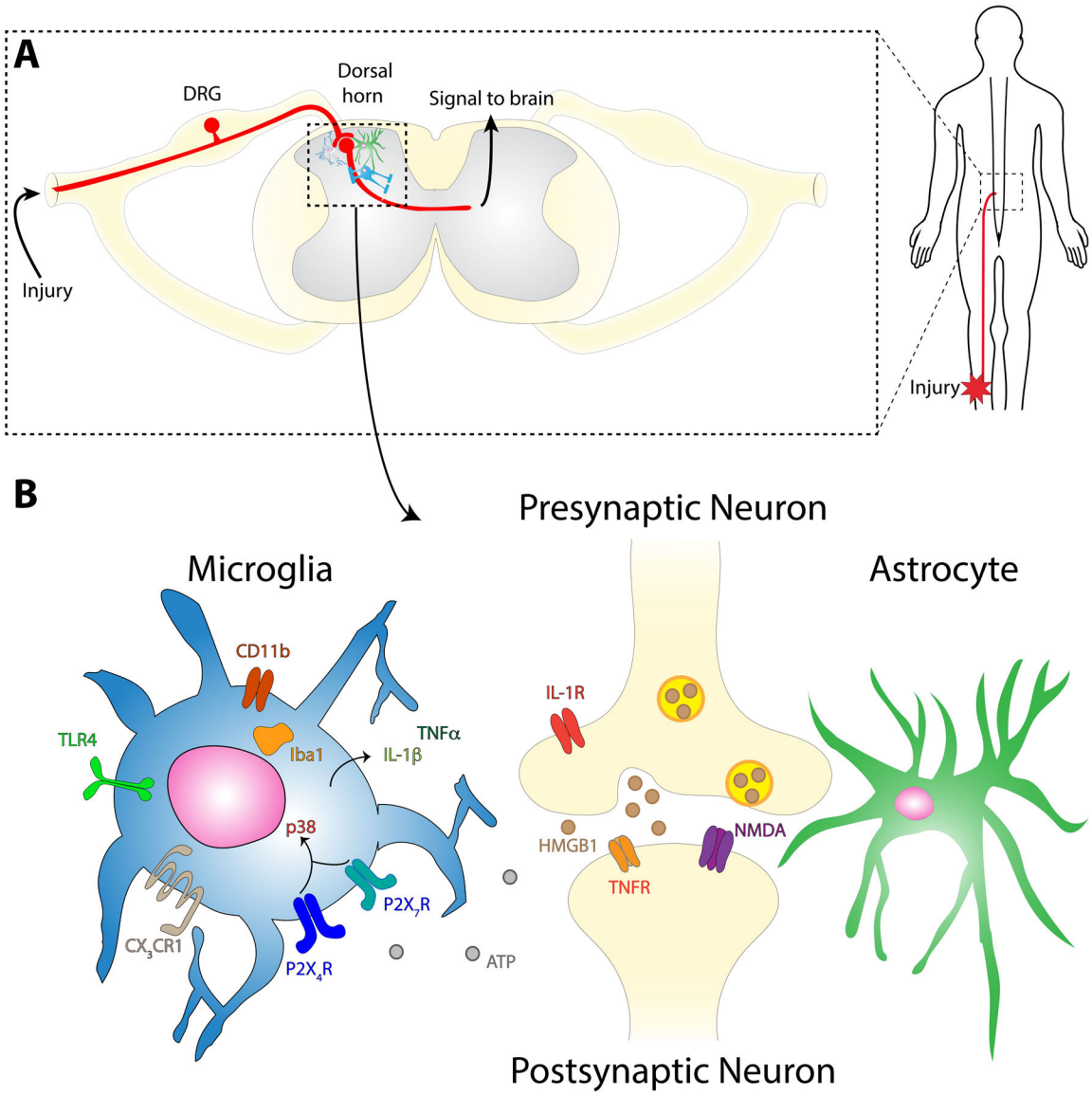

Figure 3.

Glial modulation of dorsal horn circuits is key to homeostasis and response to injury. Either direct or indirect injury to a primary afferent (presynaptic) neuron activates microglia in the dorsal horn of the spinal cord. The release of neuronal algesic mediators such as ATP and HMGB1, act on microglial P2XRs and TLR4, respectively, to activate downstream signaling through p38 MAPK and ultimately enhance local cytokine release that is the hallmark of neuroinflammation. These proinflammatory cytokines such as IL-1β and TNF-α can then act on their synaptically expressed receptors to enhance excitatory neurotransmission and produce pain. DRG, dorsal root ganglion.

Table 1.

Studies targeting glial activation in pain registered on ClinicalTrials.gov

Name of Drug	Target	Registration Number	Indication	Status of Study
Minocycline	Microglial inhibitor		Low Back Pain	Recruiting
SB-681323 (Dilmapimod)	p38 Mitogen-activated Protein Kinase Inhibitor		Neuropathic pain	Completed
Naltrexone	TLR4 Antagonist		Fibromyalgia	Completed
Naltrexone	TLR4 Antagonist		Complex Regional Pain Syndrome	Recruiting
Ibudilast	Phosphodiesterase Inhibitor		Oxycodone Self-administration	Completed
SLC022 (propentofylline)	Phosphodiesterase Inhibitor		Post-herpetic Neuralgia	Active, Not Recruiting
Oral Tetrahydrocannabinol	Cannabinoid-receptor agonist		Pain	Active, Not Recruiting