The Role of Glial Cells in Chronic Pain
The Mechanisms of Glial Activation and the Implications for Chronic Pain Treatment

INTRODUCTION

Pain management remains a significant health concern, especially for those who suffer from chronic pain. Also known as neuropathic pain, this type of pain usually follows injury, surgery, infection, or chemotherapy (Marx, 2004). Chronic pain, or pain that persists beyond the natural course of healing, affects over five million people in the United States (Tawfik et al., 2007) and costs over $100 billion annually in medical expenses and lost productive time (Department of Health and Human Services, 1998; Stewart et al., 2003). Despite advances in the pharmaceutical industry, nearly two-thirds of patients receive little to no relief from current pain treatments (Sindrup and Jensen, 1999; Collins et al., 2000; McQuay et al., 1996). Until recently, chronic pain was perceived to be a message transmitted to the brain via neurons, like any other sensory signal (Miller, 2005). However, breakthroughs in pain research have indicated that a previously ignored third factor, glia, actually contributes to chronic pain.

Glial cells, specifically microglia and astrocytes, traditionally act as the “support system” for neurons, yet more research is confirming that glial cells are also primary promoters of chronic pain (Miller, 2005; Watkins, Milligan & Maier, 2001). Conclusions from this research have shown increased activation of glial cells, particularly microglia and astrocytes, in chronic pain states. Additionally, studies concentrating on glial behavior, secretion of cytokines and neurotrophins, and possible targets of drug therapy have yielded encouraging results that may pave the way for chronic pain treatment. Current research suggests that microglia secrete cytokines and neurotrophins, which send signals to astrocytes. Astrocytes, which monitor the
passing of substances from blood vessels to the brain via the blood-brain barrier, experience increased permeability as a result of cytokine and neurotrophin expression. This increased permeability may contribute to increased neuron sensitivity, thus resulting in neuropathic pain.

Since so many people fall victim to neuropathic pain, and because there is virtually no effective treatment, support for present and future research is imperative. While research has addressed many questions concerning the relationship between glial activation and neuron sensitivity, there remain many unanswered questions regarding why glial activation occurs, the role of secreted cytokines and neurotrophins, and how glia can be used as targets of drug therapy. A thorough literature review has revealed compelling answers to these questions, answers that have furthered knowledge about the nervous system and may be able to improve chronic pain treatments in the future.

BACKGROUND

Glial Cells: Constructing the Blood-brain barrier

For the most part, the purpose of glial cells is to provide support and protection for the neuron, and to construct the blood-brain barrier (Miller, 2005). Astrocytes, a particular subset of glial cells, mainly comprise the blood-brain barrier and facilitate the movement of substances between blood vessels and the brain (Figure 1). Astrocytes essentially prevent potentially harmful substances from reaching the brain by only allowing certain small molecules to enter the nervous system. Microglia, on the other hand, will prepare an immune response and destroy any pathogens that get past the astrocytes (Figure 1). In the human body, glial cells outnumber neurons by ten to one, and they will remain in a resting state unless activated during an immune response. At the resting state astrocytes are still active in monitoring brain function, but there is still much to be learned about the resting state of microglia; since most studies focus on
disrupting microglia, there is a lack of information on the purpose and function of resting microglia (Banati, 2002). Both microglia and astrocytes are immunocompetent cells, which means that they are known to respond to the presence of bacteria, viruses, and trauma; nevertheless, research during the last decade has shown that this immune response may be prolonged after physical injury and nerve damage, which ultimately contributes to chronic pain.

Testing Chronic Pain and Measuring Glial Activation

While pain usually persists in order to relay the message that something is wrong to the brain, chronic pain exists past the healing process and has no functional purpose in alerting the body. Chronic pain manifests itself into one of two similar conditions: allodynia or hyperalgesia. Allodynia refers to exaggerated pain responses and hyperalgesia refers to hypersensitivity to pain (Wieseler-Frank, Maier, & Watkins, 2005); both are induced in animal models to represent chronic pain so that neuron and glial activity can be examined. Since there are extensive regulations that dictate ethical and safe practices of pain research involving animals, pain is
usually induced by a chronic constriction injury (CCI) or by injecting a viral protein into the spinal cord (Garrison et al., 1991). Once allodynia or hyperalgesia is induced, a common method to quantify the level of pain being experienced is by using von Frey monofilaments. Von Frey monofilaments are applied to the dorsal aspect of the hind paw of the animal and increase incrementally in pressure until a threshold response (recoiling of the paw) is observed. The more pain-sensitive the animal, the lower the threshold response to monofilament pressure. Another way to measure glial activation is to assess the expression of a protein that is known to be synthesized in a chronic pain state (Figures 2 and 3); in astrocytes, glial fibrillary acidic protein (GFAP) is measured and complement-3 receptor is measured in microglia (Garrison et al., 1991). Using these methods, scientists can examine modes of glial activation or can test the effectiveness of potential drug treatments.

Figure 2
Microglial Activation
(Intact vs. Damaged Nerve Cells)
Microglial activation measured by complement-3 receptor marker before and after peri-spinal injection of a viral protein, which causes an exaggerated pain response.

a) Intact nerve cell b) Nerve cell after peri-spinal injection of viral protein (Watkins, Milligan, & Maier, 2001)

Figure 3
Astrocyte Activation
(Intact vs. Damaged Nerve Cells)
Astrocyte activation is measured by glial fibrillary acidic protein (GFAP) marker in rat models after Chronic Constriction Injury (CCI).

a) Intact nerve cell b) Nerve cell after chronic constriction injury (Garrison et al., 1991)
GLIAL BEHAVIOR IN CHRONIC PAIN STATES

In recognizing that glial cells play a role in chronic pain, one of the most important findings was that P2X receptors resided on glial cells, not on neurons (Sutherland, 2004). P2X receptors, or pain-signaling molecules, have long been associated with pain, yet they were perceived to be associated with nerve cells. As a result of this discovery, researchers began inspecting the mechanisms of glial activation more closely. An entire series of studies concentrated on microglial receptors, and the results showed a strong connection between the Toll-like receptors and microglia activation (DeLeo, 2006). Toll-like receptors are pattern recognition receptors found on microglia that influence the microglial immune response against pathogens (Guo & Schluesener, 2007). Specifically, the activity of Toll-like receptor 4 has been isolated and seems to be the primary activator of microglia in neuropathic pain states (DeLeo, 2006). However, the function of Toll-like receptors in the central nervous system in a pain-free state is not yet understood, and more research on these receptors must be done before the receptors can become potential targets for drugs (Wieseler-Frank, Maier, & Watkins, 2005). Since drugs would aim to convert activated receptors to a resting state, researchers must first identify the form and function of Toll-like receptors in a pain-free environment.

PRO-INFLAMMATORY CYTOKINES

When microglia are activated, they release pro-inflammatory cytokines into the central nervous system. Studies first suggested that microglia released pro-inflammatory cytokines only when physiological, not pathological, pain was demonstrated in animal models. The initial rationale was that the cytokines being released in chronic pain subjects was a result of the slight trauma animals experienced during surgery or injection—cytokine release was not associated with the chronic pain itself (Wieseler-Frank, Maier, & Watkins, 2005). Nevertheless, later
reports that studied hyperalgesia in the spinal cord revealed that cytokines were indeed being secreted in a chronic pain state (Wieseler-Frank, Maier, & Watkins, 2005). These pro-inflammatory cytokines, specifically tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6), are proteins that are vital in immune-to-brain communication (Watkins & Maier, 2005). It is hypothesized that the inflammation encouraged by cytokines increases the permeability of the blood-brain barrier, and thus affects the movement of metabolites and ions from blood vessels to the brain (Hansson, 2006). Because neuron activity is a function of ion movement, increasing permeability may increase neuron excitability and sensitivity (Miller, 2005). Moreover, successful testing of drugs that suppress cytokine expression also support the idea that pro-inflammatory cytokines act in a pain state as well as during an immune response.

**NEUROTROPHIC FACTORS**

In addition to pro-inflammatory cytokines, microglia can also secrete neurotrophic factors, which may play a role in activating or deactivating astrocytes. Most notably, nerve growth factor (NGF) has been shown to contribute to inflammation-induced hyperalgesia (Fang et al., 2003). Current research is aimed at other growth factors and their potential effects, including brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). Since the behavior of cells after secretion of BDNF resembles that of NGF, this neurotrophin may function as a neurotransmitter for pain perception (Fang et al., 2003). GDNF, on the other hand, produces the opposite results; this neurotrophin is actually down-regulated in chronic pain states (Fang et al., 2003). In fact, one study showed that GDNF might even relieve neuropathic pain by regulating sodium-channel subunits in dorsal root ganglia (Boucher et al., 2000). It is suspected that GDNF may also be able to reverse chronic pain and keep patients in a pain-free state for an extended amount of time, but, for a variety of reasons, much more research
is needed to support these claims. First, there is a fine line between correlation and causation of GDNF and pain that limits the role of GDNF as a treatment. Additionally, while it is known that cytokines and neurotrophins are both secreted by microglia, there is virtually no literature addressing the relationship, if any, between the two. Learning more about GDNF may allow the use of neurotrophins as a means to reverse and alleviate chronic pain.

**TREATMENTS**

*Current Chronic Pain Treatment*

One of the reasons research in glial activation is so important is because of the severe need for drugs that can target glia. The majority of medications are ineffective because they are aimed at relieving neuron, not glia, induced pain; in fact, drugs that only target neurons, such as morphine, only alleviate pain for one out of five patients (Wieseler-Frank, Maier, & Watkins, 2005). Moreover, the relief for that one patient is short-lived because many chronic pain patients develop a tolerance to morphine (Cherny et al., 1994)—only opioids such as methadone or fentanyl, which have dramatic side effects, maintain effectiveness (Schumacher, Basbaum, & Way, 2004).

*Potential Chronic Pain Treatments*

The most promising therapeutic research presently revolves around inhibiting glial activation. Drugs such as the methylxanthine derivative, propentofylline (Tawfik et al., 2007) or ibudilast are observed not only to block glial activation, but also reverse it (Ledeboer et al., 2006; Ledeboer et al., 2007). When animal models with induced allodynia were treated with propentofylline, not only was current allodynia reversed, but a pain-free state was maintained during the two-week drug washout period (Tawfik et al., 2007). Examination at the molecular level revealed that glial activation was stemmed because cytokine expression of IL-1β, IL-6 and
TNFα was suppressed (Tawfik et al., 2007). Studies with ibudilast similarly saw attenuation of allodynia and downregulation of pro-inflammatory cytokine expression (Ledeboer et al., 2006; Ledeboer et al., 2007). Following the successes of these drugs in animal models, studies using other glial activation inhibitors have exploded with numerous pharmacological blocks being tested for allodynia attenuation and, as research continues, the most effective of these drugs may be selected to continue on in human clinical trials.

Despite this promising research, caution should be used in applying animal studies to humans because of the difference in animal and human clinical situations (Linderoth & Foreman, 2006). Humans have usually experienced pain for a much longer time than animals, so they may develop a tolerance to new glia-targeting drugs (as is seen with morphine). Furthermore, there may be some unknown physiological differences between human chronic pain and the CCI or viral protein models in animals that may alter drug effectiveness.

CONCLUSIONS

Almost a decade ago, experts in the pain field were skeptical of the idea that glial cells may play a role in chronic pain, yet today scientists have accepted the significance of glial cells in neuropathic pain—what is being questioned is how glial cells promote chronic pain. The state of current research seems to support two conclusions; first, that microglia and astrocytes are most active, with microglial activation acting as a precursor to astrocyte activation, and secondly, that targeting glial cells could lead to promising chronic pain treatments. It seems that microglia initiate pain by activation and secretion of either cytokines or neurotrophins that directly enhance neuron sensitivity. In the case of neurotrophins, the secretion of NGF or BDNF activates astrocytes and causes chronic pain, whereas GDNF alleviates chronic pain (Wieseler-Frank, Maier, & Watkins, 2005). The prevention of cytokine expression and neurotrophin secretion
indicates that treatments directed at suppressing microglial activation may be more effective than targeting neurons.

Despite this progress, much research remains to be done, especially in examining the resting state of glial cells and potential drug therapies. With regards to glial cells, research should be done to determine the resting function of microglia and Toll-like receptors in order to learn more about their deactivation. Research should be conducted that tests the relationship between neurotrophins and cytokine expression; moreover, there is a dearth of research concerning the potential pain reversal and pain relieving functions of neurotrophins. Overall, research in the field of chronic pain is both dynamic and groundbreaking, offering a greater understanding of neuropathic pain and bringing the scientific community closer to finding a sustainable treatment for chronic pain patients.
Works Cited


