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Long-term control of neuropathic pain in a non-viral gene therapy paradigm

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Abstract

Traditional approaches to treating chronic neuropathic pain largely focus on manipulations directly altering neuronal activity or neuron-to-neuron communication. Recently, however, it has become clear that glial cells (including microglia and astroglia) play a significant role in pain expression in a variety of neuropathic pain models. Multiple aspects of the inflammatory response of glial cells, commonly observed in neuropathic pain conditions, have been implicated in pain expression. Thus, glial cell inflammation has emerged as a potential therapeutic target in neuropathic pain. Our laboratory has been exploring the use of an anti-inflammatory cytokine, interleukin-10 (IL-10), to control glial inflammatory activation thereby controlling neuropathic pain. IL-10 protein delivery is limited by a short half-life and an inability to cross into the central nervous system from the periphery, making a centrally delivered gene therapy approach attractive. We have recently characterized a non-viral gene therapy approach using two injections of naked DNA to achieve long-term (>3 months) control of neuropathic pain in a peripheral nerve injury model. Timing and dose requirements leading to long-term pain control are discussed in this review, as is recent work using microparticle-encapsulated DNA to achieve long-term therapeutic efficacy with a single injection.

Keywords

IL-10; allodynia; glia; CNS

Glial activation is an important mediator of chronic pain in diverse pathologies

Chronic pain may arise from a wide variety of insults to the peripheral or central nervous system (CNS) that include infection, autoimmune disease and mechanical trauma.[1–5] Symptoms associated with chronic pain include an enhanced sensitivity to painful stimuli (hyperalgesia) and the perception of normally non-painful stimuli as painful (allodynia). The intensity of symptom expression may change over time, as may the location of the pain. The complex nature of symptom expression along with the diversity of possible origins of chronic pain have contributed to the difficulty of developing effective therapeutic interventions.

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A great deal of research in the past decade has highlighted the crucial role of microglial and astroglial cells (herein referred to as 'glia') in the inflammatory response to both injury and infection.[6–9] These cells are commonly activated following a wide variety of infectious or mechanical insults, and constitute a vital signaling network that contribute to the pathology of injury. The term 'tetrapartite synapse' has been coined by DeLeo *et al*.[10] to describe the intimate conversation that takes place between pre-synaptic neurons, post-synaptic neurons, microglia and astrocytes that ultimately defines the signaling that occurs within a neuronal network under normal circumstances, as well as during pathological conditions. The language used in this conversation among glia and neurons not only includes classic neuromodulators such as glutamate and nitric oxide, but also a large number of proteins initially identified for their role in immune signaling, namely cytokines and chemokines.[11–16] Glial activation is often accompanied by both morphological changes as well as increased production of mediators termed proinflammatory cytokines (interleukin-1 (IL-1), tumor necrosis factor α, IL-6, IL-6).

Neuronal hyperexcitability is a potential downstream consequence of glial activation.[17–19] This neuronal hyper-excitability is in turn thought to underlie the expression of classic symptoms of neuropathic pain, including allodynia and hyperalgesia.[20–22] This line of research has identified glial activation as a cornerstone of injury-induced signaling within the CNS that is responsible for symptom expression, and therefore identifies this activation as an attractive potential therapeutic target.

Glial activation is subject to regulation through cytokine signaling

The cytokine inflammatory signaling network involves not only classic proinflammatory mediators such as IL-1 and tumor necrosis factorα, but also regulatory cytokines, including IL-10, that interfere with and counteract the effects of IL-1 and tumor necrosis factorα.[23–25] IL-10 exerts its effects through a number of mechanisms including the downregulation of inflammatory second messengers such as nuclear factor κβ and the downregulation of surface receptors such as the IL-1 receptor, as well as the upregulation of some molecules such as suppressor of cytokine signaling and the activation of second messengers such as signal transducer and activator of transcription 3. The actions of IL-10 have been extensively reviewed elsewhere.[26]

Interleukin-10 is a particularly attractive therapeutic candidate in the control of glial activation for several reasons. First, IL-10 has the demonstrated capacity to suppress glial activation in a variety of contexts including infections, mechanical trauma and autoimmune inflammation.[27] The use of IL-10 has been shown *in vivo* in animal models to control inflammation in a diversity of paradigms.[26,28–30] In addition, in many CNS sites such as spinal cord, IL-10 receptors are present only on glia and not on neurons.[31] The selective targeting of glial activation offers the possibility of reducing the inflammatory input driving neuronal hyperexcitability while leaving basal neuronal functioning intact. This is a major advantage over therapies that directly target neuronal activity and are often associated with severely limiting side effects.

Chronic pain, by definition, is a condition that is prolonged in time. Therefore, it is desirable that the therapy be equally prolonged in duration. As a proof of principle for the therapeutic use of IL-10 to suppress chronic pain, bolus IL-10 protein administration is able to block and reverse neuropathic pain in multiple animal models.[32] However, the short-term efficacy of protein injections (several hours), which corresponds to the IL-10 half-life of protein in the intrathecal space, limits this approach.[32] The short half-life of the protein makes a gene therapy approach an attractive alternative. Several attempts have been made to achieve long-term therapeutic efficacy by the use of viral vector overexpression of IL-10. These include

adenoviral, adeno-associated viral and herpes simplex viral vectors through various routes of administration.[32–35] Long-term efficacy has proven difficult to achieve and repeated administration of therapeutic viral vectors are often ineffective or potentially dangerous owing to the host immune response that they may provoke.[36,37] This latter concern is especially noteworthy for CNS applications, given the negative repercussions of inflammation-induced neuronal loss.

Long-term therapeutic control of glial activation can be achieved through intrathecal non-viral IL-10 gene delivery

We have recently developed and characterized a non-viral gene therapy approach that makes use of two successive intrathecal injections of naked plasmid DNA coding for the anti-inflammatory cytokine IL-10^{F129S} (Sloane *et al*.[38] and Milligan *et al*.[39]). This cytokine contains a point mutation (serine substitution for phenylalanine at amino acid 129) from the wild-type IL-10 protein and has shown *in vivo* therapeutic efficacy in a variety of both peripheral and central inflammatory contexts. We have shown the ability of this novel procedure to control the allodynia associated with peripheral nerve injury, chemotherapy-induced neuropathy, and both the allodynia and paralysis associated with experimental autoimmune encephalomyelitis (a model of relapsing-remitting multiple sclerosis).[30,39,40] Changes in the expression of glial activation and immune markers in both spinal cord and dorsal root ganglia associated with several of these inflammatory models are also prevented by pDNA-IL-10^{F129S} therapy in these studies.

This injection protocol requires two intrathecal injections of IL-10^{F129S} DNA to be administered no less than 5 h, and no more than 3 days apart, to achieve long-term therapeutic efficacy. Injections separated by 2 h or given as a single combined dose bolus provide therapeutic benefits lasting only approximately 6 days. Maximal efficacy (>3 months) has been observed with an initial injection dose of 100 μg DNA and a second injection of 25 μg DNA separated by a 2–3 day interval (Figure 1). The timing requirements of this procedure appear to involve a sequence of events triggered by the initial DNA injection (the ‘priming’ injection). Within 6 h, a significant accumulation of innate immune cells (primarily cells strongly staining for markers of macrophages and undifferentiated monocytes) is observed in cerebrospinal fluid (CSF) at the intrathecal injection site (Figure 2). Whether these cells are recruited from the peripheral circulation or are intrinsic immune cells of the meninges that translocate to the CSF is as yet unknown. These cells appear to be of monocytic origin, with phenotypes consistent with those anticipated to be recruited following an immune challenge. It has been previously documented that innate immune cells, largely consisting of macrophages, infiltrate the CNS following local injection of various immunostimulatory substances including synthetic oligodeoxynucleotides. By 24 h following the priming DNA injection, the influx of cells into CSF peaks consists primarily of activated macrophages. This cell type is well known for its phagocytic capacity, and the increased presence of these cells would likely enhance the uptake and clearance of a subsequent DNA injection from CSF owing to the enriched local population. At 3 days following the priming DNA injection, local CSF still contains this enriched population of macrophages when the second, or ‘therapeutic’, DNA injection occurs. It is important to note that the innate immune response to the priming DNA injection occurs independent of the transgene contained in the plasmid. That is, 100 μg of a control plasmid that does not contain the IL-10 gene is perfectly capable of leading to long-term therapeutic efficacy, provided it is followed by a 25 μg therapeutic DNA injection that contains the gene of interest, IL-10^{F129S} (Sloane *et al*.[38]). The reverse injection schedule, however, does not lead to a successful long-term therapy. An initial priming injection of 100 μg pDNA-IL-10^{F129S} followed by 25 μg control plasmid leads to a therapy lasting only approximately 6 days.[38]

The similar time course of cell accumulation in CSF and the window of time in which a therapeutic DNA injection will lead to long-term efficacy appear to be more than coincidental. Cell influx following a 100 μg DNA injection can be blocked by intrathecal pretreatment with IL-10 protein and co-injection of IL-10 protein along with the DNA injection (Figure 3a). This suggests that an acute inflammatory response to the priming DNA injection is responsible for cell influx into CSF. Moreover, if cell influx is blocked through IL-10 protein pre- and co-treatment, therapeutic benefit is observed only for several days following a second pDNA-IL-10^{F129S} injection as opposed to the normal duration of greater than 3 months (Figure 3b).

The IL-10^{F129S} transgene is not only required on the second injection for long-term therapeutic efficacy, but also there appears to be an ongoing requirement for anti-inflammatory signaling. Intrathecal administration of either the IL-10 neutralizing antibody (but not control IgG) or the inflammatory HIV coat protein, gp120, induces a rapid and sustained failure of the gene therapy.[38] This finding suggests that the inclusion of an IL-10 gene or other regulatory cytokine genes along with any desired therapeutic gene of interest may be beneficial in providing long-term efficacy.

Non-viral IL-10 gene therapy may be applicable to a variety of neuropathic conditions

This two-injection paradigm, in which a priming DNA injection triggers the accumulation of phagocytic cells followed by a therapeutic DNA injection administered during the period of immune cell accumulation, has potential widespread application. This paradigm may serve as a general model in the development of other gene therapy applications and may provide a method to enhance gene uptake (viral or non-viral) by taking advantage of the inherent immunogenicity of gene therapy vectors.

The proposed mechanism underlying the effectiveness of this two-injection paradigm is that an innate immune response to an initial injection potentiates a subsequent therapeutic injection through recruitment of phagocytic innate immune cells. The accumulation of phagocytic immune cells (primarily macrophages) is predicted to enhance uptake of plasmid DNA on a subsequent injection, thereby improving transfection efficiency of the local cell population. This idea further predicts that ongoing inflammation, or immune priming, at the site of gene therapy administration should enhance phagocytic cell recruitment following an initial injection, thereby optimizing a subsequent therapeutic injection. This prediction is supported by observations of increased cell recruitment following intrathecal DNA injection in the context of sciatic nerve injury-induced neuroinflammation compared with sham-operated controls (Figure 4a). Aging is a second context known to be associated with increased CNS immune reactivity, and in accordance with the above concepts, injection of naked DNA into aged rats (approximately 24 months) provokes a substantially greater accumulation of macrophages at 24 h post-injection as compared with younger rats (approximately 6 months) (Figure 4b).

An additional prediction that can be made is that long-term therapeutic efficacy requires the initial presence of sufficient numbers of phagocytic cells as well as sufficient copies of plasmid containing the therapeutic gene of interest. If it were possible to achieve both of these ends following a single intrathecal injection, this would offer a more attractive clinical approach. We have been pursuing this possibility with the use of pDNA-IL-10^{F129S} encapsulated in microparticles consisting of a lactic and glycolic acid polymer. The primary benefit of microparticle encapsulation is the subsequent chronic release of DNA as the particles degrade over time. We have recently made the unexpected observation that microparticles injected intrathecally, which do not contain DNA, induce significant local accumulation of phagocytic immune cells.[41] By injecting particles containing pDNA-IL-10^{F129S} the requirements of

sufficient phagocytic cell presence as well as sufficient exposure of these cells to therapeutic plasmid copies may both be fulfilled. We have recently observed therapeutic efficacy following lactic and glycolic acid polymer-encapsulated pDNA-IL-10^{F129S} lasting approximately 3 months in the context of peripheral nerve injury.[41]

The principal goal driving this therapeutic approach is the control of inflammatory glial activation to control symptoms of painful neuropathy. This gene therapy has since been applied successfully to autoimmune inflammation in a model of multiple sclerosis, and has demonstrated the ability to control paralysis as well as pain in this context.[30] Glial activation as a common end point arising from diverse sources of inflammation and underlying a variety of symptoms may provide a powerful therapeutic target for multiple neuroinflammatory diseases. This family of diseases may include such clinical challenges as Alzheimer's disease, amyotrophic lateral sclerosis and diabetic neuropathy, all of which are associated with glial activation in CNS.[42–46] The development of effective and chronic therapies that target CNS inflammation holds promise in the treatment of a range of diseases, including those leading to chronic pain.

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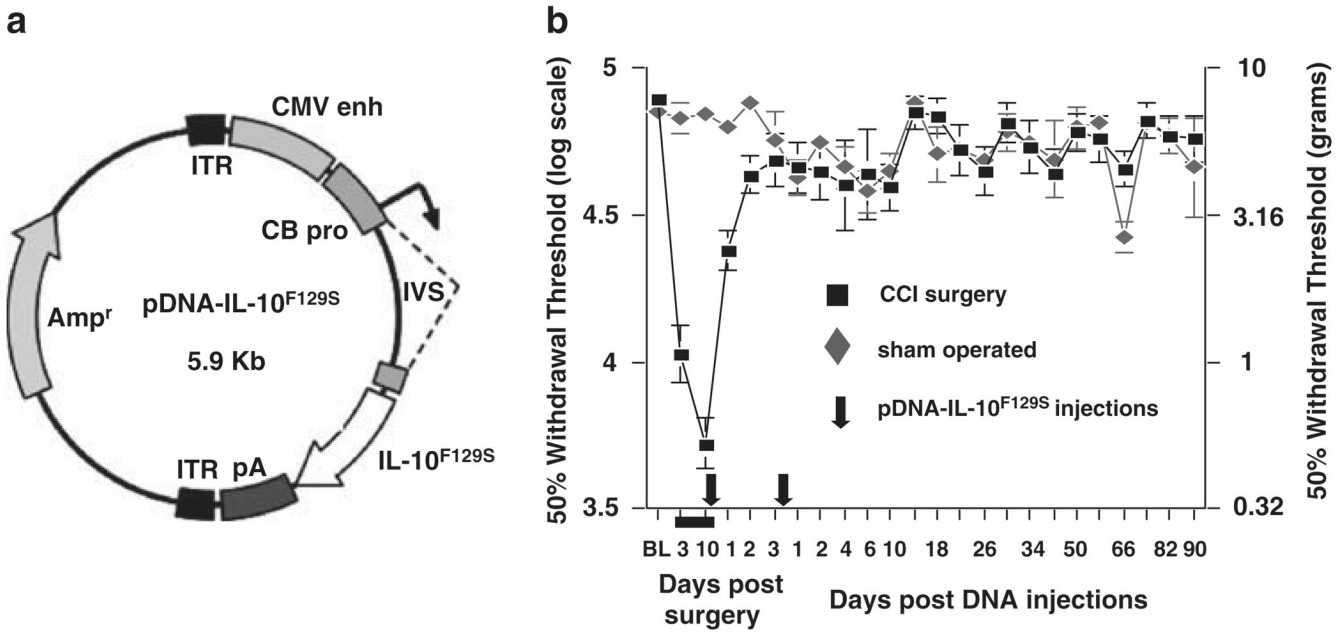

Figure 1.

(**a**) Design of the pDNA-IL-10^{F129S} construct.[39] Gene transcription is driven by a cytomegalovirus enhancer (CMV enh) and a chicken β actin promoter (CB pro).[39] The expression cassette is flanked by two inverted terminal repeat (ITR) sequences and the backbone contains an ampicillin (AMP)-resistance gene. (**b**) Two injections of pDNA-IL-10^{F129S} separated by 3 days leads to the long-term reversal of peripheral nerve injury-induced allodynia.

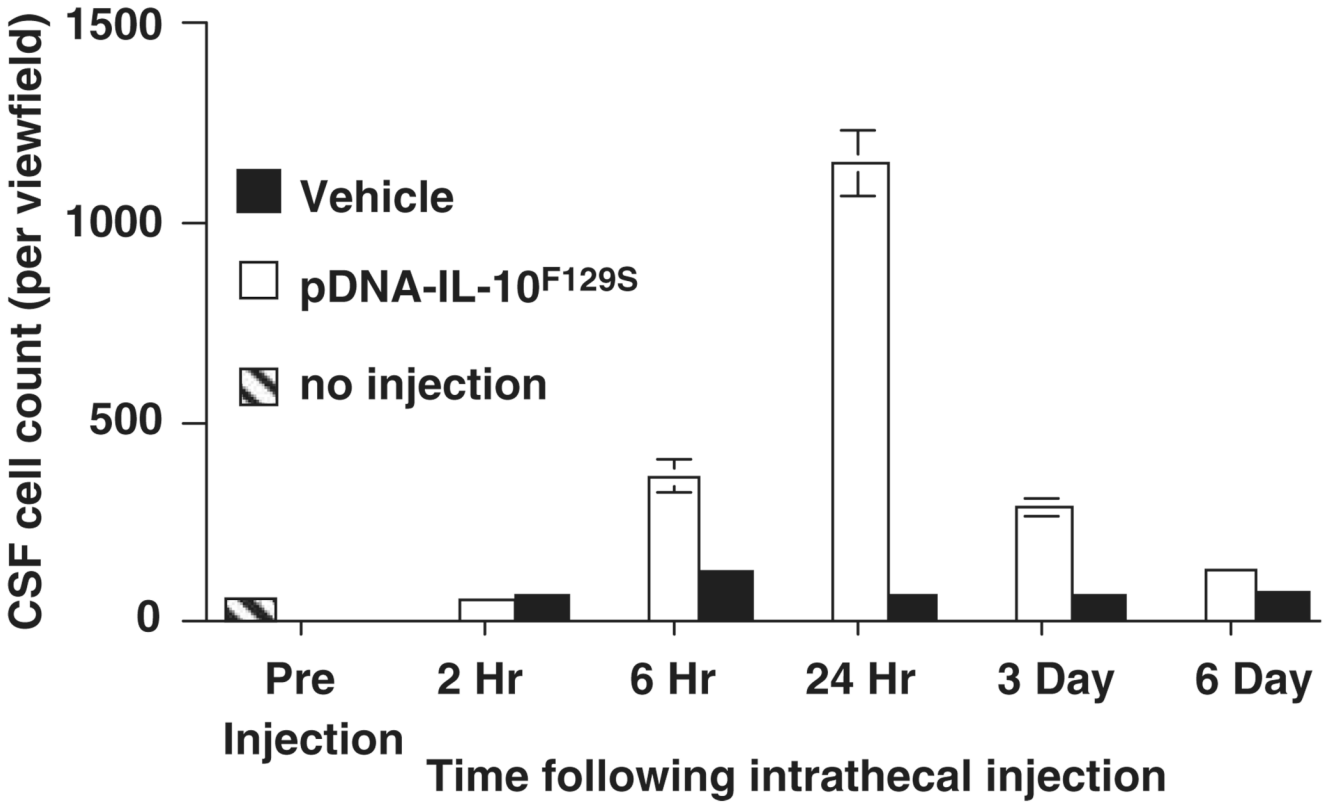

Figure 2.
Time course of cell influx following pDNA-IL-10^{F129S} injection. Between 6 h and 3 days following injection of DNA, but not vehicle, cell counts in cerebrospinal fluid (CSF) at the site of injection are significantly elevated. These cells primarily consist of immune cells of monocytic origin that positively stain for macrophage markers (ED1 and ED2, data not shown).

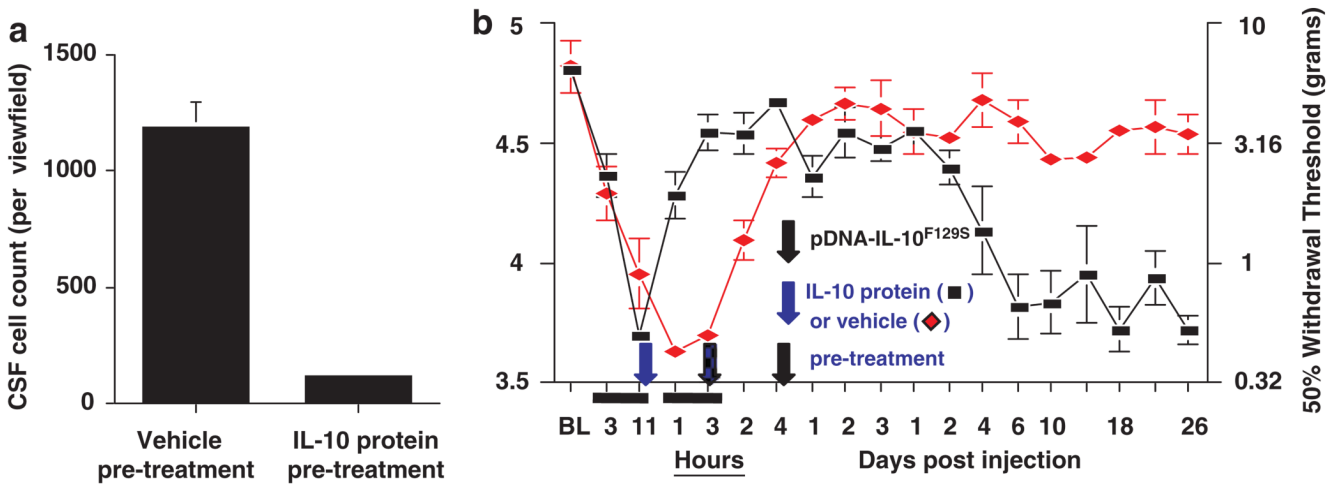

Figure 3.

Enriched cell population in cerebrospinal fluid (CSF) contributes to long-term therapeutic efficacy. (**a**) Accumulation of cells in CSF following DNA injection can be blocked by pre-treatment with interleukin (IL)-10 protein. (**b**) Pre-treatment with IL-10 protein also blocks the long-term reversal of allodynia that is normally seen in the two-injection paradigm.

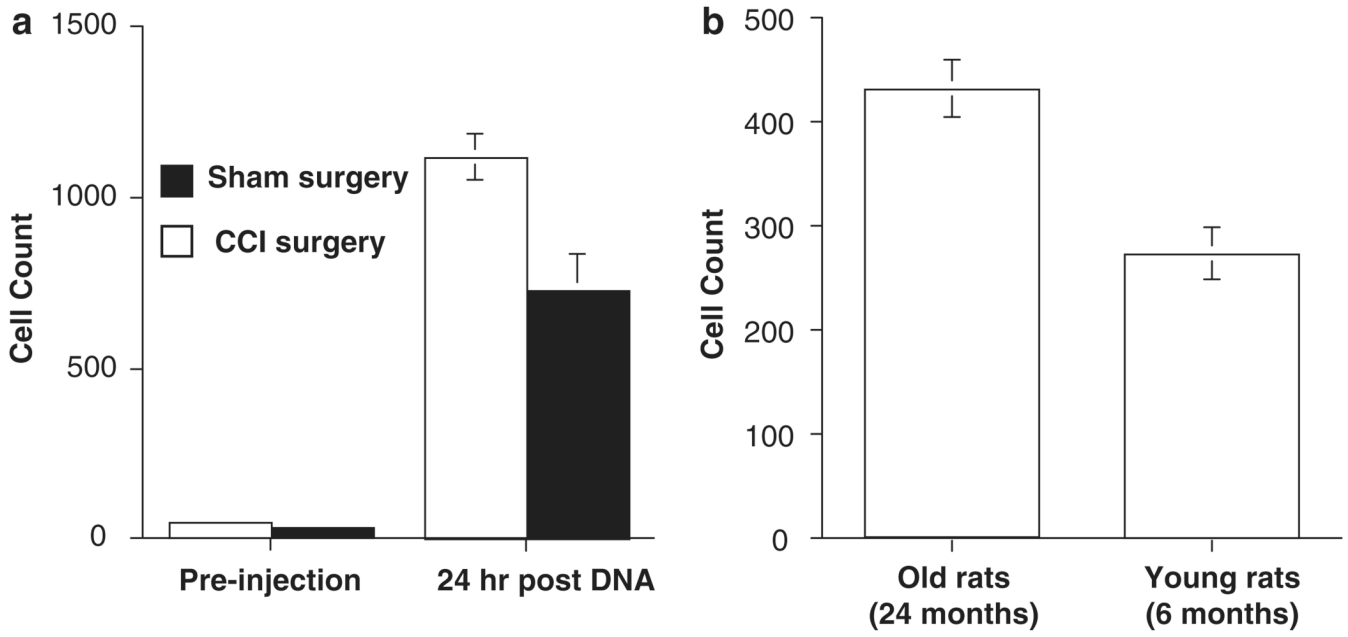

Figure 4.

Pre-existing inflammation enhances cell accumulation in cerebrospinal fluid (CSF). (**a**) Lumbar CSF contains a significantly higher number of phagocytic immune cells both before and following injection of 100 μg pDNA in animals receiving chronic constriction injury (CCI; peripheral nerve injury model) of the sciatic nerve as compared with animals receiving sham surgery. (**b**) Rats that are aged also show a greater degree of cell accumulation in CSF following 100 μg pDNA injection as compared with younger rats.