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Effects of spinal non-viral interleukin-10 gene therapy formulated with D-mannose in neuropathic interleukin-10 deficient mice: Behavioral characterization, mRNA and protein analysis in pain relevant tissues

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ABSTRACT

Studies show that spinal (intrathecal; i.t.) interleukin-10 (IL-10) gene therapy reverses neuropathic pain in animal models, and co-administration with the mannose receptor (MR; CD206) ligand D-mannose (DM) greatly improves therapeutic efficacy. However, the actions of endogenous IL-10 may be required for enduring pain control observed following i.t. IL-10 gene therapy, potentially narrowing the application of this non-viral transgene delivery approach. Here, we show that i.t. application of naked plasmid DNA expressing the IL-10 transgene co-injected with DM (DM/pDNA-IL-10) for the treatment of peripheral neuropathic pain in IL-10 deficient (IL-10 KO) mice results in a profound and prolonged bilateral pain suppression. Neuropathic pain is induced by unilateral sciatic chronic constriction injury (CCI), and while enduring relief of light touch sensitivity (mechanical allodynia) in both wild type (WT) and IL-10 KO mice was observed following DM/pDNA-IL-10 co-therapy, transient reversal from allodynia was observed following i.t. DM alone. In stably pain-relieved IL-10 KO mice given DM/pDNA-IL-10, mRNA for the IL-10 transgene is detected in the cauda equina and ipsilateral dorsal root ganglia (DRG), but not the lumbar spinal cord. Further, DM/pDNA-IL-10 application increases anti-inflammatory TGF-β1 and decreases pro-inflammatory TNF mRNA in the ipsilateral DRG compared to allodynic controls. Additionally, DM/pDNA-IL-10 treated mice exhibit decreased spinal pro-inflammatory mRNA expression for TNF, CCL2 (MCP-1), and for the microglial-specific marker TMEM119. Similarly, DM/pDNA-IL-10 treatment decreases immunoreactivity for the astrocyte activation marker GFAP in lumbar spinal cord dorsal horn. Despite transient reversal and early return to allodynia in DM-treated mice, lumbar spinal cord revealed elevated TNF, CCL2 and TMEM119 mRNA levels. Both MR (CD206) and IL-10 receptor mRNAs are increased in the DRG following CCI manipulation independent of injection treatment, suggesting that pathological conditions stimulate upregulation and availability of relevant receptors in critical anatomical regions required for the therapeutic actions of the DM/pDNA-IL-10 co-therapy. Taken together, the current report demonstrates that non-viral DM/pDNA-IL-10 gene therapy does not require endogenous IL-10 for enduring relief of peripheral neuropathic pain and does not require direct contact with the spinal cord dorsal horn for robust and enduring relief of neuropathic pain. Spinal non-viral DM/pDNA-IL-10 co-therapy may offer a framework for the development of non-viral gene therapeutic approaches for other diseases of the central nervous system.

1. Introduction

Non-viral transgene delivery is one of the least efficient methods of gene transfer for therapeutic applications (Glover et al., 2005), but due to its improved safety profile and reduced cost

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burden, it has been pursued for the treatment of diseases of the central nervous system (CNS) (Jayant et al., 2016). While non-opioid treatments for the control of chronic neuropathic pain are limited, one promising avenue is the application of spinal non-viral interleukin-10 (IL-10) gene therapy, an approach previously demonstrated to provide enduring pain relief in a variety of animal models (Milligan et al., 2006a,b, 2012; Ledeboer et al., 2007; Sloane et al., 2009b,c; Soderquist et al., 2010b; Dengler et al., 2014; Grace et al., 2017).

IL-10 is a powerful anti-inflammatory cytokine that pleiotropically inhibits the actions of many pro-inflammatory factors by mechanisms that include the destabilization of mRNA transcripts for the pro-inflammatory cytokines tumor necrosis factor (TNF) and interleukin-1β (IL-1β) (Moore et al., 2001; Lobo-Silva et al., 2016). Following peripheral nerve injury, a brief compensatory upregulation in IL-10 protein production is followed by decreased IL-10 expression below baseline levels in pain-relevant anatomic locations (Jancalek et al., 2010, 2011; Khan et al., 2015). Studies show that spinal non-viral IL-10 gene delivery in neuropathic animals produces pain relief through elevated spinal IL-10 production with corresponding reduction of pro-inflammatory mediators of pathological pain (Ledeboer et al., 2007; Sloane et al., 2009a; Soderquist et al., 2010a; Dengler et al., 2014). However, whether endogenous IL-10 is required for the long-lasting pain relief observed following i.t. spinal non-viral IL-10 gene therapy remains unknown. Additionally, the anatomical regions in the pain pathway necessary for IL-10 transgene expression that leads to pain relief are still unclear.

While a single large dose of naked plasmid DNA encoding the IL-10 transgene (pDNA-IL-10; 100 μg) or repeated doses (100 μg followed by ≥25 μg within 3–72 hr) result in transient or enduring pain relief, respectively (Milligan et al., 2006a; Ledeboer et al., 2007; Sloane et al., 2009b), the doses used render these approaches clinically unfeasible. A novel gene delivery formulation, whereby a single co-injection of as little as 1 μg of naked pDNA-IL-10 with the immune cell adjuvant D-mannose (DM), a known mannose receptor-specific (MR; CD206) ligand, greatly improves the efficacy of spinal non-viral IL-10 gene therapy in rats, allowing for stable long lasting pain relief following a single i.t. injection (Dengler et al., 2014). The MR is expressed by subpopulations of macrophages and dendritic cells, as well as by microvascular endothelial cells (Taylor et al., 2005). In the CNS, the MR is expressed by astrocytes, microglia, and some neurons (Burudi et al., 1999; Burudi and Regnier-Vigouroux, 2001), and in the PNS by Schwann cells (Baetas-da-Cruz et al., 2009). Increased MR expression is often associated with anti-inflammatory macrophages (Gordon, 2003). Macrophages and other trafficking lymphocytes (i.e. T cells), along with non-leukocytic resident cell types such as satellite glia, are present within the DRG following sciatic nerve injury and likely contribute to neuropathy (Hu et al., 2007; Hanani, 2015). Notably, MR expression is present on leukocytes (Martinez-Pomares, 2012), and MR-activation itself leads to anti-inflammatory signaling as well as transient pain relief (Dengler et al., 2014). However, the transcriptional regulation of critical pro- and anti-inflammatory cytokines and chemokines in the pain pathway following DM-mediated pDNA-IL-10 co-therapy is not known.

In the current report, we applied spinal non-viral DM/pDNA-IL-10 co-therapy to neuropathic wild type (WT) and IL-10 deficient (IL-10 KO) mice. Sciatic nerve chronic constriction injury (CCI) was induced, an established mouse model of peripheral neuropathy, resulting in reliable pathological sensitivity to light touch known as allodynia (Colleoni and Sacerdote, 2010; Jaggi et al., 2011). Both central and peripheral nervous tissues associated with the pain pathway were analyzed for IL-10 transgene mRNA, as well as transcriptional regulation of pro- vs. anti-inflammatory cytokine and chemokine mRNA and protein. The findings reported here support that DM acting as an immune adjuvant for improved spinal non-viral pDNA-IL-10 gene transfer provides a new strategy for gene therapeutics to treat chronic pain, with the potential for application to other chronic CNS diseases.

2. Materials and Methods

2.1. Animals

All experiments were performed using adult male mice (8–12 weeks of age). C57BL/6J (WT; RRID: IMSR_JAX:000664) or B6.129P2-Il10^{tm1Cgn}/J (IL-10 KO; RRID: IMSR_JAX:002251) mice were purchased from Jackson Laboratories or bred in-house from breeders also purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were maintained in specific-pathogen free conditions confirmed negative for detection of *Helicobacter* spp. Prior to handling, all animals were acclimated to the mouse colony at the University of New Mexico (UNM) Health Sciences Center Animal Facility for a minimum of 7 days. Animals were housed in groups of 3–5 at 23° ± 2 °C in light controlled rooms (12:12 light:-dark; lights on at 6:00 am) and fed standard rodent chow and water *ad libitum*. All procedures were approved by the Institutional Care and Use Committee (IACUC) of the UNM Health Sciences Center, conducted in accordance to the NIH Guidelines for the Care and Use of Laboratory Animals, and closely adhered to recommendations from the International Association for the Study of Pain for the use of animals in research.

2.2. Animal model of peripheral neuropathy

A modification of the sciatic nerve chronic constriction injury (CCI) model developed by Bennett and Xie (Bennett and Xie, 1988) was used for application in the mouse (Costa et al., 2008; Martucci et al., 2008; Liu et al., 2017) and briefly described here. Under isoflurane anesthesia (induction at 3.0 followed by 2.0–2.5 vol% in oxygen, 2.0 L/min), the lower back and dorsal left thigh were shaved and then cleaned with diluted Bacti-Stat AE (EcoLab Health Care Division, Mississauga, Ontario, Canada), followed by water, and lastly swabbed with 70% EtOH that was allowed to air dry before proceeding. Using aseptic procedures, the left sciatic nerve was carefully isolated by gentle blunt dissection through the fascia between the *gluteus superficialis* and *biceps femoris* muscles. The exposed sciatic nerve was snuggly ligated with three segments of sterile 4–0 chromic gut suture (Ethicon; Cat#:635H) proximal to the nerve's trifurcation and without pinching of the nerve. To allow enhanced malleability of the thick suture material, thereby reducing the risk of unintended damage, segments of chromic gut material were briefly soaked in a bath of isotonic sterile saline *(Hospira; Cat#:NDC 0404-4888-10)* prior to application. Additionally, great attention was paid to keeping the sciatic nerve moist via regular irrigations with isotonic sterile saline. Sham surgery was identical to the CCI surgery but without nerve ligation. The overlying muscle was sutured closed with one 3–0 sterile silk suture (Ethicon; Cat#:K572H). The overlying skin was closed using two Reflex™ wound clips (Kent Scientific Corp.; Cat#:INS750344). Full recovery from anesthesia was observed within 10–15 min following surgery. At this time, mice that had undergone CCI showed minor ventroflexion of the ipsilateral hindpaw while Sham mice revealed no postural abnormalities. Animal body weights were monitored prior to and following surgery. One day after surgery, animals were monitored for wound condition, hindpaw health, and general activity level. If autotomy was present at any point during the experiments, mice were immediately euthanized (less than 1.0% of all mice).

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