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Lack of interleukin-17 leads to a modulated micro-environment and amelioration of mechanical hypersensitivity after peripheral nerve injury in mice

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ABSTRACT

Interleukin-17 (IL-17) is involved in a wide range of inflammatory disorders and in recruitment of inflammatory cells to injury sites. A recent study of IL-17 knock-out mice revealed that IL-17 contributes to neuroinflammation and neuropathic pain after peripheral nerve injury. Surprisingly, little is known of micro-environment modulation by IL-17 in injured sites and in pathologically related neuroinflammation and chronic neuropathic pain. Therefore, we investigated nociceptive sensitization, immune cell infiltration, myeloperoxidase (MPO) activity, and expression of multiple cytokines and opioid peptides in damaged nerves of wild-type (IL-17$^{+/+}$) and IL-17 knock-out (IL-17$-/-$) mice after partial sciatic nerve ligation. Our results demonstrated that the IL-17$-/-$ mice had less behavioral hypersensitivity after partial sciatic nerve ligation, and inflammatory cell infiltration and pro-inflammatory cytokine (tumor necrosis factor–α, IL-6, and interferon-γ) levels in damaged nerves were significantly decreased, with the levels of anti-inflammatory cytokines IL-10 and IL-13, and expressions of enkephalin, β-endorphin, and dynorphin were also decreased compared to those in wild-type control mice. In conclusion, we provided evidence that IL-17 modulates the micro-environment at the level of the peripheral injured nerve site and regulates progression of behavioral hypersensitivity in a murine chronic neuropathic pain model. The attenuated behavioral hypersensitivity in IL-17$-/-$ mice could be a result of decreased inflammatory cell infiltration to the injured site, resulting in modulation of the pro- and anti-inflammatory cytokine milieu within the injured nerve. Therefore, IL-17 may be a critical component for neuropathic pain pathogenesis and a novel target for therapeutic intervention for this and other chronic pain states.

1. Introduction

There is growing evidence that interleukin-17 (IL-17) plays an essential role in the recruitment of inflammatory cells to sites of injury, and inhibition of IL-17 function has been suggested as a very promising therapeutic approach for modulation of inflammatory diseases and tumor metastasis [11,21,25,37,39]. Interestingly, a recently published paper concluded that IL-17 is a pleiotropic cytokine with both pro- and anti-inflammatory effects, depending on the micro-environment context, degree of inflammation, and stability of heterogeneous IL-17–producing cells [30]. A previous report also states that IL-17 had monophasic expression in degenerating nerves after chronic constriction injury, and that IL-17–positive T cells were detectable within the endoneurium of injured nerves of inflammation [11]. In addition, using multiple animal models, Noma et al. concluded that IL-17 has a limited role in the acute phase of nerve injury and the associated acute pain but

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may have a role in later phases of development of neuropathic pain [23]. A recent study using IL-17 knock-out (IL-17−/−) mice demonstrated that IL-17 contributes to neuroinflammation and neuropathic pain after peripheral nerve injury [9]. Although these data suggest that peripheral IL-17 is implicated in the processing of pain information, possible mechanisms for the involvement of peripheral IL-17 in modulation of the micro-environment context and nociceptive processing in neuropathic pain remain unknown. Therefore, this study, using a chronic murine neuropathic pain model, was designed to test the hypothesis that lack of IL-17 would modulate the recruitment of inflammatory cells to painful inflamed sites, alter the micro-environment context, and attenuate pain. In this study, we aimed to determine whether neuropathic pain from partial sciatic nerve ligation (PSNL) would be altered in IL-17−/− mice by analyzing nociceptive sensitization, immune cell infiltration, myeloperoxidase (MPO) activity, multiple cytokine expression, and endogenous opioid peptide expression in peripheral injured nerves.

2. Methods

2.1. Animals and preparation

All animal experimental procedures in this study were reviewed and approved by the Institutional Animal Care and Use Committee at Chang Gung Memorial Hospital, and all animals in these experiments were treated in accordance with the "Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals" issued by the International Association for the Study of Pain [42]. Adult male C57BL/6C (B6) mice were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). Breeding pairs for ubiquitous IL-17 knock-out mice (IL-17−/−) on the C57BL/6J background were kindly provided by Prof. Yoichiro Iwakura (Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo, Japan) and were maintained in a pathogen-free environment. IL-17−/− mice were backcrossed onto the B6 strain for at least 8 generations. Then the heterozygous offspring were intercrossed to produce the wild-type (IL-17+/+) and IL-17−/− mice that were used in this study. All experiments were carried out in 8- to 10-week-old male mice weighing 15 to 20 g. Both strains of mice were randomly assigned to 1 of 2 groups that received either sham surgery only or right-side partial sciatic nerve ligation (PSNL). Thermal hyperalgesia and tactile allodynia were estimated before surgery (day 0) as well as on days 1, 3, 7, and 14 after surgery. At those same times, infiltration of several inflammatory cell types into the injured site was detected by immunohistochemistry staining and quantified by flow cytometry, using specific antibodies. The production in inflamed sciatic nerves of IL-17, pro-inflammatory cytokines tumor necrosis factor–α (TNFα), IL-1β, IL-6, and interferon-γ (IFNγ), and anti-inflammatory cytokines IL-1Ra, IL-4, IL-10, and IL-13 was determined using enzyme-linked immunosorbent assay (ELISA). The expression of opioid peptides in the injured nerve was determined by immunohistochemistry and quantified by real-time polymerase chain reaction (PCR). During experiments, animals were housed in individual ventilation cages with a qualified satellite facility. The postoperative wound was observed daily, and animals with signs of wound infection or dehiscence were excluded from the studies. At the end of the protocol, all animals were killed by carbon dioxide intoxication and subsequent cervical dislocation.

2.2. Surgical procedures

The chronic neuropathic pain model for mice was performed as previously reported [15,17]. Briefly, all animals were anesthetized with 1.5% to 2% isoflurane. The right sciatic nerve was exposed at the high thigh level, and half of the diameter of the nerve was tightly ligated by a 6-0 polydioxanone suture (Ethicon, Arista, NY). Sham surgeries consisted of exposing the sciatic nerve without ligation. The muscle and skin layers were closed, and the animals were allowed to recover in their cages before the indicated experimental studies.

2.3. Nociceptive behavioral analysis

The measurement of nociceptive behaviors (mechanical and thermal-evoked withdrawal responses) was performed as previously reported [16,17]. In brief, animals (n = 12 per group) were placed individually on an elevated plastic cage and were allowed to familiarize to the environment for at least 60 minutes before testing. Withdrawal latencies to heat stimulation were assessed by applying a focused radiant heat source (Model-336 IITC Paw and Tail Flick Analgesia Meter; IITC Inc., Woodland Hills, CA) to unrestrained animals. The heat stimulus was applied from beneath a heat-tempered glass floor on the distal portion of the plantar aspect of the hind paw. The intensity of radiant heat was adjusted to achieve the basal withdrawal latencies of approximately 7 to 10 seconds. The cut-off value was set at 15 seconds to avoid tissue damage in the case of failure to remove the paw. The paw withdrawal latency to heat stimulus was measured automatically and recorded as the time from onset of the thermal stimulus to withdrawal response. Thermal baseline measurements were obtained from animals before sciatic nerve ligation. Three experiments for each mouse with 5 to 10 minutes between each test were scheduled to obtain the average paw withdrawal latency. Withdrawal responses to punctuate mechanical stimulations applied from beneath the cage to the distal portion of the plantar aspect of the hind paw were determined using a calibrated Electronic von Frey Anesthesiometer (model 2290CE, IITC Life Science, Inc., Woodland Hills, CA). The device automatically recorded and displayed the force (in grams) that elicited a withdrawal response. Each stimulus was applied until a withdrawal response was observed or until the cutoff value of 20 g was reached. These experiments were repeated more than 3 times for each mouse with a 5-minute resting period between tests, and the mean was calculated.

2.4. Immunohistochemistry

The immunoreactivity of several inflammatory cell types, including neutrophils, macrophages, and T cells, was detected by specific antibodies to obtain the inflammatory profile of the different injury conditions. Animals (IL-17+/+ or IL-17−/−, n = 4 per each time point per group) were anesthetized with isoflurane and sacrificed on days 1, 3, 7, and 14 after nerve ligation. The injured sciatic nerves (~6 mm long, including sites proximal and distal to the ligation) were removed and fixed in 4% paraformaldehyde. After subsequent dehydration processing, samples were embedded in paraffin, sliced into 5-μm sections with an automatic microtome, and then soaked with xylene to remove paraffin. Rehydrated tissue sections were incubated at 95°C to 100°C for 30 minutes in sodium citrate buffer (10 mmol/L sodium citrate, 0.05% Tween-20, pH 6.0) for the antigen retrieval procedure, followed by blocking with 0.3% hydrogen peroxide (H_2O_2) and 10% normal goat serum (NGS; Vector Laboratories, Burlingame, CA). Subsequently, sections were incubated in a rat anti-mouse Ly-6G antibody (for neutrophils) (1:500; BD Biosciences Pharmingen, San Diego, CA), a rabbit polyclonal Iba1 antibody (for macrophages) (1:500; Biocare Medical, Concord, CA), or rat anti-CD3 antibody (1:200; AbD Serotec, Oxford, UK), diluted in phosphate buffered saline (PBS) containing 0.3% Triton-X 100 and 3% NGS, overnight at 4°C. The sections were then incubated in biotinylated goat anti-rat or anti-rabbit IgG secondary antibody (all 1:200; Vector Laboratories, Burlingame, CA)

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