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ORIGINAL RESEARCH

Low-Dose IL-2 Attenuates Neuropathic Pain via Treg Expansion in Rats

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Background: Neuropathic pain (NP) involves complex neuroimmune interactions. Regulatory T cells (Tregs) have been implicated in immune homeostasis, but their role in NP pathogenesis and therapeutic potential remains poorly understood.

Aim: This study aimed to investigate the immunomodulatory effects of low-dose interleukin-2 (IL-2) on Treg populations and neuropathic pain behavior in a rat model of chronic constriction injury (CCI).

Methods: CCI was induced in Sprague-Dawley rats, followed by daily intraperitoneal administration of low-dose IL-2 (5000 U/day) for three consecutive days, administered either three days prior to or following CCI. Pain behaviors were assessed by measuring mechanical withdrawal threshold and thermal withdrawal latency. Treg and T conventional (Tconv) cell subsets were quantified by flow cytometry in blood, spleen, dorsal root ganglia (DRG), and spinal cord. Functional markers (CD62L, ICOS) and serum IL-10 concentrations were also examined.

Results: CCI increased T cell infiltration in the DRG, with limited endogenous Treg expansion. Low-dose IL-2 significantly elevated Treg proportions in blood, spleen, and DRG, without promoting Tconv expansion. In the DRG, IL-2–treated CCI rats showed a marked increase in Treg proportions, peaking at day 3 (20.73 ± 2.83% vs 6.74 ± 0.67% in controls) and remaining significantly elevated through day 21. IL-2 also enhanced Treg expression of ICOS and CD62L and increased serum IL-10 levels. Rats receiving IL-2 treatment demonstrated significant improvements in mechanical pain thresholds (61.53 ± 8.46% reduction) and thermal pain thresholds (52.74 ± 6.73% reduction) relative to controls on day 21. Preventive IL-2 injection (pre-CCI) was less effective than post-CCI administration.

Conclusion: Low-dose IL-2 selectively augments functional Tregs and mitigates neuropathic pain through modulation of peripheral and neural immune responses. Further Treg-specific mechanistic validations are required to confirm its potential for translational clinical therapy.

Keywords: interleukin-2, regulatory T cells, neuropathic pain, chronic constriction injury, neuro-immune crosstalk

Introduction

Neuropathic pain (NP) affects 8–10% of the total population and is a condition caused by lesions in the somatosensory nervous system.[1] NP encompasses various conditions, including peripheral nerve injury, chemotherapy-induced neuropathy, and central pain syndromes.[2] It is characterized by spontaneous pain (eg, stabbing, cutting) and abnormal sensations such as allodynia.[3] However, current treatment options for NP are limited, and their effectiveness is often insufficient. If left untreated or inadequately managed, NP may transition into chronic NP, which imposes a significant socioeconomic burden due to increased healthcare costs and the impact on patients' quality of life.[4]

The normal nervous system is primarily composed of neurons and glial cells, with a small number of resident immune cells that generally serve a surveillance role under normal conditions.[5] Following nerve injury, peripheral immune cells infiltrate and critically contribute to NP initiation and persistence.[6] As research advances, the importance of peripheral immune cells in the pathogenesis of NP is becoming increasingly evident.

Although T cells do not initially enter the nervous system, the process of T cells migrating from the peripheral immune system to the nervous system plays a critical role in the initiation, development, maintenance, and chronic progression of NP.[7] Neutrophils appear within hours after injury, releasing mediators that recruit macrophages and T cells.[8]

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Once NP is established, T cells interact directly or indirectly with other nervous system cells, including neurons and glial cells. Damaged neurons and activated glial cells release cytokines and chemokines, which promote T cell proliferation and function.[9] Clinical studies have shown that patients with NP who exhibit high gene expression also display upregulation of several genes associated with T cell cycle regulation, differentiation, and survival, underscoring the critical role of T cells in NP.[10]

Regulatory T cells (Tregs) are a distinct subset of immune cells different from conventional T cells (Tconv). Their principal mechanisms include secreting immunosuppressive cytokines such as IL-10 and TGF-β, expressing high-affinity IL-2 receptors to compete for growth factors, and directly modulating the activity of antigen-presenting cells.[11] Through these pathways, Tregs play a critical role in maintaining immune homeostasis and suppressing excessive inflammation, making them potential mediators in controlling T cell–driven neuroinflammation.

Effector T cells such as Th1 and Th17 release inflammatory cytokines that aggravate neuroinflammation and sustain pain. In contrast, regulatory T cells (Tregs) suppress excessive immune activation by secreting inhibitory cytokines including IL-10 and TGF-β, thereby limiting glial cell activation and reducing neuroinflammation.[12] Thus, the dynamic balance between T cell subsets is essential in shaping the initiation and persistence of pain, and modulation of Tregs represents a promising therapeutic strategy.

Low-dose interleukin-2 (IL-2) has the unique ability to selectively expand Tregs without activating conventional T cells. This approach has been well established in multiple disease models, including systemic lupus erythematosus, rheumatoid arthritis, and transplant rejection, and has already advanced to clinical trials, where it has demonstrated favorable safety and feasibility.[13] Within this context, findings derived from rat models of chronic nerve injury not only provide mechanistic insights into the role of Tregs in neuropathic pain but also offer important translational value. They may help to inform the design of future human studies and the development of novel therapeutic strategies aimed at modulating immune responses for clinical benefit.

This study investigated the temporal dynamics of Tconv and Treg populations in SD rats following chronic constriction injury (CCI) and evaluated the effects of low-dose IL-2 on neuropathic pain behavior. The focus was on the distribution of these cell populations in the peripheral immune system (including blood and spleen), the peripheral nervous system (such as the dorsal root ganglia), and the central nervous system (specifically the spinal dorsal horn). Additionally, the effects of low-dose interleukin-2 (IL-2) injections on pain behavior in rats were examined, along with the temporal changes in CD4+ Tconv and Treg cells in the mentioned tissues. To our knowledge, this work adds novel in vivo evidence suggesting that low-dose IL-2 may improve Treg function and mitigate neuropathic pain by influencing immune activity in both the periphery and the DRG. Although further validation is needed, insights from this rat model may offer valuable guidance for subsequent human trials and the development of IL-2–based therapeutic approaches.

Materials and Methods

Animals and Models

Adult male Sprague-Dawley (SD) rats weighing 130–150g were used in this study. The rats were acclimatized to their environment for 3–5 days after being housed in cages. All animal procedures in this study were reviewed and approved by the Institutional Animal Care and Use Committee of Peking Union Medical College Hospital (Beijing, China) and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and reported following the ARRIVE guidelines (https://arriveguidelines.org/). In this study, n refers to the number of animals. Each experimental group consists of 6–8 animals, including the naïve, sham, and other experimental groups mentioned below. A total of 106 animals were used in the whole procedure. Baseline pain behavior was measured one day before establishing the model. Lottery method for randomization was used in the experiment, all animals were assigned a unique identification number. On the experimental day, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (40mg/kg) under sterile conditions. The fur was removed, and a small incision was made at the desired nerve ligation site. Using blunt dissection, the target nerve was carefully exposed. The sciatic nerve was exposed and loosely ligated with three non-absorbable sutures to induce chronic constriction injury (CCI).[14] The sham surgery group underwent the same surgical procedure, including nerve incision and exposure, with sutures passing through the nerve but without tying a loose knot. The surgical incision was closed using sutures after the procedure.

Behavioral Test

All behavioral measurements were conducted at a relatively fixed time each day. The data was collected on day 1 before and day 1, 3, 5, 7, 10, 14, 18, 21 after CCI, respectively. All behavioral data were collected with no exclusions. Mechanical withdrawal thresholds (MWT) were assessed using the von Frey method. Rats were first acclimated to a suspended cage with a metal mesh, and the probe was vertically placed under the paw, maintaining it for approximately 6–8 seconds. A positive response was considered if the rat's paw retracted sharply, and the corresponding value was read and recorded.[15] Animals were placed on a glass surface, and radiant heat was directed directly below the rat's paw. The time required for the animal to withdraw its paw in response to heat stimulation was recorded as the thermal withdrawal latency (TWL).[16] Prior to testing, animals underwent a habituation period to the environment. Subsequently, all behavioral measurements were conducted by an independent experimenter blinded to group assignments.To minimise potential confounders, cages and test position were randomized. During the whole behavioral test process, animal showed no uncontrollable pain or distress including persistent vocalization, self-mutilation and excessive grooming leading to lesions.

Interleukin-2 Injection

After successfully establishing the chronic constriction injury (CCI) model, different concentrations of interleukin-2 (IL-2) were continuously administered via intraperitoneal injection for three consecutive days under sterile conditions, with a total injection volume of 0.2 mL. The control group received an equivalent volume of normal saline. IL-2 administration began on day 1 post-CCI surgery and continued for three consecutive days (day 1, 2 and 3, respectively). A series concentration was used in the titration. Low-dose IL-2 was administered at a concentration of 25,000 U/mL, with each rat receiving 5000 U IL-2 per day in a 200 μL volume for three consecutive days.[17] The dose was selected based on prior studies showing effective Treg expansion without inducing significant pro-inflammatory side effects. Injections were given daily at the same time each day. On the days when pain behavior tests were performed, intraperitoneal injections were administered after the tests were completed. For preventive injections, 5000 U IL-2 was administered for three days prior to the establishment of the CCI model (day −3, −2 and −1, respectively).

Flow Cytometry Test

Blood (0.3–0.5mL) was collected from rats using tail vein or cardiac puncture methods and immediately transferred to anticoagulant tubes. After diluting the sample with an equal volume of PBS, it was centrifuged, and the supernatant containing white blood cells was collected and stored at −80°C for subsequent analysis. After washing and counting the cells, the concentration was adjusted to 1x10^6/mL for flow cytometry staining. Single-cell suspensions were prepared and stained with fluorescently-labeled antibodies targeting surface markers CD3, CD4, CD25, CD62L, CD127, and ICOS (BD PharMingen, BioLegend) for 30 minutes in the dark. After fixation, the cells were permeabilized using a permeabilization buffer. Then incubate the cells with the anti-FOXP3 primary antibody, for 30 minutes in the dark. After washing with PBS, the cells were resuspended in 300–500 μL of PBS for analysis on the LSR Fortessa flow cytometer (BD Bioscience) and analyzed using FlowJo software (Tree Star, Ashland, OR).

Following a similar protocol, rat spleens were digested with collagenase II (Sigma) at a final concentration of 400 U/mL. After digestion at 37°C for 20 minutes, the tissue was filtered through a 70 μm cell strainer, centrifuged at 800 rpm for 2 minutes, washed three times with cell wash solution, and centrifuged again to collect the cell pellet. Cell counting was performed, and the concentration was adjusted to 1x10^7/mL for staining and analysis as described above.[18]

Collagenase II (Sigma) was diluted to a final concentration of 400U/mL and used to digest L4-L6 dorsal root ganglia (DRG) and spinal dorsal horn tissue. After digestion at 37°C for 30 minutes, the tissue was filtered through a 70μm cell strainer, centrifuged at 800rpm for 2 minutes, washed three times with cell wash solution, and then centrifuged to obtain a cell pellet. Cell counting was performed, and the cell concentration was adjusted to 1e7/mL for the same staining procedures as described above.[19]

Cytokine Beads Assay

Standard samples containing IL-10 started from 2500pg/mL and were serially diluted by 1:2 for a total of 10 dilutions, with the lowest concentration being 4.88 pg/mL. Fifty microliters of serum were added to 0.5ul absorbent particles and

incubated at room temperature on a shaker for 1 hour. Then, secondary antibodies containing PE were added, and the incubation continued for an additional 2 hours in the dark. Finally, after washing and centrifugation, the pellet was resuspended in 300ul PBS, and parameters were recorded on the flow cytometer (LSR Fortessa, BD Bioscience). Analysis of cytokine concentration was performed using FlowJo software.[20]

Statistical Analysis

Statistical results were expressed as mean ± standard deviation for each group, and statistical analysis and chart plotting were performed using GraphPad Prism 6 and SPSS 17.0. Flow cytometry results were analyzed and plotted using FlowJo 10.0. Chi-square tests were used to compare the incidence rates of two or more events.

Prior to the study, an a priori power analysis for a two-way repeated measures ANOVA (Group × Time) was performed using G*Power 3.1. Parameters were set as follows: effect size f = 0.4 (large effect), $\alpha = 0.05$, statistical power = 0.80, number of groups = 2, number of time points = 6, correlation among repeated measures = 0.5, and nonsphericity correction $\varepsilon = 0.75$. The analysis indicated that at least 4–5 animals per group would be required to achieve sufficient power. Considering potential dropouts, we included 4–6 animals per group in the present study.

To assess the effects of group (experimental vs control), time (days 0, 3, 7, 10, 14, and 21), and their interaction on cell counts and behavioral measures, a two-way repeated measures ANOVA was conducted. Prior to ANOVA, residuals were tested for normality using the Shapiro–Wilk test, confirming that data met the assumptions for parametric analysis. Post hoc multiple comparisons were corrected using Bonferroni HSD test. Statistical significance was set at two-tailed $p < 0.05$.

Results

Time Curve of Tconv and Treg Absolute Number and Percentage

After the successful establishment of the CCI model, blood was collected at six time points: 1 day before model establishment, and 3, 7, 10, 14, and 21 days after model establishment. The curve depicting the changes in the content of Tconv in blood over time remained relatively constant. The proportion of Tconv in total T cells remained around 55–65% without significant differences between groups or at various time points.

While the proportion of Tregs in blood remained within a 4–10% range throughout the 21-day observation period, the absolute quantity of Tregs and the variability in their numbers have slightly increased on days 14 and 21, although not reached a statistical significance. The Tconv cells in the spleen followed a similar pattern, with their absolute numbers remaining consistent over the 21-day period. Treg numbers in the spleen also stayed relatively constant, with proportions ranging from 4–8%.

In the dorsal root ganglion (DRG), starting on day 3 after CCI establishment, the absolute number of Tconv cells began to increase, with a notable rise from 108.67±12.01 cells initially to 405.36±10.02 cells at its peak on day 10. After day 10, the number of Tconv cells gradually decreased, but remained significantly elevated on days 14 (328.33 ±53.54) and 21 (311.83±20.74). Two-way ANOVA revealed a significant main effect of Group ($F(1, 36) = 941.33$, $p < 0.0001$), a significant main effect of Day ($F(5, 36) = 94.67$, $p < 0.0001$), and a significant Group × Day interaction ($F(5, 36) = 99.97$, $p < 0.0001$). However, the proportion of $CD4^+$ T cells in the DRG showed less fluctuation, consistently constituting about 30–45% of the total T cells across all time points.

Treg numbers in the DRG showed a marked increase from day 3 onwards, with a significant difference compared to the sham surgery group from day 7 (27.67±2.08 vs 2.67±0.58). Two-way ANOVA revealed a significant main effect of Group ($F(1,36) = 941.33$, $p < 0.0001$), a significant main effect of Day ($F(5,36) = 94.67$, $p < 0.0001$), and a significant Group × Day interaction ($F(5,36) = 99.97$, $p < 0.0001$). Treg proportions in the CCI group remained between 5–8% from day 7 to day 21, with no significant fluctuations (Figure 1).

It was observed that Tconv began to significantly increase from the 7th day (43.35±6.51 vs 5.67±2.52), and the difference remained significant compared to the control group until the 21st day (37.67±6.11 vs 8.67±4.04). However, due to their lower absolute values, the proportion of Tconv showed greater variability, and there was no significant difference compared to the control group (Figure 2).

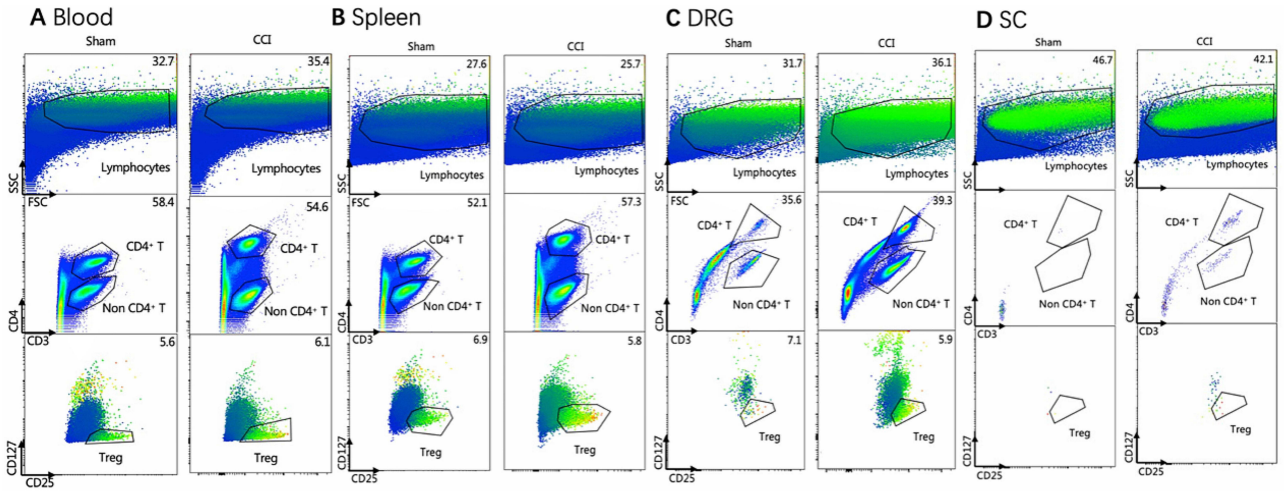

Figure 1 Illustration of selection of Tconv and Treg in the Blood (**A**), Spleen (**B**), DRG (**C**) and SC (**D**) by Flow Cytometry After Establishing the CCI Model. DRG: dorsal root ganglia, SC: spinal cord.

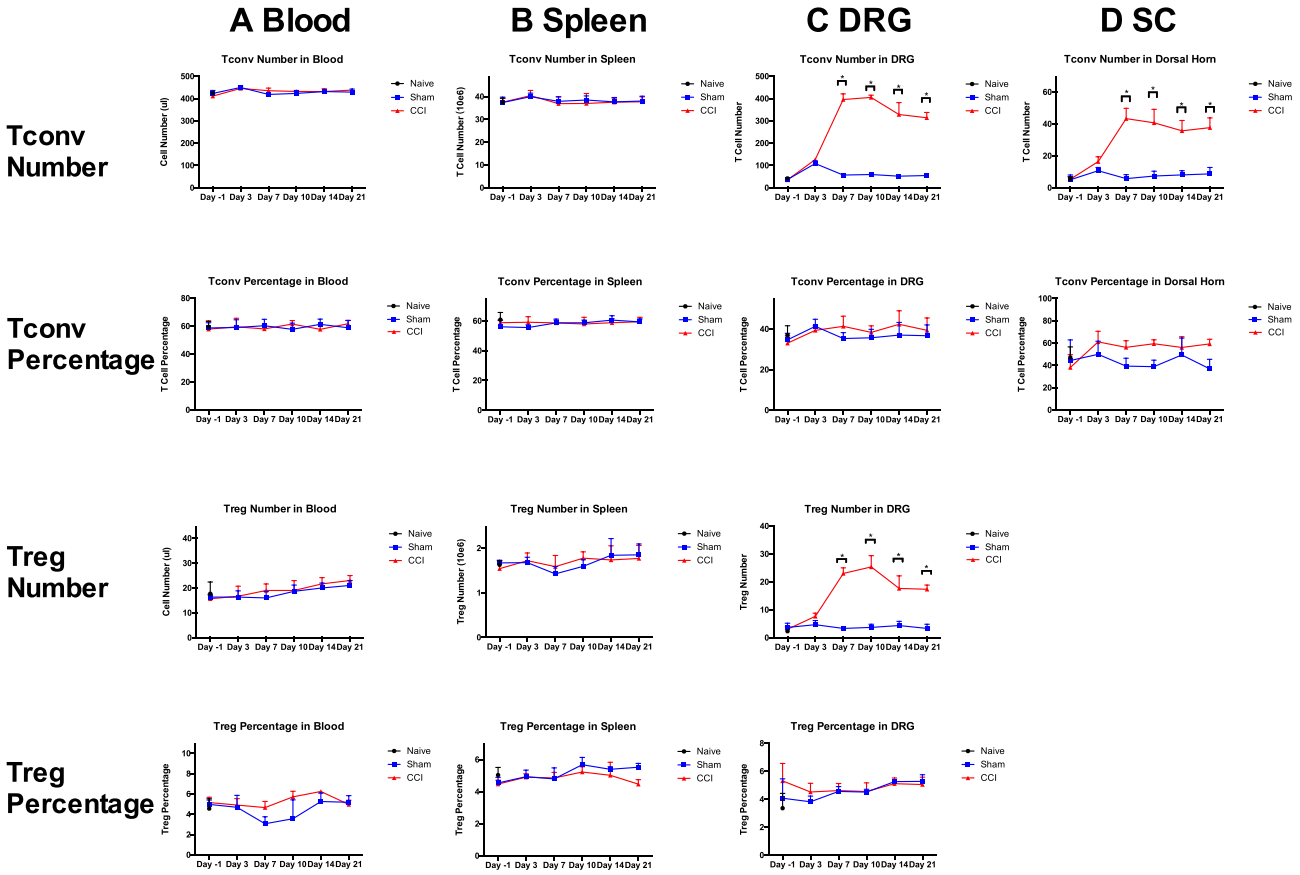

Figure 2 Changes in absolute value and percentage of Tconv and Tregs after establishing the CCI model. The curves represent the temporal changes in Tconv and Treg absolute numbers and proportions in blood (**A**), spleen (**B**), DRG (**C**), and spinal cord (**D**). Data are presented as mean ± SEM; *p < 0.05, p < 0.01. DRG: dorsal root ganglia, SC: spinal cord.

Since Treg cells typically constitute only 5–10% of $CD4^+$ T cells, their absolute values in the spinal dorsal horn are very low. The impact is significant when calculating proportions, and therefore, this experiment was unable to confirm the presence of Treg cells in the spinal dorsal horn through flow cytometry. Figure 1 illustrates the gating strategy for identifying Tconv and Treg populations in the spinal dorsal horn using flow cytometry.

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Low Dose IL-2 Improves Treg Number and Function in Peripheral and DRG

Based on previous literature and the specific circumstances of the reagents, the concentration range of 2000–10000U was selected for exploring low-dose IL-2 in rats. Specifically, 1000U, 2500U, 5000U, 7500U, and 10000U were chosen as concentrations for the preliminary test group. IL-2 was injected at these doses the next day after the model was established (post-CCI injection) and for three consecutive days thereafter (day 1, day 2 and day 3). Blood was collected after model establishment and on the third day to observe the changes in absolute values of $CD4^+$ Tconv and Treg. The results indicated that at a dose of 2500U, the number of Tregs increased by 1.53±0.14 times, and at a dose of 10000U, the number of $CD4^+$ Tconvs increased by 1.25±0.12 times (p=0.03). At doses of 5000U and 7500U, the number of Tregs increased by 3.38±0.49 times and 3.59±0.40 times, respectively, while the number of Tconvs did not significantly increase (1.03±0.10 times and 1.13±0.05 times). At this dose, no significant changes in body temperature or febrile responses were observed in the rats. Therefore, based on the experimental data, the concentration of low-dose IL-2 for continuous intraperitoneal injection was determined to be 5000U (Figure 3).

The pre-CCI injection were also performed, which low dose IL-2 was injected 3 days before CCI model was established (day −3, day −2, day −1) (Figure 3). The behavioral test were also performed at the same timepoint as the post-CCI group and the control group. However, the behavioral test in post-CCI group was better than the preventative injection, indicating a a better timing for injection (Figure 3).

In the CCI group with IL-2 injection, the absolute values of Treg cells started to show a significant difference from the control group on day 3 (3.71±0.31 vs 2.34±0.20) (10^6), reached the highest cell count on day 7 (6.56±0.33) (10^6), and then rapidly declined. However, from day 10 to day 21, Treg cell counts remained significantly elevated, comparable to the cell counts on day 3. In the CCI group with IL-2 injection, the proportion of Treg cells, compared to the control group, began to rise

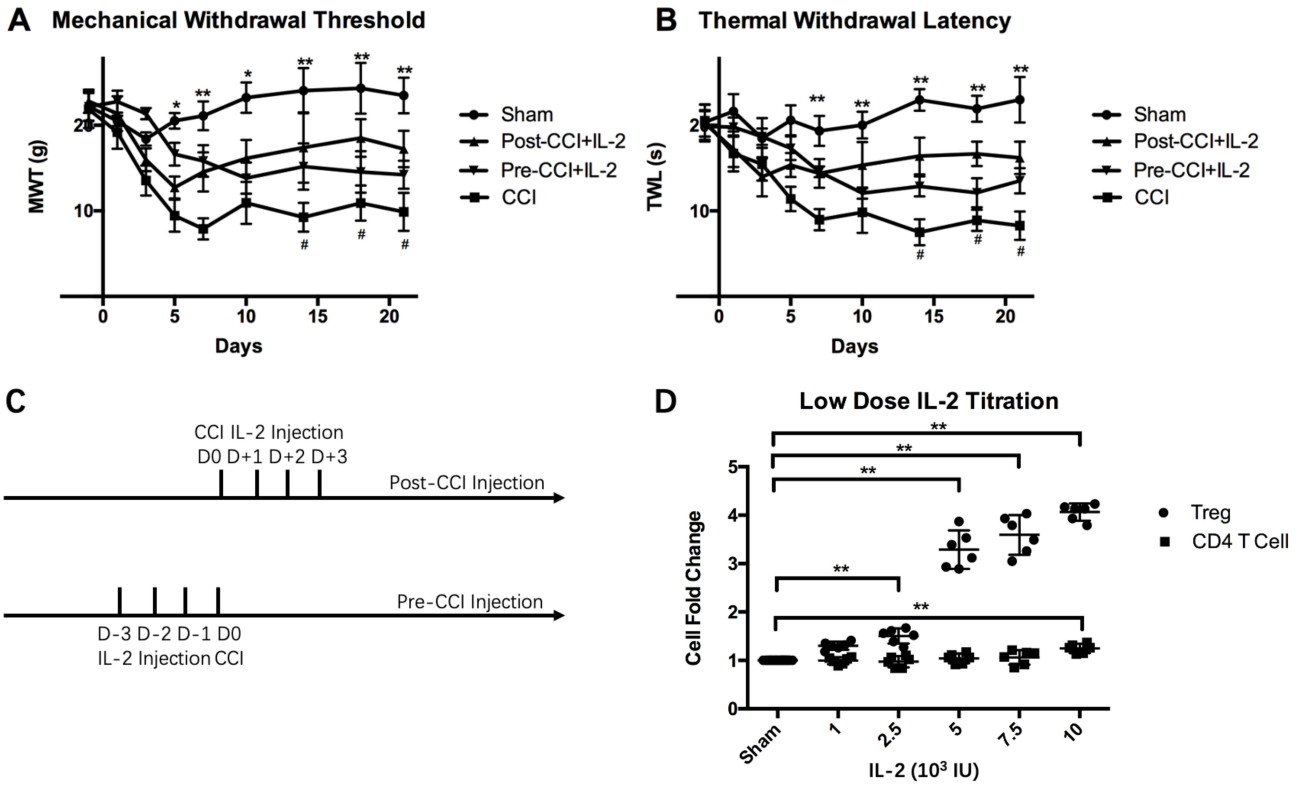

Figure 3 Behavioral test of IL-2 injection in CCI model. The mechanical withdrawal threshold (MWT, (**A**) and thermal withdrawal latency (TWL, (**B**) of the rats were observed at nine time points from one day before model establishment to 21 days post-establishment. (n=6 per group, * P<0.05, ** P<0.01; * indicates a significant difference between the CCI group and the sham group, and # P<0.05 indicates a significant difference between the CCI group and the post-CCI+IL-2 group.) For post-CCI injection, IL-2 administration began on day 1 post-CCI surgery and continued for three consecutive days (day 1, 2 and 3, respectively). For pre-CCI injections, IL-2 was administered for three days prior to the establishment of the CCI model (day −3, −2 and −1, respectively) (**C**). A series concentration was used in the titration, with a suitable concentration that each rat receiving 5000 U IL-2 per day for three consecutive days (**D**).

from day 3 (10.20±1.03% vs 6.75±0.95%) and reached its peak on day 7 (17.91±1.21% vs 7.43±0.33%). Subsequently, the proportion of Treg cells gradually decreased, with no significant differences from the control group on days 14 and 21.

For Tconv cells in the DRG, their numbers remained low in the sham groups, while in the CCI groups, Tconv numbers increased significantly starting from day 7. However, the proportion of Tconv cells among total T cells remained relatively constant across all four groups, with no significant differences observed at any time point.

In the IL-2-treated CCI group, the absolute number of Tregs in the DRG significantly increased from day 3 (25.23±3.24 vs 7.86±1.07), peaking on day 7 (43.15±10.58). Although Treg numbers declined after day 7, they remained significantly elevated on days 14 and 21. In contrast, Treg numbers were minimal in the sham surgery groups. The proportion of Tregs in the IL-2-treated CCI group was also significantly higher than the control group starting from day 3, peaking on day 7. Although Treg proportions gradually declined, they remained significantly elevated compared to the control group, except on day 14 (Figure 4).

Flow cytometry analysis of Treg surface molecule expression in blood on day 7 revealed upregulated expression of markers associated with Treg migration (CD62L) and markers related to Treg stability, proliferation, and suppressive function (ICOS). Both IL-2 injection groups showed significant increases in CD62L and ICOS expression (Figure 5A and B). Additionally, IL-10 levels in the serum were elevated in the IL-2 injection group (Figure 5C), indicating enhanced Treg function and anti-inflammatory activity.

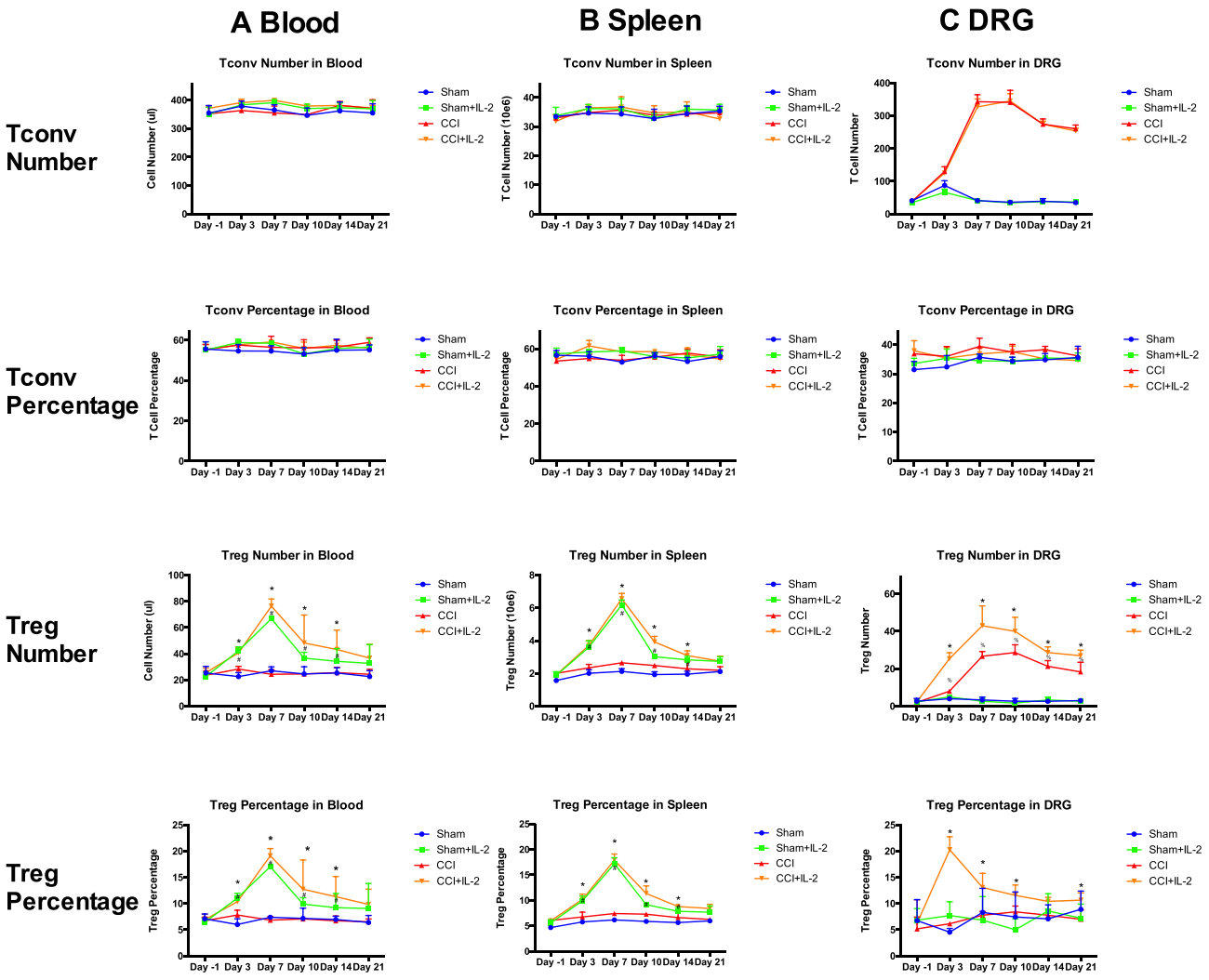

Figure 4 Changes in absolute value and percentage of Tconv and Tregs after IL-2 injection. The curves represent the changes over time in the absolute values and proportions of Tconv and Tregs in blood (**A**), spleen (**B**) and DRG (**C**) relative to the total T cell population and total CD4+ T cells, respectively. CD25 expression at day 7 were also illustrated. (Each group n=8, * P<0.05, ** P<0.01).

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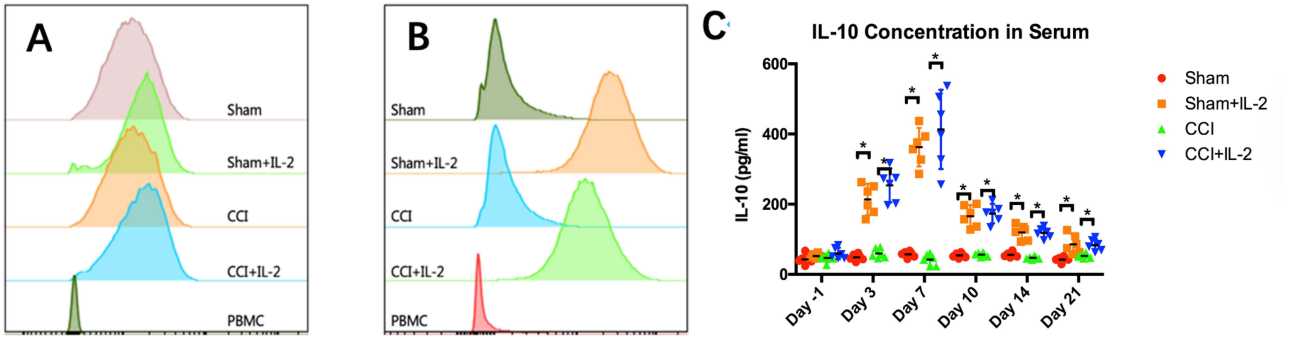

Figure 5 Expression of markers in blood Treg cells on the 7th day after continuous intraperitoneal injection of low-dose IL-2. Flow cytometry was used to determine the expression of ICOS (**A**) and CD62L (**B**) in Treg cells in the following groups: Sham surgery group with IL-2 injection (Sham+IL-2), CCI group with IL-2 injection (CCI+IL-2), Sham surgery group with saline injection (Sham), and CCI group with saline injection (CCI). The concentration of IL-10 was displayed in (**C**).

Discussion

In this study, an increase in the quantity and proportion of Treg was observed in the dorsal root ganglia (DRG) and peripheral immune system after interleukin-2 (IL-2) intervention, while the proportion of Tconv remained relatively constant. The theoretical discussion suggests that a portion of Tconv may have transformed into Treg cells, as traditional Tconv proportions would decrease if their quantity remained unchanged. Although there is some divergence in the current understanding of Treg origins, there is a general consensus that Treg originates from the peripheral immune system, infiltrating the relevant lesion site, and specializing into tissue-specific Treg.

This study considers several potential sources of Tregs. First, Treg expansion may primarily occur through peripheral expansion. Second, a small number of Tregs may reside in tissues and, following nerve injury, could self-amplify within nerve cells without penetrating the blood-brain barrier. Third, there is a possibility of conversion between Tregs and Tconvs, such as the conversion between Th17 and Tregs. Based on the constant proportion of Tconvs observed in this study, the increase in Tregs could also result from such conversions. However, the study did not confirm the exact source of Tregs. Future research could verify Treg sources by profiling blood Tregs, increasing the difficulty of blood-brain barrier penetration, or distinguishing Tregs through the transfer of externally labeled cells.[21]

To increase the quantity and proportion of Treg, several strategies can be considered. Firstly, expanding the quantity of blood Treg, similar to the experimental approach in this study, could be employed. Simultaneously, confirming the synchronous increase in Treg in the DRG, as observed in this study, strengthens this approach. Secondly, a key focus of future research involves identifying tissue-specific expression molecules that can selectively expand tissue-resident Treg. Thirdly, promoting the conversion of Th17 and other T cell types into Treg is another potential strategy. Although this method results in Treg with variable characteristics and poses some theoretical risks, increasing their proportion can enhance the immunoprotective effects of Treg and promote the formation of an anti-inflammatory immune microenvironment.

The presence and residence of Tconv in the DRG may be one of the contributing factors to chronic NP.[22] T cells can produce and release various inflammatory mediators, such as cytokines and chemokines, promoting the occurrence of inflammatory reactions. Inflammatory mediators released by T cells, such as TNF, IL-1β, and IL-17, can activate glial cells. Activated glial cells can further release inflammatory mediators, such as prostaglandins, chemokines, and neurotrophic factors, influencing neuronal function and participating in the pain transmission process.[23] These inflammatory mediators can increase neuronal excitability and pain sensitization, leading to the generation and exacerbation of pain sensations. Some studies suggest that T cells can directly interact with neurons and release pain-related molecules, such as neuropeptides and neurotransmitters. Additionally, T cells can modulate the levels of neurotransmitters like gamma-aminobutyric acid (GABA) and glutamate, affecting both excitatory and inhibitory functions of neurons. These molecules play a role in the occurrence and maintenance of neuropathic pain by influencing neuronal excitability and pain transmission, altering synaptic connections, and synaptic plasticity.

In the present study, after IL-2 injection, the quantity and proportion of Treg in the DRG increased. Therefore, Treg can play a reparative role in various aspects of DRG damage caused by Tconv. Firstly, under the influence of Treg, the

downregulation of inflammatory factors can lead to an improvement in neuronal excitability and pain sensitization. Simultaneously, the downregulation of neuropeptides and neurotransmitters released by T cells, directly or indirectly, reduces the hyperexcitable state of neurons and downregulates the activity of ion channels. Changes in synaptic connections and plasticity, as well as significant alterations in synaptic transmission strength and frequency, are expected. However, evidence for direct interactions between T cells/Treg cells and glial cells/neurons is currently lacking, necessitating new methods to establish such a direct connection.[24]

This study confirms the presence of Tconv cells in the spinal cord of the CCI neuropathic pain model. However, due to experimental constraints, the simultaneous presence of Tregs in the spinal cord was not verified. Nonetheless, the study did confirm the infiltration of peripheral immune cells into the central nervous system. Although there is ongoing debate in the literature about T cell activation, prevailing evidence suggests that astrocytes play a significant role in immune protection at the spinal cord level.[25] T cells and Tregs are scarce in the spinal cord, morphological data or alternative experimental approaches may be required to better assess their numbers, localization, and functions.

Our findings are consistent with and extend prior work by Hu et al,[26] who demonstrated that low-dose IL-2 alleviated neuropathic pain in a mouse spared nerve injury model primarily through Treg expansion and adoptive transfer. While both studies highlight the analgesic potential of IL-2-mediated Treg modulation, there are notable differences. Hu et al established direct causality by using Treg transfer, whereas our study focused on dose optimization, timing (pre- vs post-injury), and the characterization of Treg distribution and functional markers (ICOS, CD62L, IL-10) in a rat chronic constriction injury model. Taken together, these complementary approaches strengthen the evidence that low-dose IL-2 selectively augments Tregs and supports their translational relevance as a therapeutic strategy for neuropathic pain. Traditionally, Treg was mainly considered to function in the peripheral circulation and the immune system, associated with immune diseases and immune dysregulation disorders such as tumors and autoimmune diseases.[27] However, as research on Treg has advanced, its role is not limited to the immune system, and it extends beyond immune diseases. In various non-immune and non-inflammatory diseases, Treg plays an essential role—in essence, exerting immunoregulatory effects in specific tissues at disease sites.[28] Broadly speaking, whenever there is microscopic tissue damage, Treg is involved in repair. Although there is some divergence in the current understanding of Treg origins, there is a general consensus that Treg originates from the peripheral immune system, infiltrating the relevant lesion site, and specializing into tissue-specific Treg.[29]

Contrary to previous perceptions, Treg are widespread in the nervous system, although their numbers are relatively low in normal states. However, they play a crucial role in monitoring and immune functions. Tregs are present in both the central and peripheral nervous systems, not only in brain parenchyma but also in the meninges and cerebrospinal fluid. Improper regulation, such as limiting the activation and function of immune cells like Tconv and microglial cells, may lead to tissue damage. Dysregulation of Tregs in the nervous system is linked to various neurological disorders. In conditions such as multiple sclerosis (MS), compromised Treg function leads to uncontrolled immune responses against myelin phospholipids, which are essential for protecting nerve fibers. Enhancing Treg activity and restoring their function could facilitate the repair of neurological functions.

The process and role of Tregs crossing the blood-brain barrier (BBB) are crucial for their functionality.[30] The interaction between Tregs and the BBB, especially endothelial cells, during this process is one of the key steps determining Treg migration, differentiation, and capabilities after crossing the barrier. Only after crossing the BBB can Tregs interact with various cell types within the nervous system. The heterogeneity of Tregs is also an important aspect. Different Treg subsets have distinct roles in anti-inflammatory responses and tissue repair. For example, Tregs that secrete IL-10 are thought to inhibit neural inflammation and promote neural tissue repair.[31]

Research on Tregs in the nervous system is still ongoing, and key research directions include their precise mechanisms of action, interactions with neural cells, and potential therapeutic applications.[32] Understanding the specific mechanisms of Tregs in immune regulation within the nervous system is crucial for utilizing them to regulate immune responses and treat neurological disorders. Some studies indicate that Treg cells may play a negative regulatory role in the pain process, meaning a decrease or dysfunction of Treg cells may be correlated with an increase in pain severity.[33] Treg cells help inhibit the transmission of pain signals and the pain processes associated with inflammation by suppressing inflammatory reactions, regulating the activation of immune cells, and reducing the production of

inflammatory mediators.[17] Besides, beyond changes in quantity, the functionality of Treg cells may also be affected. Clinical studies have observed changes in both the quantity and function of Treg cells in chronic pain patients.

This study investigated the effects of low-dose interleukin-2 (IL-2) in a rat chronic constriction injury (CCI) model of neuropathic pain. The results demonstrated that IL-2 selectively expanded Tregs in the periphery and dorsal root ganglia (DRG) without promoting Tconv expansion, enhanced functional markers (ICOS, CD62L) and serum IL-10, and improved mechanical and thermal pain thresholds. These findings suggest that low-dose IL-2 alleviates neuropathic pain through modulation of peripheral and neural immune responses (Figure 6). Building on its clinical application in autoimmune diseases and evidence from other disease models, low-dose IL-2 deserves further investigation in human trials for neuropathic pain.

This study provides new evidence that low-dose IL-2 alleviates neuropathic pain through Treg modulation; however, several limitations remain. First, while the observed analgesic effects correlate with Treg expansion, further studies involving Treg depletion or adoptive transfer are necessary to establish a direct causal relationship. Further studies are warranted to determine whether low-dose IL-2 selectively targets Tregs or also modulates other immune cell populations, such as Th1, Th2, and Th17 cells Second, while we employed a chronic constriction injury model, it remains to be determined whether these findings extend to other forms of neuropathic pain, such as chemotherapy-induced or diabetic neuropathy. Expanding to diverse models would enhance the impact of the research. In addition, future work should include sex-based analyses and examine T cell subsets across sexes. Finally, long-term follow-up assessing the durability and safety of low-dose IL-2 will be necessary to strengthen the generalizability and translational value of our findings.

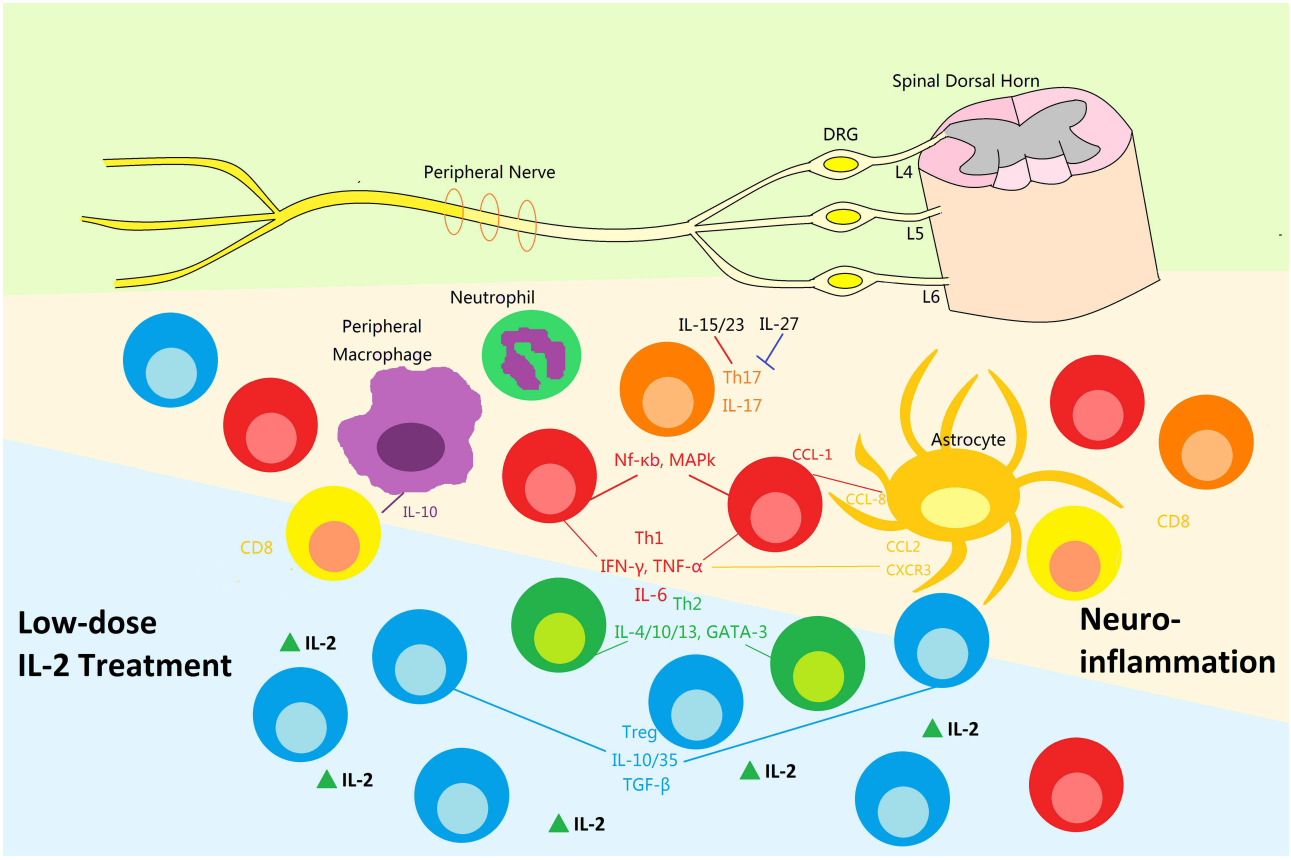

Figure 6 Schematic diagram of the proposed model for this study. Peripheral T cells play a crucial role in the peripheral nervous system, where an inflammatory cell predominance increases the severity of neuropathic pain. Conversely, Tregs and other protective cells, through the cytokines they release, can suppress excessive immune activation and contribute to the formation of an immune-protective microenvironment.

Conclusions

Tregs in the nervous system have diverse roles, not only performing immunoregulatory functions but also as crucial messengers between the nervous system and the peripheral immune system. Tregs, in appropriate quantities, may guide nervous system repair mechanisms influenced by the peripheral immune system. Neuropathic pain, primarily caused by external injuries leading to alterations in the peripheral nervous system, involves a cascade of changes in neural systems beyond neuronal impact and damage, with glial and immune cells playing critical roles in damage generation and maintenance (Figure 6). In conclusion, while our findings highlight the potential of low-dose IL-2 in alleviating neuropathic pain via Treg modulation, future studies addressing causality, other immune subsets, diverse pain models, sex-based differences, and long-term safety are needed to strengthen its generalizability and translational relevance.

Data Sharing Statement

Data available on request from the corresponding author.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Colloca L, Ludman T, Bouhassira D, et al. Neuropathic pain. *Nat Rev Dis Primers*. 2017;3(1):17002. doi:10.1038/nrdp.2017.2
2. Scholz J, Finnerup NB, Attal N, et al. The IASP classification of chronic pain for ICD-11: chronic neuropathic pain. *Pain*. 2019;160(1):53–59. doi:10.1097/j.pain.0000000000001365
3. Finnerup NB, Kuner R, Jensen TS. Neuropathic pain: from mechanisms to treatment. *Physiol Rev*. 2021;101(1):259–301. doi:10.1152/physrev.00045.2019
4. Attal N, Lanteri-Minet M, Laurent B, Fermanian J, Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey. *Pain*. 2011;152(12):2836–2843. doi:10.1016/j.pain.2011.09.014
5. Greenhalgh AD, David S, Bennett FC. Immune cell regulation of glia during CNS injury and disease. *Nat Rev Neurosci*. 2020;21(3):139–152. doi:10.1038/s41583-020-0263-9
6. Hore Z, Denk F. Neuroimmune interactions in chronic pain - An interdisciplinary perspective. *Brain Behav Immun*. 2019;79:56–62. doi:10.1016/j.bbi.2019.04.033
7. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci*. 2007;10(11):1361–1368. doi:10.1038/nn1992
8. Morin N, Owolabi SA, Harty MW, et al. Neutrophils invade lumbar dorsal root ganglia after chronic constriction injury of the sciatic nerve. *J Neuroimmunol*. 2007;184(1–2):164–171. doi:10.1016/j.jneuroim.2006.12.009
9. Gómez-Nicola D, Valle-Argos B, Suardiaz M, Taylor JS, Nieto-Sampedro M. Role of IL-15 in spinal cord and sciatic nerve after chronic constriction injury: regulation of macrophage and T-cell infiltration. *J Neurochem*. 2008;107(6):1741–1752. doi:10.1111/j.1471-4159.2008.05746.x
10. He X, Fan L, Wu Z, He J, Cheng B. Gene expression profiles reveal key pathways and genes associated with neuropathic pain in patients with spinal cord injury. *Mol Med Rep*. 2017;15(4):2120–2128. doi:10.3892/mmr.2017.6231
11. Attias M, Al-Aubodah T, Piccirillo CA. Mechanisms of human FoxP3(+) T(reg) cell development and function in health and disease. *Clin Exp Immunol*. 2019;197(1):36–51. doi:10.1111/cei.13290
12. Luchting B, Rachinger-Adam B, Heyn J, Hinske LC, Kreth S, Azad SC. Anti-inflammatory T-cell shift in neuropathic pain. *J Neuroinflammation*. 2015;12(1):12. doi:10.1186/s12974-014-0225-0
13. Tahvildari M, Dana R. Low-dose IL-2 therapy in transplantation, autoimmunity, and inflammatory diseases. *J Immunol*. 2019;203(11):2749–2755. doi:10.4049/jimmunol.1900733
14. Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*. 1988;33(1):87–107. doi:10.1016/0304-3959(88)90209-6
15. Liu F, Zhang L, Su S, et al. Neuronal C-reactive protein/FcgammaRI positive feedback proinflammatory signaling contributes to nerve injury induced neuropathic pain. *Adv Sci*. 2023;10(10):e2205397. doi:10.1002/advs.202205397
16. Liu F, Wang Z, Qiu Y, et al. Suppression of MyD88-dependent signaling alleviates neuropathic pain induced by peripheral nerve injury in the rat. *J Neuroinflammation*. 2017;14(1):70. doi:10.1186/s12974-017-0822-9
17. Araujo LM, Jouhault Q, Fert I, Bouiller I, Chiocchia G, Breban M. Effects of a low-dose IL-2 treatment in HLA-B27 transgenic rat model of spondyloarthritis. *Arthritis Res Ther*. 2021;23(1):193. doi:10.1186/s13075-021-02559-y
18. Bai R, Gao H, Han Z, et al. Flow cytometric characterization of T cell subsets and microglia after repetitive mild traumatic brain injury in rats. *Neurochem Res*. 2017;42(10):2892–2901. doi:10.1007/s11064-017-2310-0
19. Liu L, Yin Y, Li F, Malhotra C, Cheng J. Flow cytometry analysis of inflammatory cells isolated from the sciatic nerve and DRG after chronic constriction injury in mice. *J Neurosci Methods*. 2017;284:47–56. doi:10.1016/j.jneumeth.2017.04.012
20. Liu Q, Dwyer GK, Zhao Y, et al. IL-33-mediated IL-13 secretion by ST2+ tregs controls inflammation after lung injury. *JCI Insight*. 2019;4(6).

21. Park T-Y, Jeon J, Lee N, et al. Co-transplantation of autologous Treg cells in a cell therapy for Parkinson's disease. *Nature*. 2023;619 (7970):606–615. doi:10.1038/s41586-023-06300-4
22. Ray PR, Shiers S, Caruso JP, et al. RNA profiling of human dorsal root ganglia reveals sex differences in mechanisms promoting neuropathic pain. *Brain*. 2023;146(2):749–766. doi:10.1093/brain/awac266
23. Brandolini L, d'Angelo M, Antonosante A, Cimini A, Allegretti M. Chemokine signaling in chemotherapy-induced neuropathic pain. *Int J Mol Sci*. 2019;20(12):2904. doi:10.3390/ijms20122904
24. Hernandez R, Poder J, LaPorte KM, Malek TR. Engineering IL-2 for immunotherapy of autoimmunity and cancer. *Nat Rev Immunol*. 2022;22 (10):614–628. doi:10.1038/s41577-022-00680-w
25. Sun C, Zhang J, Chen L, et al. IL-17 contributed to the neuropathic pain following peripheral nerve injury by promoting astrocyte proliferation and secretion of proinflammatory cytokines. *Mol Med Rep*. 2017;15(1):89–96. doi:10.3892/mmr.2016.6018
26. Hu R, Zhang J, Liu X, Huang D, Cao YQ. Low-dose interleukin-2 and regulatory T cell treatments attenuate punctate and dynamic mechanical allodynia in a mouse model of sciatic nerve injury. *J Pain Res*. 2021;14:893–906. doi:10.2147/JPR.S301343
27. Duffy SS, Keating BA, Perera CJ, et al. Regulatory T cells and their derived cytokine, interleukin-35, reduce pain in experimental autoimmune encephalomyelitis. *J Neurosci*. 2019;39(12):2326–2346. doi:10.1523/JNEUROSCI.1815-18.2019
28. Ito M, Komai K, Mise-Omata S, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature*. 2019;565 (7738):246–250. doi:10.1038/s41586-018-0824-5
29. Lisi L, Navarra P, Cirocchi R, et al. Rapamycin reduces clinical signs and neuropathic pain in a chronic model of experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2012;243(1–2):43–51. doi:10.1016/j.jneuroim.2011.12.018
30. Montague-Cardoso K, Malcangio M. Changes in blood-spinal cord barrier permeability and neuroimmune interactions in the underlying mechanisms of chronic pain. *Pain Rep*. 2021;6(1):e879. doi:10.1097/PR9.0000000000000879
31. Fonseca MM, Davoli-Ferreira M, Santa-Cecília F, et al. IL-27 counteracts neuropathic pain development through induction of IL-10. *Front Immunol*. 2020;10:3059. doi:10.3389/fimmu.2019.03059
32. Luo H, Liu HZ, Zhang WW, et al. Interleukin-17 regulates neuron-glial communications, synaptic transmission, and neuropathic pain after chemotherapy. *Cell Rep*. 2019;29(8):2384–97e5. doi:10.1016/j.celrep.2019.10.085
33. Singh SK, Krukowski K, Laumet GO, et al. CD8+ T cell-derived IL-13 increases macrophage IL-10 to resolve neuropathic pain. *JCI Insight*. 2022;7(5). doi:10.1172/jci.insight.154194.