Neuroscience Letters 666 (2018) 17–23

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Long-lasting ameliorating effects of the oligodeoxynucleotide IMT504 on mechanical allodynia and hindpaw edema in rats with chronic hindpaw inflammation

Candelaria Leiguarda[a], María Florencia Coronel[b], Alejandro Daniel Montaner[c], Marcelo José Villar[a], Pablo Rodolfo Brumovsky[a,*]

[a] Instituto de Investigaciones en Medicina Traslacional (IIMT), Universidad Austral-CONICET, Av. Juan D. Perón 1500, Derqui, Pilar, B1629AHJ, Buenos Aires, Argentina
[b] Instituto de Biología y Medicina Experimental (IBYME), CONICET, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, C1428ADN, Argentina
[c] Instituto de Ciencia y Tecnología "Dr. César Milstein", CONICET, Fundación Pablo Cassará, Ciudad Autónoma de Buenos Aires, C1440FFX, Argentina

ARTICLE INFO

Keywords:
Pain
Allodynia
Rats
Oligodeoxynucleotide
Systemic
Inflammation

ABSTRACT

Purpose: Previously we showed that systemic administration of IMT504 prevents or ameliorates mechanical and thermal allodynia in rats with sciatic nerve crush. Here we analyzed if IMT504 is also effective in reducing mechanical allodynia and inflammation in rats undergoing hindpaw inflammation. Materials and methods: Male Sprague-Dawley rats received unilateral intraplantar injection of complete Freunds adjuvant (CFA), and were grouped into: 1) untreated CFA, 2) vehicle-treated CFA, 3) IMT504-treated CFA (5 daily (5*) doses of 20, 2 or 0.2 mg/kg, or 3*2 mg/kg). Naïve groups were also included. Finally, early (immediately after intraplantar CFA) and late (7 days after intraplantar CFA) IMT504 treatment protocols were also tested. Hindpaw mechanical allodynia, dorsoventral thickness, edema and cellular infiltration of ipsilateral hindpaws were evaluated in all groups. Results: Untreated CFA rats exhibited mechanical allodynia of quick onset (day 1) and long duration (7 weeks inclusive). Early and late treatments with 5*20 mg/kg IMT504 to CFA rats resulted in both quick and long-lasting antiallodynic effects, as compared to untreated CFA rats. This was also the case in CFA rats undergoing late IMT504 treatment at lower doses (3* and 5*2 mg/kg). Very low doses of IMT504 (5*0.2 mg/kg) only showed a mild improvement in withdrawal threshold, never reaching basal levels. Finally, rats treated with 3* or 5*2 mg/kg or 5*0.2 mg/kg exhibited significant decreases in dorsoventral thickness, edema, and inflammatory cell infiltration of the inflamed hindpaw. Conclusion: Early and late administration of IMT504 results in quick and long-lasting reductions in mechanical allodynia and hindpaw edema. While the mechanisms behind these effects remain to be established, data suggests that IMT504 administration could be a promising strategy in the control of inflammatory pain.

1. Introduction

Chronic pain is a commonly observed multifaceted health condition with major clinical and social consequences [1,2]. It is estimated that, only in the United States and Europe, around 20% of the population suffers chronic pain [3,4]. Chronic pain is difficult to manage, it is associated with high levels of disability, poor health and depression [5], and over time it results in high healthcare costs [6].

Peripheral neuropathy and chronic tissue inflammation commonly cause chronic pain [5], and patients suffering these conditions present clinical manifestations such as allodynia (pain induced by innocuous stimuli), hyperalgesia (exaggerated pain induced by noxious stimuli) and paresthesia (e.g. stabbing, burning sensations) [7]. Unfortunately, many patients with inflammatory or neuropathic pain remain refractory to treatment using the analgesic drugs currently available in the market. Moreover, the severity of adverse effects observed with such drugs is often the reason for treatment abandonment [[7],8].

The situation described above is driving efforts worldwide to improve the quality of life of chronic pain patients, through search and development of new effective and safe analgesic drugs. One of these drugs, IMT504, begins to emerge as a potentially interesting option. IMT504 is a PyNTTTTGT immunostimulatory oligodeoxynucleotide (ODN) lacking CpG motifs [9]. These ODNs are synthetic molecules that modulate cells of the immune system, such as B cells, plasmocytoid dendritic cells, CD56+ cells and mesenchymal stem cells (MSCs). Particularly for the latter, IMT504 has been shown to induce their

* Corresponding author.
E-mail address: pbrumovs@austral.edu.ar (P.R. Brumovsky).

https://doi.org/10.1016/j.neulet.2017.12.032
Received 15 August 2017; Received in revised form 10 December 2017; Accepted 13 December 2017
Available online 14 December 2017

activation, proliferation, differentiation, secretion of immunoglobulins and expression of co-stimulatory molecules [10,11]. For this reason, ODNs such as IMT504 have been assayed as adjuvants in vaccines [12] and for the treatment of allergy and cancer [13]. However, in recent years it has been shown that IMT504 also modulates pain. Thus, in a study in rats with unilateral sciatic nerve crush, which causes mechanical and cold allodynia of up to 2–3 weeks duration, 5 daily subcutaneous injections of IMT504 were shown to prevent or ameliorate both pain-like behaviors [14].

Based on these findings, here we evaluated if IMT504 (tested at different concentrations and dosage schemes) is also capable of preventing or ameliorating the occurrence of mechanical allodynia, acute thermal nociception and inflammation in rats with unilateral complete Freunds adjuvant (CFA)-induced hindpaw inflammation.

2. Methods

2.1. Experimental animals

Adult Sprague–Dawley male rats (200–300 g, BioFucal, Argentina) were kept in a 12 h light-cycle, with water and food *ad libitum*. All experiments performed were approved by the Institutional Animal Care and Use Committee (IACUC; #16-02) of the IIMT, and were carried out according to the policy of the Society for Neuroscience and the International Association for the Study of Pain for the use of animals in pain research.

2.2. Hindpaw inflammation

In fifty-two (52) rats anaesthetized with Isoflurane (5% induction, 2.5% maintenance, 0.8 l/min O2 flow rate; Piramal Healthcare, UK), the right hindpaw received an intradermal injection of 100 µl of CFA (1:1, dissolved in normal saline; Sigma-Aldrich, MO, USA), using a 1 ml syringe with a 25G needle attached. The animals (from now on, called CFA rats) were left to recover from anesthesia in a warm and quiet environment before relocating in their corresponding cages.

2.3. Experimental drug

In all experiments, the ODN IMT504, with sequence 5′-TCATCATT TTGTCATTTTGTCATT-3′ (developed by Immunotech SA, Argentina) was used. The HPLC-grade phosphorothioate ODN (Oligos etc. Inc., Integrated DNA Technologies, OR, USA) was suspended in sterile saline (0.9% NaCl; 20 mg/ml; storage concentration), and assayed for LPS contamination. For some experimental protocols the ODN was dissolved in saline solution to working concentrations (2 mg/ml (rats treated with 2 mg/kg) or 0.2 mg/ml (rats treated with 0.2 mg/kg)), and administered at a final volume of 200–250 µl, depending on the animal weight.

2.4. Treatment protocols using IMT504

A pilot test was performed in groups of rats allocated to early or late treatment protocols (ET and LT, respectively), using 5 daily injections (5*) of 20 mg/kg IMT504 (Fig. 1A). The ET protocol (n = 5) was performed immediately after the intradermal injection of CFA in the hindpaw; the LT protocol (n = 5) was initiated 7 days after the induction of hindpaw inflammation (Fig. 1A). In all cases, animals where tested for pain-like behavior during 4 weeks after the induction of hindpaw inflammation (days 1, 3, 7; weeks 2, 3 and 4).

In a second set of experiments, different concentrations and dosages of IMT504 were administered subcutaneously using the LT protocol (Fig. 2A). Rats were separated in 3 groups (n = 5 per group) and treated as follows: i) 5*0.2 mg/kg, ii) 5*2 mg/kg, and iii) 3*2 mg/kg of the ODN IMT504. All these groups were tested for pain-like behavior up to 7 weeks after induction of hindpaw inflammation.

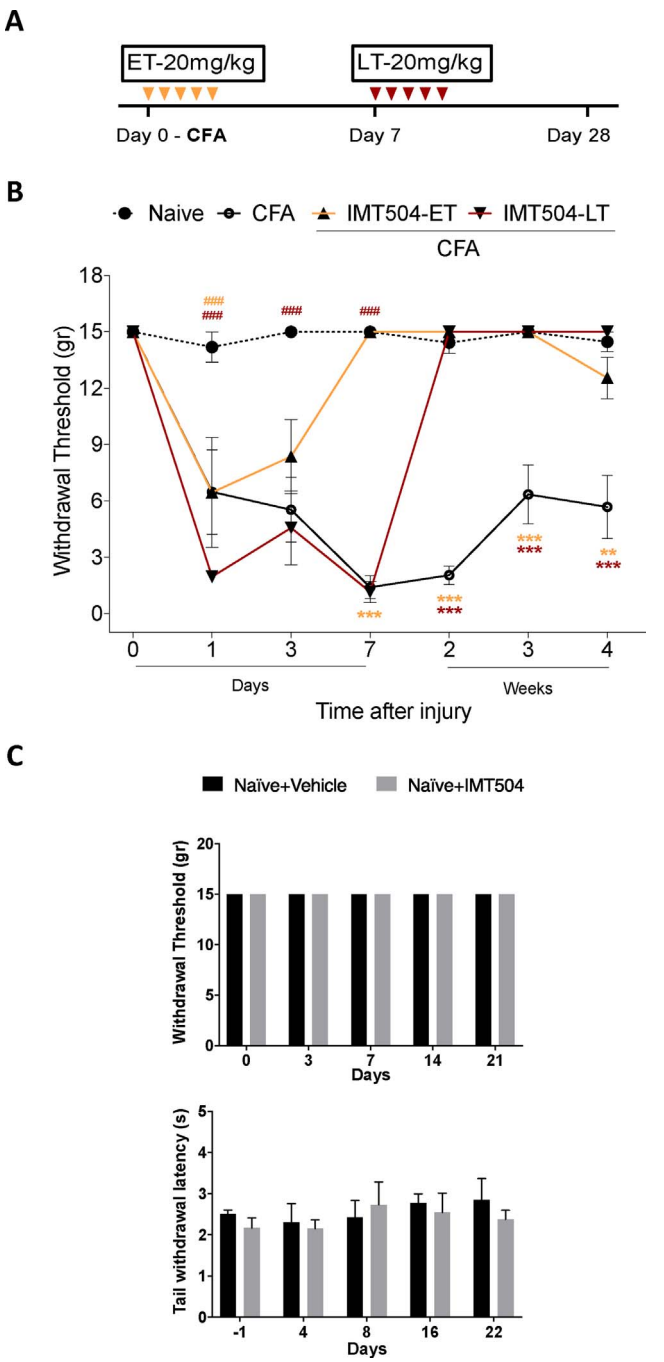

Fig. 1. Effects of ET and LT of high concentration IMT504 on pain-like behavior. A. Timeline of CFA-induced hindpaw inflammation, ET and LT protocols initiation and behavioral tests ending. B. While naïve rats (n = 3) virtually always exhibited basal withdrawal thresholds, all injured animal groups showed ipsilateral mechanical allodynia already 1 day after intraplantar CFA. Untreated CFA rats (n = 5) remained allodynic throughout the tested period. Rats on ET protocol (n = 5) exhibited progressive recovery, reaching basal levels 7 days after treatment initiation. Rats on LT protocol (n = 5) remained allodynic the first week after injury, and begun recovery towards basal levels, fully achieved 7 days after treatment initiation. Both ET and LT groups maintained basal withdrawal thresholds once IMT504 treatment took effect. C. Naïve rats treated with 5*20 mg/kg IMT504 and observed for up to 22 days, did not shown changes in mechanical withdrawal thresholds (upper panel) or tail withdrawal latencies (lower panel). Statistically significant differences are shown between naïve and IMT504-treated rats (#) and untreated CFA and IMT504-treated rats (*).

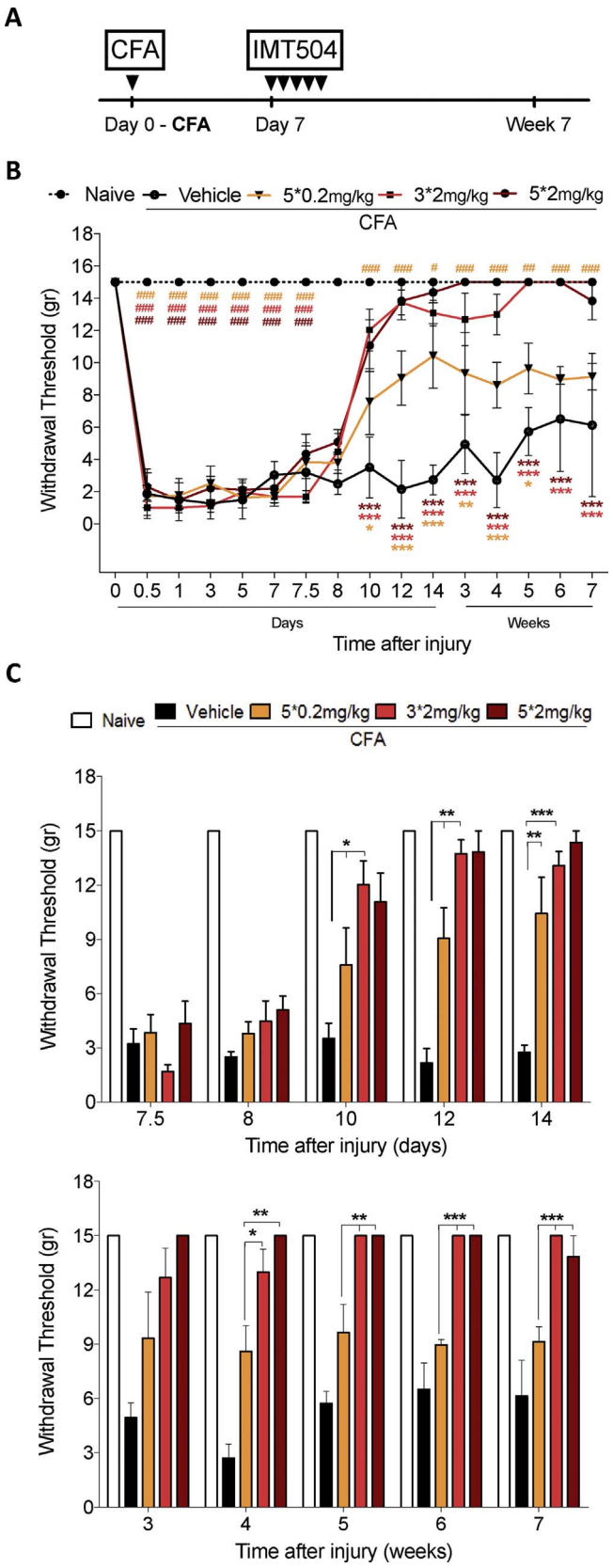

Fig. 2. Effects of LT using different low concentrations of IMT504 on pain-like behavior. A. Timeline of CFA-induced hindpaw inflammation, LT protocol initiation and behavioral tests ending. B. While naïve rats (n = 5) virtually always exhibited basal withdrawal thresholds, all injured animal groups showed ipsilateral mechanical allodynia already 12 h after intraplantar CFA. Vehicle-treated CFA rats (n = 9) remained allodynic throughout the whole tested period. Rats on LT protocol receiving 3* (n = 5) or 5* (n = 5) 2 mg/kg IMT504 remained allodynic only during the first week after injury, beginning recovery 3 days after treatment initiation, and reaching basal ipsilateral withdrawal thresholds from 5 days and onwards. Rats receiving 5*0.2 mg/kg IMT504 (n = 5) showed a slower, incomplete recovery, never reaching basal withdrawal thresholds. Rats receiving 3* or 5*2 mg/kg IMT504 maintained basal withdrawal thresholds throughout the whole tested period. C. Comparison between treatment protocols showed significant differences 10-14 days, and 4–7 weeks after injury, between rats receiving 3* or 5*2 mg/kg IMT504 and those receiving 5*0.2 mg/kg IMT504. Statistically significant differences are shown between naïve and IMT504 treated rats (#) and untreated CFA and IMT504 treated rats, or in between IMT504-treated groups (*).

2.5. Control groups

Naïve (uninjured and untreated; n = 16), untreated CFA (n = 10), and vehicle-treated CFA rats (n = 9) were used as controls. Eight naïve rats remained untreated, 4 were vehicle-treated, and 4 received 5*20 mg/kg IMT504. Untreated CFA rats received an intradermal hindpaw injection of CFA and no further treatment. Vehicle-treated CFA rats received 5 subcutaneous injections of saline (200 µl), once daily, starting 7 days after injury.

2.6. Behavioral assessment

Behavioral assessment was performed during daytime in all animals before any intervention (basal responses) and at different time-points after injury and IMT504 or vehicle administration.

For mechanical allodynia assessment, a set of von Frey filaments (Stoelting, IL, USA) and the modified up-down method of Dixon were used, as described by Chaplan and cols. [15], to establish the 50% withdrawal threshold. A withdrawal threshold of 6 g or lesser was considered an allodynic response.

For acute thermal nociception assessment, the tail immersion test was used, as previously described [16]. The latency of tail-flick reflex to swift immersion of the last 3 cm of the tip of the tail of each rat on a hot bath (52 °C) was measured using a stopwatch (resolution of 0.01 s). This was repeated three times, with 10 s intervals, and an average response obtained.

2.7. Hindpaw dorsoventral thickness measurement

Hindpaw dorsoventral thickness was measured in awake rats, and as previously described [17]. Briefly, once the animals were calm and allowed the positioning of a caliper touching both the ventral and dorsal surfaces of the hindpaw, the dorsoventral thickness was measured. This approach was performed immediately prior to CFA injection and after every time-point evaluated for mechanical allodynia. Care was taken not to compress the hindpaw during measurement.

2.8. Histological analysis

Seven weeks after injury, naïve, vehicle- and IMT504-treated CFA rats were deeply anaesthetized and perfused using Lanás fixative as previously described [18]. Contra- and ipsilateral hindpaws were dissected out, cryo-preserved, embedded in Cryoplast OCT compound (Biopack, Argentina), deep-frozen and transversally sectioned (20 µm) using a cryostat (Thermo Scientific HM525 NX, MA, USA). Tissue sections were mounted on glass slides and stained with standard hematoxylin and eosin for light microscopy analysis using a Nikon Eclipse E-800 photomicroscope (Nikon, Tokyo, Japan).

The public domain NIH program ImageJ (developed at the U.S. National Institutes of Health; http://rsb.info.nih.gov/nihimage/) was

used to measure the thickness of the dermis and the epidermis (without stratum corneum) in the ipsilateral hindpaw. This was performed on photomicrographs taken from 5 serial sections (4 sections in between) of the hindpaw skin per rat, at 15X magnification (visualizing the epidermis, the dermis and part of the hypodermis). A 1750 µm length proximal to the hindpaw pads was chosen for thickness measurement (in injured rats, this area showed clear signs of inflammation). Two measurements per section per layer were taken, 500 µm to each side of the center of the mentioned length, and averaged for analysis.

Inflammatory cell infiltration (leukocytes) was quantified in 10 fields positioned on the dermis at the center of the above mentioned 1750 µm using a 100× oil objective (2 fields along 5 serial sections per rat). Inflammatory cells were recognized by the presence (granulocytes) or absence (lymphocytes) of granules in their cytoplasm. The number of inflammatory cells/mm^2 was calculated.

2.9. Statistical analysis

All data was expressed as mean ± S.E.M and evaluated using GraphPad Prism 7.0a (all data underwent standard normality analysis). Behavioral and dorsoventral thickness data was statistically analyzed using two-way repeated measures analysis of variance (two-way ANOVA), followed by the Bonferroni post-hoc test. Dermal and epidermal width and number of infiltrating cells were statistically analyzed using one-way ANOVA, followed by Tukey's post-hoc test. *P* values are presented as follows: ns, $p > 0.05$; *$0.05 > p > 0.01$; **$0.01 > p > 0.001$, ***$p < 0.001$ and ****$p < 0.0001$.

3. Results

3.1. Effects of 5 × 20 mg/kg IMT504 in ET and LT protocols on mechanical withdrawal threshold

A pilot test was conducted to address the effect of IMT504 at a concentration of 20 mg/kg (5 daily injections) on hindpaw mechanical withdrawal thresholds.

All CFA rats, regardless of their treatment, exhibited a clear reduction in ipsilateral mechanical withdrawal thresholds, reaching allodynic levels, as early as 1 day after injury (Fig. 1B). Untreated CFA rats remained allodynic throughout the entire tested period.

In contrast, CFA rats on ET protocol exhibited progressively increasing withdrawal thresholds, starting on the third day after treatment initiation and reaching basal thresholds 7 days after injury and onwards. On the other hand, CFA rats on LT protocol showed recovery of basal mechanical withdrawal thresholds starting 1 week after treatment (2 weeks after injury), and remaining normal from there on. Thus, significant differences were observed between untreated and IMT504-treated CFA animals, starting 7 days after administration of the ODN ($p < 0.001$) (Fig. 1B).

Finally, in naïve rats (Fig. 1B) and in the contralateral hindpaws (data not shown) of IMT504- or untreated-CFA rats, withdrawal thresholds remained normal during this pilot test.

3.2. Effects of 5*20 mg/kg IMT504 in naïve rats on hindpaw mechanical withdrawal threshold and tail heat nociception

Naïve rats receiving 5*20 mg/kg IMT504 did not show any evident changes in hindpaw mechanical withdrawal or tail thermal withdrawal latencies, remaining always comparable to saline-treated naïve rats (Fig. 1C).

3.3. Effects of different concentrations of IMT504 in LT protocols, using 3 or 5 injections on mechanical withdrawal threshold

We focused next on the LT with IMT504, exploring different concentrations and number of injections on mechanical withdrawal thresholds in CFA rats. As observed during the pilot test, all animals undergoing plantar CFA injection exhibited a dramatic decrease in withdrawal thresholds, showing mechanical allodynia already 12 h after injury (Fig. 2B). Vehicle-treated CFA rats maintained the same degree of allodynic behavior throughout the entire experimental time, as compared to IMT504-treated or naïve rats ($p < 0.001$) (Fig. 2B).

In contrast, CFA rats on LT protocols exhibited a progressive recovery towards basal mechanical withdrawal thresholds from day 10 after injury ($p < 0.001$); the effect seemed to be dose-dependent. Thus, rats treated with 3* or 5*2 mg/kg IMT504 showed a considerably fast recovery of basal withdrawal thresholds, reaching basal levels between 10 and 12 days after injury and maintaining such condition throughout the entire experimental time. On the other hand, CFA rats receiving 5*0.2 mg/kg IMT504 also showed an increase in mechanical withdrawal threshold, albeit not reaching basal levels. Moreover, these rats showed no significant differences with the vehicle-treated CFA rats ($p > 0.05$) towards the end of the experimental time (Fig. 2B).

Comparisons between treatment protocols showed differences between groups receiving 5*0.2 mg/kg IMT504, and 3* or 5*2 mg/kg IMT504, starting 10 days after injury and persisting throughout the experimental time ($p < 0.05$) (Fig. 2C). In all cases, the highest doses were effective in eliminating mechanical allodynia, in contrast to the smallest dose used.

Finally, and as observed during the pilot test, in naïve rats (Fig. 2B and C), and in the contralateral hindpaws (data not shown) of IMT504- or vehicle-treated CFA rats, the withdrawal thresholds exhibited normal basal values.

3.4. Effects of different concentrations of IMT504 in LT protocols, using 3 or 5 injections on hindpaw inflammation

All CFA rats exhibited a considerable increase in hindpaw dorsoventral thickness, showing significant differences with naïve rats, 12 h after injury and onwards ($p < 0.001$) (Fig. 3A). However, while vehicle-treated CFA rats maintained increased hindpaw dorsovental thickness as compared to naïve rats, all rats receiving LT IMT504 at different doses showed a reduction that started 3 days after treatment initiation ($p < 0.001$) (Fig. 3A). Such reduction was maintained even up to 7 weeks after injury, and no significant differences were observed between treatments ($p > 0.05$).

Histological analysis of the hindpaw 7 weeks after injury shows that in fact, rats receiving IMT504 at different doses exhibit a reduction in hindpaw thickness, when compared to vehicle-treated CFA rats (Fig. 3B). Such effect appeared strongly dependent on modifications in dermal thickness (Fig. 3C-upper); however, epidermal effects were also present (Fig. 3C-lower).

Finally, rats treated with 3* or 5*2 mg/kg (but not 5*0.2 mg/kg) IMT504 exhibited statistically significant reductions in inflammatory cell infiltration 7 weeks after injury, compared to the high number of infiltrating cells observed in vehicle-treated CFA rats (Fig. 4). However, none of the IMT504-treated rats returned to basal cell infiltration levels, as shown by comparison with naïve rats (Fig. 4).

4. Discussion

The present study in rats with cutaneous inflammatory injury shows that both early and late administration of the ODN IMT504 dose-dependently reduces mechanical allodynia and hindpaw inflammation. Importantly, the effect of IMT504 exhibits a rather fast onset, with significant recovery from mechanical allodynia and hindpaw inflammation observed within the first 3 days after treatment initiation, and exhibiting long-lasting effects.

The effects on mechanical allodynia presented here agree with our previous study in rats with sciatic nerve crush, where we showed that early or late administration of 5 daily doses of 20 mg/kg of IMT504, respectively, prevented or strongly ameliorated the occurrence of

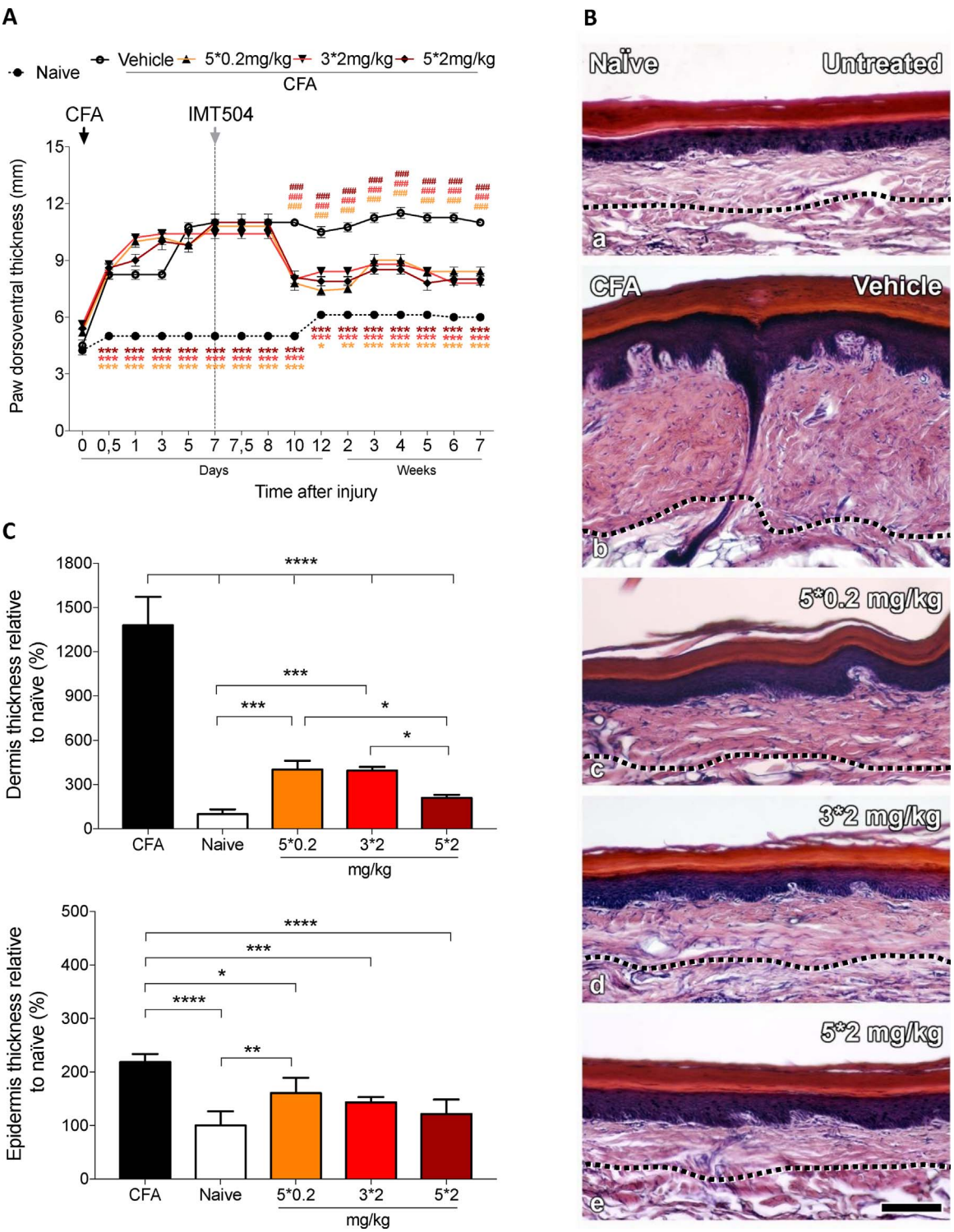

Fig. 3. Effects of LT using different low concentrations of IMT504 on hindpaw dorsoventral thickness and edema. A. While naïve rats (n = 5) only showed mild increases in hindpaw dorsoventral thickness (never higher than 6 mm), all rats receiving intraplantar CFA exhibited fast increases in hindpaw dorsoventral thickness, starting 12 h after injury. Vehicle-treated CFA rats (n = 4) maintained high dorsoventral thickness throughout the whole tested period. In contrast, rats under the LT protocol and receiving 3*2 mg/kg (n = 5) or 5*0.2 mg/kg IMT504 (n = 5) exhibited a considerable and long-lasting decrease in hindpaw dorsoventral thickness, towards basal levels; however, none of these groups reached basal values at any of the survival times tested. B. Bright-field photomicrographs of the hindpaw skin of naïve (a; n = 4) and vehicle-treated CFA rats (b; n = 4), or CFA rats receiving 5*0.2 mg/kg (c; n = 5), 3* (d; n = 5) or 5* (e; n = 5) 2 mg/kg IMT504. In contrast to vehicle-treated CFA rats, those receiving IMT504 exhibited considerable decreases in hindpaw edema. C. Skin thickness quantification revealed that IMT504 strongly reduces the edema in the dermis (upper figure); the ODN also appeared to reduce the thickness of the epidermal layer (lower figure). In A, statistically significant differences are shown between naïve and IMT504 treated rats (#) and untreated CFA and IMT504 treated rats (*). In B, statistically significant differences are shown between all groups of rats (*). Scale bar: 100 µm (e = a-d).

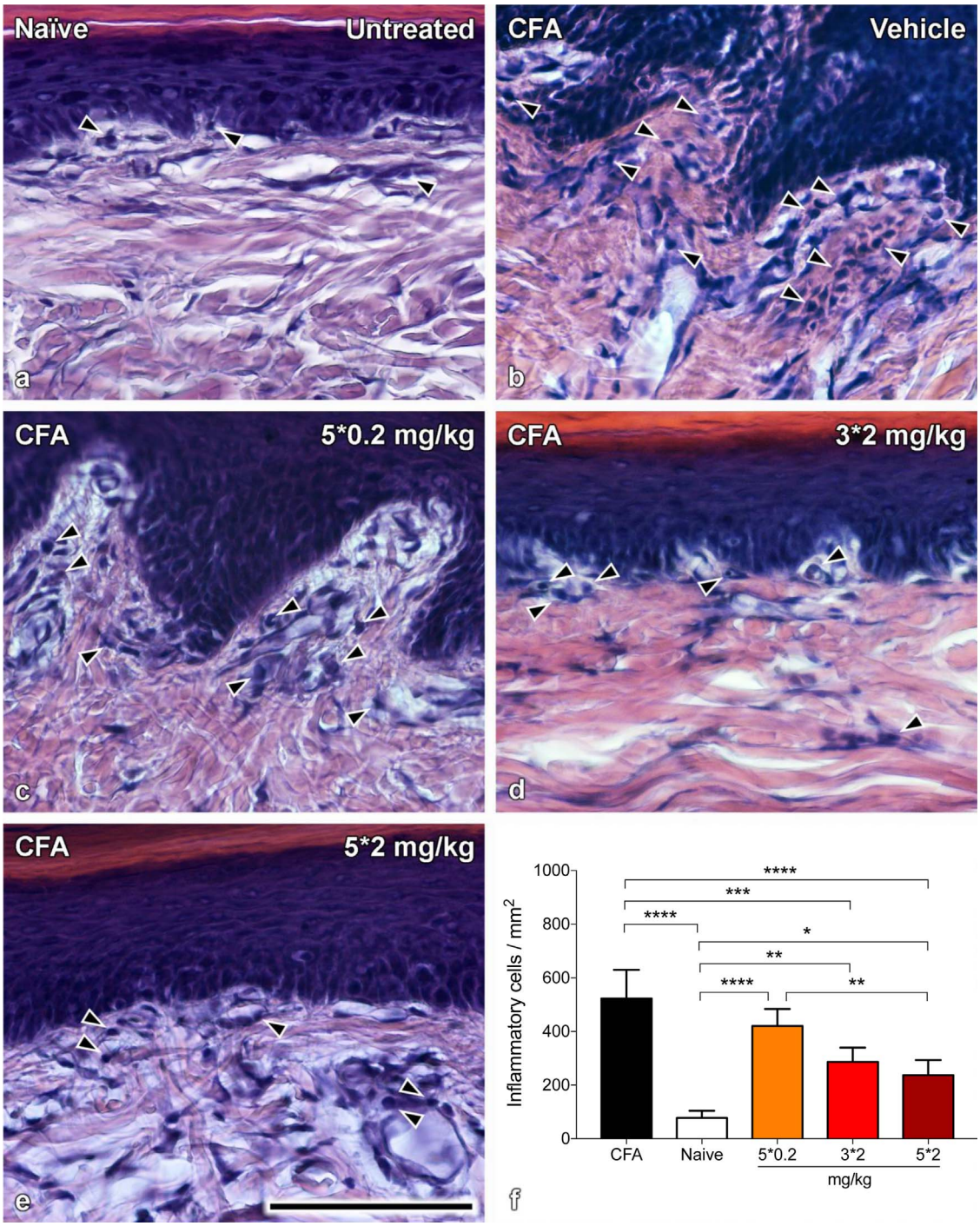

Fig. 4. Effects of LT using different low concentrations of IMT504 on inflammatory cell infiltration 7 weeks after injury. A-E. Bright-field photomicrographs of the hindpaw skin of naïve (a; n = 4) and vehicle-treated CFA rats (b; n = 4), or CFA rats receiving 5*0.2 mg/kg (c; n = 5), 3* (d; n = 5) or 5* (e; n = 5) 2 mg/kg IMT504. In contrast to vehicle-treated CFA rats, those receiving IMT504 exhibited a decrease in the presence of inflammatory cells, the effect appearing stronger in rats treated with high IMT504 doses. F. Rats treated with IMT504 showed considerable reductions in inflammatory cell infiltration in the dermis, compared with vehicle-treated rats. However, rats treated with 5*0.2 mg/kg IMT504 did not show significant reductions in cell infiltration. Statistically significant differences are shown between all groups of rats (*). Scale bar: 100 μm (e = a–d).

mechanical and cold allodynia [14]. In addition, here we show that antiallodynic effects can be obtained using lower doses than previously tested. Moreover, a dose-dependent effect was also exposed, as shown by the incomplete reversal of mechanical allodynia when using very low doses of IMT504 (5*0.2 mg/kg).

The mechanisms behind the antiallodynic and anti-inflammatory effects of IMT504 reported here remain to be established. However, one possibility is that the ODN exerted immunomodulatory actions [19] during peripheral inflammation. On one hand, several inflammatory mediators that excite peripheral nerve endings are normally released by infiltrating inflammatory cells during tissue inflammation, which are known to increase the excitability of peripheral nerve endings [20,21]. On the other hand, IMT504 has been shown to influence the proliferation and cytokine production of human immature B cells, plasmocytoid dendritic cells, natural killer, natural killer T cells [[19],11], and MSCs [10,22]. In line with such influences, here we show that treatment with IMT504 causes considerable decrease in inflammatory cells infiltration that, in addition, are accompanied by important

reductions in hindpaw edema.

The anti-inflammatory effect of IMT504 is not complete, as shown in rats receiving 3* or 5*2 mg/kg IMT504 treatments, where considerable cell infiltration and a certain degree of edema remain even 7 weeks after injury. Interestingly, at this time-point, rats do not show any allodynic behavior, suggesting that the antiallodynic effect is not entirely dependent on the full absence of inflammation. It has been suggested, *in vitro*, that IMT504 induces immature human B cells to secrete interleuquin (IL)-6 and IL-10 [9]. While IL-6 is a known proinflammatory IL that induces pain [23], IL-10 has a proven role as an antinociceptive molecule [24], both at the spinal cord level [25,26], as well as in the periphery [27,28]. Therefore, it could be hypothesized that the maintenance of a certain degree of cell infiltration in rats treated with IMT504 was a positive occurrence, potentially associated with a change of phenotype of B cells from a pro-inflammatory (IL-6-dependent) to an anti-inflammatory (IL-10-dependent) state [29], in turn favoring antiallodynic effects.

Finally, the long-lasting effects of IMT504 could also depend on actions upon MSC progenitors in bone marrow and peripheral blood. *In vitro* studies demonstrated that, when rat and human bone marrow mononuclear cells are cultured in the presence of IMT504, the mean number of fibroblastic adherent colonies and fibroblast colonies forming units (CFU-Fs) that originate MSCs significantly augment [10,22]. Interestingly, an increasing body of evidence strongly suggests that endogenous mobilization of MSCs, and also their exogenous transplantation, exert antiallodynic effects in models of acute [14,30] and chronic [31,32] peripheral nerve injury. Also, intrathecal administration of human umbilical cord-derived MSCs in rats with acute spinal cord inflammation results in a reduction in mechanical allodynia and thermal hyperalgesia [33]. This effect appears to relate to the inhibition of neuroinflammation, as shown by the suppression of activated astrocytes and microglia, a significant reduction of pro-inflammatory cytokines such as IL-1β and IL-17A, and the up-regulation of anti-inflammatory IL-10 [33].

In conclusion, while the exact mechanisms of action of IMT504 during painful peripheral inflammation remain to be established, the present results suggest that by modifying the inflammatory milieu, the ODN may alter the natural course of cellular and molecular events taking place at the site of lesion, and as a consequence, the electrophysiological changes of sensory neurons and pain sensitivity resulting from the intradermal injection of an inflammatory agent. With such scenario, the results presented here suggest that IMT504 has an interesting potential for the treatment of inflammatory chronic pain.

Acknowledgements

We would like to thank Pablo Tate and Jorge Aquino for excellent technical assistance and Guillermo Gastón, Franco Puebla and Santiago Cabrera for animal care and manipulation. This study was supported by Austral Grants T10-15 and 80020160200010UA, and Agencia Nacional de Promoción Científica y Tecnológica [PICT-0296-2008].

References

[1] N. Henschke, S.J. Kamper, C.G. Maher, The epidemiology and economic consequences of pain, Mayo Clin. Proc. 90 (2015) 139–147.
[2] K. Kawai, A.T. Kawai, P. Wollan, B.P. Yawn, Adverse impacts of chronic pain on health-related quality of life, work productivity, depression and anxiety in a community-based study, Fam. Pract. (2017) 1–6.
[3] R.L. Nahin, Estimates of pain prevalence and severity in adults: United States, 2012, J. Pain. 16 (2015) 769–780.
[4] H. Breivik, E. Eisenberg, T. O'Brien, The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care, BMC Public Health 13 (2013) 1229.
[5] A. Litwic, M.H. Edwards, E.M. Dennison, C. Cooper, Epidemiology and burden of osteoarthritis, Br. Med. Bull. 105 (2013) 185–199.
[6] O. Van Hecke, S.K. Austin, R.A. Khan, B.H. Smith, N. Torrance, Neuropathic pain in the general population: a systematic review of epidemiological studies, Pain 155 (2014) 654–662.
[7] I. Gilron, R. Baron, T. Jensen, Neuropathic pain: principles of diagnosis and treatment, Mayo Clin. Proc. 90 (2015) 532–545.
[8] W. Rahman, A.H. Dickenson, Emerging targets and therapeutic approaches for the treatment of osteoarthritis pain, Curr. Opin. Support. Palliat. Care 9 (2015) 124–130.
[9] F. Elias, J. Flo, R.A. Lopez, J. Zorzopulos, A. Montaner, J.M. Rodriguez, Strong cytosine-guanosine-independent immunostimulation in humans and other primates by synthetic oligodeoxynucleotides with PyNTTTTGT motifs, J. Immunol. 171 (2003) 3697–3704.
[10] J. Zorzopulos, S.M. Opal, A. Hernando-Insúa, J.M. Rodriguez, F. Elias, J. Flo, R.A. Lopez, N.A. Chasseing, V.A. Lux-Lantos, M.F. Coronel, R. Franco, A.D. Montaner, Immunomodulatory oligonucleotide IMT504: effects on mesenchymal stem cells as first-in-class immunoprotective/immunoregenerative therapy, World J. Stem Cells (2017) 45–67 World Jour.
[11] J.M. Rodriguez, J. Marchicio, M. López, A. Ziblat, F. Elias, J. Fló, R.A. López, D. Horn, J. Zorzopulos, A.D. Montaner, PyNTTTTGT and CpG immunostimulatory oligonucleotides: effect on Granulocyte/Monocyte colony-Stimulating factor (GM-CSF) secretion by human CD56+ (NK and NKT) cells, PLoS One 10 (2015).
[12] F. Elias, J. Flo, J.M. Rodriguez, A. De Nichilo, R.A. Lopez, J. Zorzopulos, C. Nagle, M. Lahoz, A. Montaner, PyNTTTTGT prototype oligonucleotide IMT504 is a potent adjuvant for the recombinant Hepatitis B vaccine that enhances the Th1 response, Vaccine 23 (2005) 3597–3603.
[13] J.M. Rodriguez, F. Elias, A.D. Montaner, J. Flo, R.A. Lopez, J. Zorzopulos, R. Franco, Oligonucleotide IMT504 induces an immunogenic phenotype and apoptosis in chronic lymphocytic leukemia cells, Medicina (B. Aires) 66 (2006) 9–16.
[14] M.F. Coronel, A. Hernando-Insúa, J.M. Rodriguez, F. Elias, N.A. Chasseing, A.D. Montaner, M.J. Villar, Oligonucleotide IMT504 reduces neuropathic pain after peripheral nerve injury, Neurosci. Lett. 444 (2008) 69–73.
[15] S.R. Chaplan, F.W. Bach, J.W. Pogrel, J.M. Chung, T.L. Yaksh, Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Methods 53 (1994) 55–63.
[16] Q. Zhou, Y. Bao, X. Zhang, L. Zeng, L. Wang, J. Wang, W. Jiang, Optimal interval for hot water immersion tail-flick test in rats, Acta Neuropsychiatr. 26 (2014) 218–222.
[17] L.L. Nwidu, B. Airhihen, A. Ahmadu, Anti-inflammatory and anti-nociceptive activities of stem-Bark extracts and fractions of carpolobia lutea (Polygalaceae), J. Basic Clin. Pharm. 8 (2016) 25–32.
[18] C.J. McCarthy, E. Tomasella, M. Malet, K.B. Seroogy, T. Hökfelt, M.J. Villar, G.F. Gebhart, P.R. Brumovsky, Axotomy of tributaries of the pelvic and pudendal nerves induces changes in the neurochemistry of mouse dorsal root ganglion neurons and the spinal cord, Brain Struct. Funct. 221 (2016) 1985–2004.
[19] A. Hernando-Insúa, A.D. Montaner, J.M. Rodriguez, F. Elias, J. Flo, R.A. Lopez, J. Zorzopulos, J. Hofer, N.A. Chasseing, IMT504, the prototype of the immunostimulatory oligonucleotides of the PyNTTTTGT class, increases the number of progenitors of mesenchymal stem cells both in vitro and in vivo: potential use in tissue repair therapy, Stem Cells 25 (2007) 1047–1054.
[20] S.B. Mcmahon, J.V. Priestley, Nociceptor plasticity, in: M.K.S. Hunt (Ed.), Neurobiol. Pain Mol. Cell. Neurobiol, Oxford University Press, Oxford, United Kingdom, 2010, pp. 35–64.
[21] J. Szolcsanyi, Complete freund's adjuvant, carrageenan, and zymosan models, in: L. Handwerker, H.O. Arendt-Nielsen (Eds.), Pain Model. – Transl. Relev. Appl. IASP Press, Washington, USA, 2010, pp. 133–148.
[22] A. Hernando-Insua, J.M. Rodriguez, F. Elias, J. Flo, R. Lopez, R. Franco, N. Lago, J. Zorzopulos, A.D. Montaner, A high dose of IMT504 the PyNTTTTGT prototype immunostimulatory oligonucleotide, does not alter embryonic development in rats, Oligonucleotides 20 (2010) 33–36.
[23] Y.-Q. Zhou, Z. Liu, Z.-H. Liu, S.-P. Chen, M. Li, A. Shahveranov, D.-W. Ye, Y.-K. Tian, Interleukin-6: an emerging regulator of pathological pain, J. Neuroinflammation. 13 (2016) 141.
[24] A.J. Kwilasz, P.M. Grace, P. Serbedzija, S.F. Maier, L.R. Watkins, The therapeutic potential of interleukin-10 in neuroimmune diseases, Neuropharmacology 96 (2015) 55–69.
[25] E. Milligan, V. Zapata, D. Schoeniger, M. Chacur, P. Green, S. Poole, D. Martin, S.F. Maier, L.R. Watkins, An initial investigation of spinal mechanisms underlying pain enhancement induced by fractalkine, a neuronally released chemokine, Eur. J. Neurosci. 22 (2005) 2775–2782.
[26] B.S. Lee, I.G. Jun, S.H. Kim, J.Y. Park, Intrathecal gabapentin increases interleukin-10 expression and inhibits pro-inflammatory cytokine in a rat model of neuropathic pain, J. Korean Med. Sci. 28 (2013) 308–314.
[27] R. Wagner, M. Janjigian, R.R. Myers, Anti-inflammatory interleukin-10 therapy in CCI neuropathy decreases thermal hyperalgesia, macrophage recruitment, and endoneurial TNF-α expression, Pain 74 (1998) 35–42.
[28] K. Krukowski, N. Eijkelkamp, G. Laumet, C.E. Hack, Y. Li, P.M. Dougherty, C.J. Heijnen, A. Kavelaars, CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-Induced neuropathic pain, J. Neurosci. 36 (2016) 11074–11083.
[29] E.C. Rosser, C. Mauri, Regulatory B cells: origin, phenotype, and function, Immunity 42 (2015) 607–612.
[30] V. Usach, M. Malet, M. Lopez, L. Lavalle, G. Piero, M. Saccoliti, A. Cueto, P. Brumovsky, A. Brusco, P. Setton-Avruj, Systemic transplantation of bone marrow mononuclear cells promotes axonal regeneration and analgesia in a model of wallerian degeneration, Transplantation 101 (2016) 1573–1586.
[31] M.F. Coronel, P.L. Musolino, M.J. Villar, Selective migration and engraftment of bone marrow mesenchymal stem cells in rat lumbar dorsal root ganglia after sciatic nerve constriction, Neurosci. Lett. 405 (2006) 5–9.
[32] P.L. Musolino, M.F. Coronel, T. Hokfelt, M.J. Villar, Bone marrow stromal cells induce changes in pain behavior after sciatic nerve constriction, Neurosci. Lett. 418 (2007) 97–101.
[33] C. Chen, F. Chen, C. Yao, S. Shu, J. Feng, X. Hu, Q. Hai, S. Yao, X. Chen, Intrathecal injection of human umbilical cord-Derived mesenchymal stem cells ameliorates neuropathic pain in rats, Neurochem. Res. 41 (2016) 3250–3260.