

## Role of TNF-alpha during central sensitization in preclinical studies

Pablo Andrade · Veerle Visser-Vandewalle ·  
Carolyn Hoffmann · Harry W. M. Steinbusch ·  
Marc A. Daemen · Govert Hoogland

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**Abstract** Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a principal mediator in pro-inflammatory processes that involve necrosis, apoptosis and proliferation. Experimental and clinical evidence demonstrate that peripheral nerve injury results in activation and morphological changes of microglial cells in the spinal cord. These adjustments occur in order to initiate an inflammatory cascade in response to the damage. Between the agents involved in this reaction, TNF- $\alpha$  is recognized as a key player in this process as it not only modulates lesion formation, but also because it is suggested to induce nociceptive signals. Nowadays, even though the function of TNF- $\alpha$  in inflammation and pain production seems to be generally accepted, diverse sources of literature point to different pathways and outcomes. In this review, we systematically searched and reviewed

original articles from the past 10 years on animal models of peripheral nervous injury describing TNF- $\alpha$  expression in neural tissue and pain behavior.

**Keywords** TNF- $\alpha$  · Central sensitization · Neuropathic pain · Preclinical studies · Neuroinflammation

### Introduction

Neuropathic pain (NP) is a chronic and extensive condition secondary to dysfunction or nerve damage, usually originated in the periphery. If the injurious stimulus perpetuates, dorsal horn neurons in the spinal cord become sensitized, enduring pain perception; process known as central sensitization (CS) [1–3]. Because inflammation is essential for NP development and that apoptosis is involved in CS, tumor necrosis factor-alpha (TNF- $\alpha$ ) becomes an interesting study target [4, 5]. Several studies have investigated the relation between TNF- $\alpha$  expression in different nervous tissues and pain behavior; hitherto these studies have not been conclusive. Though in general, TNF- $\alpha$  is associated with cellular harm and pain behavior, it has also been shown that these effects are bound by temporal and spatial resolutions.

For decades, animal models have reproduced cardinal aspects of human pain, offering essential features for the preclinical study of NP. The main classification of NP models divides them into central pain, peripheral nerve injury, and secondary disease neuropathy models. In this review, we will focus on the peripheral nerve injury models in order to study the effect of these lesions on the process of central sensitization. The primary anatomical structures involved in these injury procedures are the sciatic nerve and dorsal root ganglion (DRG). These methods comprise

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P. Andrade (✉) · C. Hoffmann · H. W. M. Steinbusch ·  
G. Hoogland

Department of Neuroscience, Faculty of Health, Medicine  
and Life Sciences, Maastricht University, Universiteitssingel 50,  
Box 38, 6200 MD Maastricht, The Netherlands  
e-mail: p.andrade@maastrichtuniversity.nl

P. Andrade · V. Visser-Vandewalle · G. Hoogland  
Department of Neurosurgery, Maastricht University Medical  
Center, Maastricht, The Netherlands

P. Andrade · V. Visser-Vandewalle · H. W. M. Steinbusch ·  
G. Hoogland  
European Graduate School of Neuroscience (EURON),  
Maastricht, The Netherlands

V. Visser-Vandewalle  
Maastricht Institute of Neuromodulative Development (MIND),  
Maastricht, The Netherlands

M. A. Daemen  
Department of Neurosurgery, Kliniek Klein Rosendaal,  
Rosendaal, The Netherlands

partial ligature, complete ligature, root or nerve pinching, crushing, transection, and local inflammatory stimulus. The most representative examples include the chronic constriction injury model (Bennett model), partial sciatic nerve ligation model (Seltzer model), spinal nerve ligation, plexus avulsion, compression of the DRG/root, laminectomy, and disc injury [6–12].

TNF- $\alpha$  is considered to be one of the principal mediators in pro-inflammatory processes that involve necrosis, apoptosis and proliferation [5, 13–15]. It is produced essentially by macrophages and T-lymphocytes in response to stressed or damaged tissue, and it may therefore serve as a systemic marker for tissue injury. For instance, complications found in inflammatory and autoimmune diseases are often accompanied by increased serum levels. In case of increased levels, a systemic inflammatory reaction leading to septic shock might occur. However, if TNF- $\alpha$  is present in low quantities it would generate a process responsible for local inflammatory symptoms, depending on the damaged structure [16–18]. In the nervous system, when a local peripheral injury is produced, it generates activation and morphological changes in dorsal horn microglia [19–23]. This process includes P2X4 receptors activation, followed by Ca<sup>2+</sup> influx and p38 MAPK activation. As a consequence, microglia releases diffusible factors like TNF- $\alpha$ , modifying neuronal excitability and thereby pain perception [24–26]. TNF- $\alpha$  has two distinct receptors, TNF receptor-1 (TNFR1 or p55) and TNF receptor-2 (TNFR2 or p75). The main effect of TNFR1 is related to apoptosis and is present in practically every cell; whereas TNFR2 has a limited constitutive expression and is basically associated with cell survival [27, 28]. This is particularly important in pain attributable to different effects depending on specific receptor activation [29–31].

In this review, we present the functional and biochemical changes after peripheral nerve injury in animal models, focused on the role of TNF- $\alpha$  in the development of NP.

### Search methods

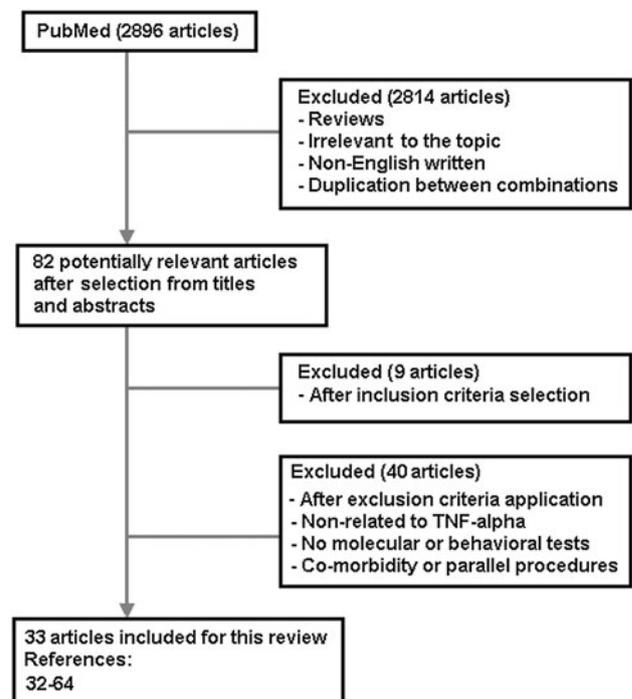
Articles were collected via PubMed from 1999 until July 2010; in order to analyze the understanding progress in the last decade, a period with increased standardized methods since the initial works associating TNF- $\alpha$  and neuropathic pain were performed. The following keywords and their combinations were used: chronic neuropathic pain, tumor necrosis factor, dorsal horn apoptosis, central sensitization, peripheral nerve injury, and neuropathic pain model. The obtained citations were examined by two independent reviewers in order to assure the application of the inclusion and exclusion criteria.

The inclusion criteria were: original full-text articles, preclinical reports, written in English, quantitative data on inflammatory response and pain production, mechanical compression or irritation to neural tissue, involvement of peripheral nervous system injury, and detailed measurement instrument with objective examination protocol. The exclusion criteria comprised: omission of TNF- $\alpha$  levels measurements, absence of standardized behavioral evaluations, presence of co-morbidity, use of TNF- $\alpha$  as inductor of lesion or inflammatory procedure, trauma-related or spinal cord injury models, and involvement of other cytokines.

### Results

From the primary search a total of 2,896 publications were identified. Based on titles and abstracts, 2,814 articles were discarded due to irrelevance to the topic or duplication between different combinations. From the 82 prospective studies, 73 were selected according to the inclusion criteria. After applying the exclusion criteria we obtained 33 articles that were finally chosen for this review (Fig. 1) [32–64].

From these 33 studies, 25 used rats of different strains and 8 applied genetically modified mice. The sample size of the experiments ranged from 7 to 360 animals, with a mean of 77 rodents per study. The most reproduced model



**Fig. 1** Flow diagram representative of search strategy, identification, inclusion and exclusion of articles

was the ligation of the sciatic nerve denominated chronic constriction injury ( $n = 14$ ), followed by nerve crushing ( $n = 8$ ) and nerve transection ( $n = 4$ ). The most common location to induce the inflammatory process was the sciatic nerve ( $n = 18$ ) followed by the spinal nerves ( $n = 5$ ). Tissue samples were obtained from diverse structures, mostly the DRG ( $n = 16$ ), sciatic nerve ( $n = 13$ ) and spinal cord ( $n = 10$ ) (Table 1). Laboratory techniques used for TNF- $\alpha$  determination in these biopsies included primarily immunohistochemistry ( $n = 22$ ), and secondary ELISA ( $n = 8$ ), qPCR ( $n = 7$ ), and immunoblotting ( $n = 5$ ). Follow-up of pain behavior monitoring ranged from 5 days to 12 weeks, 14 days being the most repeated period ( $n = 11$ ). Not all of the included studies in this review applied behavioral testing related to allodynia and hyperalgesia ( $n = 11$ ); the rest assessed mechanical sensitive thresholds primarily with von Frey filaments ( $n = 18$ ), and thermal responsiveness with the Hargreaves test ( $n = 10$ ). Some other conduct assessment techniques comprising hot-plate ( $n = 2$ ) and Randall–Selitto tests ( $n = 2$ ).

Mechanical allodynia was assessed in 20 studies through application of pressure in affected and non-affected limbs in order to evaluate painful responses. Each one of these studies showed a significant decrease in all cases in nociceptive thresholds as a consequence of the injury procedure when compared to sham groups. Initiation of pain typically started 24 h after the lesion intervention, reached maximum levels at 1–2 weeks, and returned close to presurgical control levels after 4 weeks. In two reports bilateral allodynia was present, in one case only for 7 days and referred in the second one as significant and long-lasting [42, 64]. Nine articles evaluated pharmacotherapy and found the highest effectiveness when the drugs were applied within 7 days after injury. In a specific case of cannabinoid administration, an antinociceptive effect was achieved after applying high doses from day 7 to 14 after injury (20 mg/kg) [36]. Opioid administration also reverted successfully the acute nociceptive effects, but only at increased doses and tolerance development (10 mg/kg b.i.d. for 5 days) [55].

Thermal hyperalgesia followed a similar pattern as mechanical allodynia, showing lower thresholds at short-term measurements, starting to decrease and even reaching baseline levels after 14 days. In one article TNFR1-deficient rodents did not exhibit significant painful behavior after heating stimulation at any time point after injury [63] (Table 2).

All models show increased TNF- $\alpha$  levels after injury. Pharmacological treatments that failed to decrease these levels included monotherapy of paracetamol, tramadol, cannabidiol, morphine, soluble TNF receptor, and IL-1 receptor antagonist. On the other hand, successful

interventions that lower TNF- $\alpha$  expression included NMDA receptor antagonist MK-801, thalidomide, combined therapy of soluble TNF receptor with IL-1 receptor antagonist, chronic administration of morphine (dose-dependant with aggravation after interruption),  $\alpha$ 2-macroglobulin, nimesulide, a combination of paracetamol with tramadol, recombinant human erythropoietin and triamcinolone [32, 34–36, 43, 45, 55, 61, 62, 64].

Chronologically, TNF- $\alpha$  expression was characterized by an immediate increase in affected structures starting as soon as 1 h after injury [45, 56, 59]. Peak levels are detected around 24 h, sustain for about 3 days, and in many cases then decreased, occasionally to baseline levels [40, 41, 45, 47]. Some publications describe a lowering of TNF- $\alpha$  expression after this point, but the mainstream pointed out the maintenance of a plateau phase up to 1–2 weeks, followed by a periodical decline. Measurements performed at 3 and 4 weeks reveal weak signaling of the inflammatory process [37, 39–41, 45, 47, 54, 56, 59, 60]. Only one study documented prolonged increased TNF- $\alpha$  levels lasting at least 6 and 12 weeks, not only in nervous tissue but also in connective tissue, after applying the high force reaching task model [39].

The degree to which an altered TNFR1 and TNFR2 ratio contributes to pain behavior is still not clear. One study observed an increased TNFR1 expression several days after dorsal root lesion, while TNFR2 levels remained unchanged [44]. In contrast, a similar follow-up after sciatic nerve injury resulted in a twofold increased TNFR1 level and a sevenfold elevated TNFR2 level [41]. Yet another report showed that immediate measurement only after 6 h post-spinal nerve ligation revealed that both receptors showed similar increased expression in the dorsal root ganglia and remained at comparable levels up to the maximum follow-up [59].

## Discussion

In this review we have summarized the reports that appeared over the past 10 years and described peripheral injury-induced TNF- $\alpha$  expression in the nervous tissue and its role in the production of CS. To date, there is a considerable amount of evidence to support the hypothesis that inflammatory process following tissue damage can lower pain thresholds. In this respect, many pathways and cytokines have been described to contribute in the development of chronic nociceptive signaling, including the three pro-inflammatory cytokines that induce activation of p38 MAPK: TNF- $\alpha$ , IL-1 $\beta$  and IL-6 [25]. Focus on TNF- $\alpha$  is because it is regarded as the archetypical proinflammatory agent, attributable to its function as an inductor of diverse processes that initiate and perpetuate the inflammatory

**Table 1** Demographic data from all included papers in this review

Study no.	First author	Year	No. of animals (species)	Model	Maximum Follow-up	Type of control	Type of sample	Location of inflammatory procedure
1	Arandjelovic et al. [32]	2007	28 (C57BL/6J wild-type mice)	Nerve crush injury and chronic constriction injury	5 days	Control and drug administration	Sciatic nerve	Sciatic nerve
2	Bianchi et al. [33]	2004	48 (Sprague–Dawley rats)	Formalin tail injection	24 h	Control group	Blood and skin	Distal part of the tail (intradermally)
3	Bianchi et al. [34]	2007	48 (Sprague–Dawley rats)	Intraplantar CFA injection	7 days	Control and drug administration	CSF	Hindpaw plantar surface
4	Campana et al. [35]	2006	77 (Sprague–Dawley rats)	Chronic constriction injury	14 days	Control and drug administration	Sciatic nerve	Sciatic nerve
5	Costa et al. [36]	2007	33 (Wistar rats)	Chronic constriction injury and intraplantar CFA injection	14 days	Control, sham and drug administration	DRG and sciatic nerve	Hindpaw plantar surface and sciatic nerve
6	Covey et al. [37]	2002	73 (Sprague–Dawley rats)	Chronic constriction injury	14 days	Control and sham	Sciatic nerve, spinal cord (thoracolumbar and cervical), plasma and locus coeruleus	Sciatic nerve
7	Dubový et al. [38]	2006	32 (Wistar rats)	Unilateral sciatic and spinal nerve ligations, and sciatic nerve transection	14 days	Control and sham	DRG	Sciatic nerve and L4–L5 spinal nerves
8	Elliott et al. [39]	2009	51 (Sprague–Dawley rats)	High force reaching task	12 weeks	Untrained and trained control groups	Blood, muscles, tendons and bones	Median nerve and forearm tissue
9	George et al. [40]	1999	60 (Sprague–Dawley rats)	Chronic constriction injury	14 days	Sham group	Sciatic nerve	Sciatic nerve
10	George et al. [41]	2005	360 (C57BL/6 mice)	Nerve crush injury and chronic constriction injury	28 days	Control and sham	Sciatic nerve	Sciatic nerve
11	Hatashita et al. [42]	2008	132 (Sprague–Dawley rats)	Spinal nerve crush	21 days	Sham group	DRG and spinal cord	L5 spinal nerve
12	Hayashi et al. [43]	2008	11 (Sprague–Dawley rats)	Chronic constriction injury	14 days	Control, sham and drug administration	Sciatic nerve	Sciatic nerve
13	Holmes GM et al. [44]	2004	44 (Long–Evans rats)	Rhizotomy	7 days	Control and sham	Spinal cord, DRG and medullary brainstem	Thoracic or lumbar dorsal roots
14	Kato et al. [45]	2009	52 (Sprague–Dawley rats)	Sciatic nerve crush	12 days	Control, sham and drug administration	Sciatic nerve	Sciatic nerve
15	Kleinschmitz et al. [46]	2005	200 (C7BL/6 mice)	Nerve crush injury and chronic constriction injury	28 days	Control, sham and drug administration	DRG and sciatic nerve	Sciatic nerve
16	Lee et al. [47]	2004	66 (Sprague–Dawley rats)	Chronic constriction injury	28 days	Control and sham	DRG and spinal cord	Sciatic nerve
17	Li et al. [48]	2008	16 (Sprague–Dawley rats)	Spared nerve injury	14 days	Sham group	Brain sections (red nucleus)	Ligation and section of 3 nervous ramifications of sciatic nerve

**Table 1** continued

Study no.	First author	Year	No. of animals (species)	Model	Maximum Follow-up	Type of control	Type of sample	Location of inflammatory procedure
18	Murata et al. [49]	2004	51 (Sprague–Dawley rats)	Disc incision	21 days	Sham group	DRG and NP	L4–L5 disc
19	Murata et al. [50]	2004	175 (Sprague–Dawley rats)	Disc incision	21 days	Control, sham and drug administration	DRG and NP	L4–L5 disc
20	Murata et al. [51]	2006	82 (Sprague–Dawley rats)	Disc incision	24 h	Sham group	DRG	L4–L5 disc
21	Ohtori et al. [52]	2004	18 (BCL6 mice)	Sciatic nerve crush	14 days	Control and sham	DRG and spinal cord	Sciatic nerve
22	Okamoto et al. [53]	2001	42 (Sprague–Dawley rats)	Chronic constriction injury	45 days	Sham group	Sciatic nerve	Sciatic nerve
23	Otoshi et al. [54]	2010	100 (Sprague–Dawley rats)	NP implantation into the DRG	56 days	Control and sham	DRG	DRG
24	Raghavendra et al. [55]	2002	100 (Sprague–Dawley rats)	Spinal nerve transection	11 days	Control, sham and drug administration	Spinal cord	L5 spinal nerve
25	Sacedorte et al. [56]	2008	36 (C57BL/6 mice)	Chronic constriction injury	14 days	Control and sham	Sciatic nerve, DRG and spinal cord	Sciatic nerve
26	Sakuma et al. [57]	2007	20 (Sprague–Dawley rats)	Facet joints capsulotomy	14 days	Control group	DRG	DRG
27	Schäfers et al. [58]	2001	16 (C57BL/6 mice)	Chronic constriction injury	10 days	Control group	Sciatic nerve	Sciatic nerve
28	Schäfers et al. [59]	2003	12 (Sprague–Dawley rats)	Spinal nerve ligation	5 days	Sham group	DRG	L5–L6 spinal nerves
29	Sekiguchi et al. [60]	2009	129 (Sprague–Dawley rats)	Nerve root crush	28 days	Sham group	DRG	L5 nerve root crush (proximal or distal to DRG)
30	Sweitzer et al. [61]	2001	91 (Holtzman rats)	Spinal nerve transection	14 days	Control, sham and drug administration	Spinal cord	L5 spinal nerve
31	Üçeyler et al. [62]	2008	130 (C57BL/6 mice)	Chronic constriction injury	7 days	Control, sham and drug administration	Spinal cord, cortex, hippocampus hypothalamus and thalamus	Sciatic nerve
32	Vogel et al. [63]	2006	98 (C57BL/6 mice)	Chronic constriction injury	36 days	Control group	Sciatic nerve	Sciatic nerve
33	Xu et al. [64]	2006	118 (Sprague–Dawley rats)	Ventral root transection	45 days	Sham group and drug administration	Spinal cord and DRG	L5 ventral root

CFA complete Freund's adjuvant, CSF cerebrospinal fluid, DRG dorsal root ganglion, NP nucleus pulposus

**Table 2** Detection methods to assess TNF- $\alpha$  expression and behavioral tests to measure pain

Study no.	TNF detection method and behavioral test	TNF- $\alpha$ levels	Pain behavioral data
1	Immunohistochemistry and qPCR	Ninefold increase of TNF- $\alpha$ mRNA in control animals. $\alpha$ 2-macroglobulin activated for cytokine binding decreases levels of mRNA expression after injection	None
2	ELISA and Hargreaves test	Significant increase of TNF- $\alpha$ levels in the hindpaw and forepaw at all time-points compared with control animals after formalin injection	Significant hyperalgesic hindpaw response after formalin administration, but not in the forepaw
3	ELISA and Randall–Selitto paw-withdrawal test	TNF- $\alpha$ levels significantly increased in non-treated animals. TNF- $\alpha$ concentrations prevented with nimesulide or paracetamol and tramadol combined, but not by paracetamol and tramadol used independently	Significant decrease of nociceptive thresholds in non-treated animals. Mechanical allodynia was prevented by oral administration of nimesulide, tramadol and paracetamol
4	Immunohistochemistry, histology, qPCR, von Frey filaments and Hargreaves test	TNF- $\alpha$ was present in endoneurial cells, including macrophages, axons and Schwann cells cytoplasm in non-treated animals. rhEpo decreased TNF- $\alpha$ mRNA at injury site, and also decreased the extent of nerve degeneration	Significant mechanical allodynia and thermal hyperalgesia increased in non-treated group, but significantly decreased after rhEpo administration
5	ELISA, Randall–Selitto paw-withdrawal test and Hargreaves test	Increased TNF- $\alpha$ levels were present in injured DRG when compared to sham, but no changes in injured sciatic nerve rats. High doses of cannabidiol did not modify the elevated levels present in DRG samples	Significant increase in mechanical and thermal hyperalgesia in non-treated group; attenuated both by cannabidiol, but with dose-dependent behavior and only after using high doses
6	Immunohistochemistry, in situ hybridization, bioassay and hot-plate test	Increased TNF- $\alpha$ levels in sciatic nerve until day 14, and dissipated after that, with unchanged levels in contralateral nerve; thoracolumbar spinal cord showed elevated TNF- $\alpha$ levels; no elevation in cervical spinal cord; significant accumulation increase of mRNA specific for TNF- $\alpha$ in locus coeruleus	Significant decrease of paw-withdrawal latency when compared to sham and control, with highest value on day 12 and return to baseline levels on day 14
7	Immunohistochemistry	Increased TNF- $\alpha$ levels in neuronal bodies and satellite cells compared to control and sham rats (ipsi- and contralateral, but higher in contralateral side). Levels reached highest peak 1 week after surgery, decreasing after 2 weeks; with TNFR1 expression ipsi- and contralateral after 2 weeks	None
8	ELISA and von Frey filaments	Increased TNF- $\alpha$ levels at week 6, but not significant at week 12 in serum. High levels detected in distal bone at week 12 after repetitive task performance	Withdrawal threshold decreased progressively at weeks 6 and 12 for cutaneous sensitivity and grip strength at week 12
9	Immunohistochemistry, Immunoblotting and ELISA	TNF- $\alpha$ levels increased rapidly after CCI with highest peak at 12 h, up to day 3. Rapid increase of endoneurial TNF with plateau phase back to baseline on day 14	None
10	ELISA, von Frey filaments and Hargreaves test	TNF was increased on day 1 and 3 after injury (CCI and crush) but it was not detectable on day 7 or after. TNFR1 increased twofold between days 1 and 7; and TNFR2 elevated sevenfold on days 3 and 7	Thermal hyperalgesia and mechanical allodynia are present in both CCI and crush; thermal sensitivity maximum peak on week 2, and mechanical allodynia throughout all measures
11	Immunohistochemistry, immunoblotting and von Frey filaments	TNF- $\alpha$ expression in the contralateral DRG significantly increased from day 7 to 21	Ipsilateral withdrawal threshold was significantly decreased for 21 days; contralateral threshold decreased also, but for 7 days

**Table 2** continued

Study no.	TNF detection method and behavioral test	TNF- $\alpha$ levels	Pain behavioral data
12	Immunohistochemistry, von Frey filaments, hot-plate test, pin-prick test and acetone drop test	TNF- $\alpha$ staining was significantly increased after injury; especially prominent in Schwann cells and cells with cytological mast cells characteristics. TNF- $\alpha$ -positive mast cells significant reduction after triamcinolone administration	Significant increase in heat-hyperalgesia, mechano- and cold-allodynia throughout the tests. Triamcinolone had a significant reduction on mechanical allodynia and thermal hyperalgesia
13	Immunohistochemistry and histology	Presence of constitutive TNFR1 on DRG, spinal afferences, solitary tract, vagal and spinal trigeminal tract in naive and operated animals. No TNFR2 shown in the solitary tract or dorsal horn afferences.	None
14	Immunohistochemistry, immunoblotting and von Frey filaments	1 h after the injury, TNF immunoreactivity was shown in Schwann cells, endothelial cells, fibroblasts and intact axons. Increasing expression until a peak at 48 h; after that at 96 h very weak activity.	Mechanical hyperalgesia was detected from 6 to 12 days after injury. Etanercept inhibited pain related behaviors significantly
15	qPCR	TNF- $\alpha$ levels remained unchanged in contralateral nerves. Significant ipsilateral increase at day 3 in TNF- $\alpha$ levels	None
16	qPCR, von Frey filaments and Hargreaves test	TNF- $\alpha$ , TNFR1 and TNFR2 mRNA levels in ipsilateral DRG increased significantly after 1 day post-injury, levels decreased after day 1, and returned to baseline on days 7 and 14; in the spinal cord the maximal level of TNF- $\alpha$ and TNFR1 was reached on day 3, and also decreased to baseline at days 7 and 14	Mechanical allodynia increased significantly 1 day after injury with maximum levels at day 7. Thermal hyperalgesia had its peak at day 3. Both persisted for at least 28 days
17	Immunohistochemistry and von Frey filaments	Significantly higher TNF- $\alpha$ cells and average staining intensity compared to sham	Threshold significantly reduced when compared to sham group
18	Immunohistochemistry	Significantly higher cells in disc incision group compared to sham	None
19	Immunohistochemistry and histology	Significant increase of TNF- $\alpha$ immunoreactive cells between day 1 and 3, medium levels at days 7 and 14, and very decreased on day 21. No cells found after TNF- $\alpha$ inhibitor treatment at any time point	None
20	Immunohistochemistry and electron microscopy	Significant increase pixels in endoneurium and inflammatory crescent	None
21	Immunohistochemistry	Weak to moderate immunoreactivity in DRG when compared to non-lesion groups. Increased immunoreactivity in spinal cord with its highest peak after 5 days	None
22	qPCR, von Frey filaments and Hargreaves test	Gene expression statistical significance only at day 14	CCI group showed reduced sensory threshold for thermal and mechanical allodynia (mainly at short-term periods)
23	Immunohistochemistry, immunoblotting and von Frey filaments	Immunohistochemistry colocalized TNF- $\alpha$ with endoneurial macrophages and GFAP-IR glial cells. Immunoblotting showed significant expression on day 7 in the NP group, but any difference at day 28	Threshold significantly reduced in NP group with lowest point at day 14, with gradual increase after it
24	Immunohistochemistry, ELISA, von Frey filaments and tail-flick test	High amounts of cytokines and mRNA expression after spinal nerve transection, with no effect of acute morphine administration, tolerance with chronic treatment and worsening with withdrawal	Significant increase of allodynia in both tests, reverted effect in both with opioids application (high doses)
25	qPCR, von Frey filaments and Hargreaves test	High levels of mRNA in the sciatic nerve at 6 and 24 h; in DRG at 24 and 72 h; and no expression at any moment in spinal cord	CCI group showed reduced sensory threshold for thermal and mechanical allodynia (lowest point at day 14)

**Table 2** continued

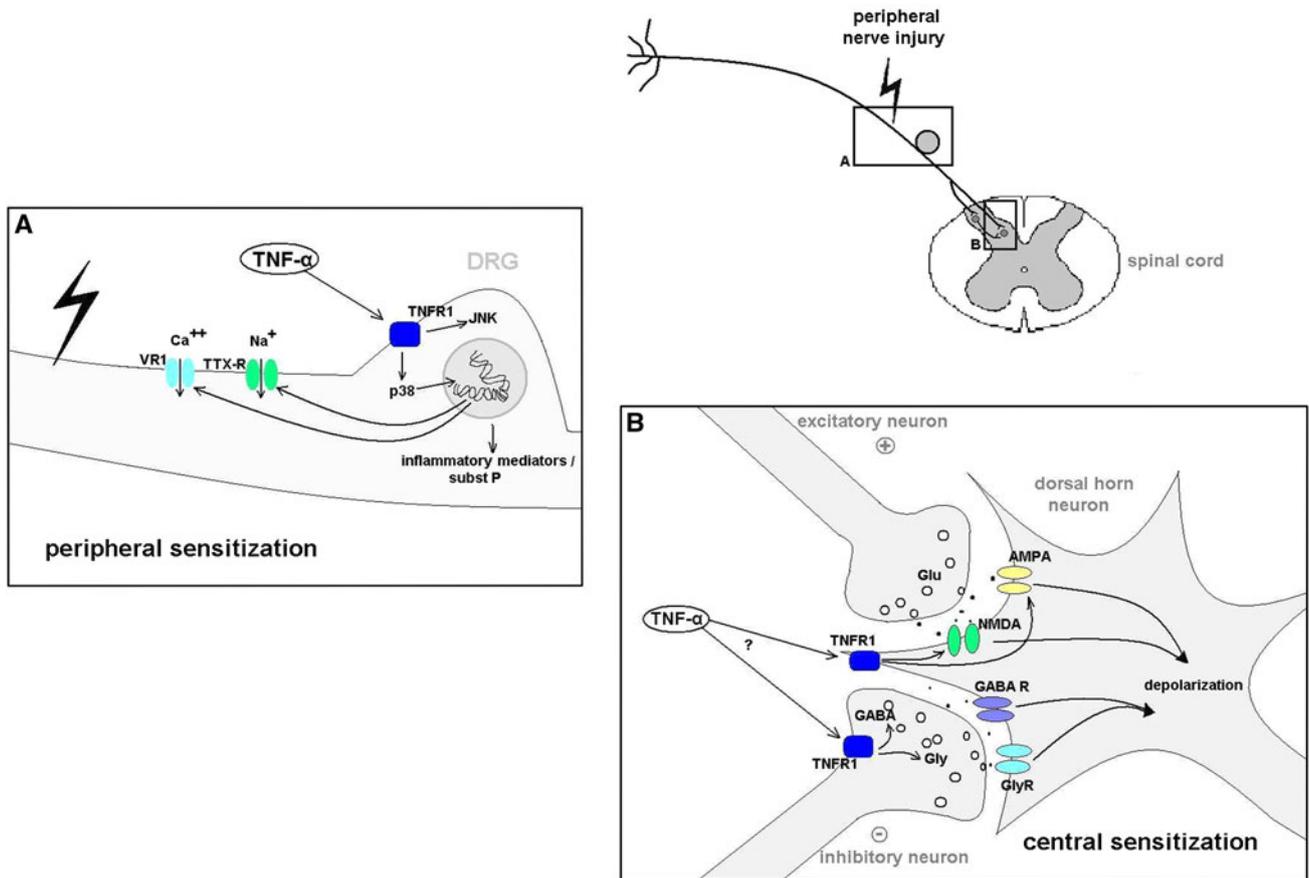
Study no.	TNF detection method and behavioral test	TNF- $\alpha$ levels	Pain behavioral data
26	Immunohistochemistry	Weak cell expression of TNFR1 in non-injured neurons; elevated immunoreactivity for TNFR1 in injured neurons. Higher receptor expression in higher levels of DRGs compared to lower spinal levels.	None
27	Immunohistochemistry, von Frey filaments and Hargreaves test	Immunohistochemistry showed no statistical differences between treated and untreated animals after 10 days (no differences between treatments)	Significant increase of mechanical and thermal allodynia in CCI group (anti-TNF Ab reduces thermal hyperalgesia)
28	Immunohistochemistry and von Frey filaments	Low levels of TNFR1 and TNFR2 in sham animals. Increased expression of both receptors after 6 h of lesion, with a second peak at 5 days. Uninjured DRG showed early TNFR1 expression similar to ligated DRGs animals	Significant mechanical allodynia showed in ligated animals when compared to sham, starting at 6 h with a peak at 24 h
29	Immunohistochemistry, Immunoblotting and von Frey filaments	Immunoblotting showed significant expression in the distal crush group at every time-point (always higher than any other group). Proximal crush group showed significance on days 2, 7 and 14. Both groups indicated signs of apoptosis in stainings	At day 14 proximal and distal crush groups showed reduced thresholds; at day 28 only the distal crush group showed significant mechanical allodynia
30	Immunohistochemistry, ELISA and von Frey filaments	Moderate to intense activation expression in the spinal cord of all animals, any treatment showed to be effective to change these findings	Significant increase of allodynia in saline treated rats, high response rate when treated with sTNFR
31	qPCR	mRNA was detectable in controls in all studied tissues. Gene expression decreased within the first 6 h after surgery in all areas. NMDA receptor antagonist inhibited early gene expression decrease	None
32	Histology, Electrophysiology, von Frey filaments, Hargreaves test and cold sensitivity test	TNFR1(-) mice showed significant increase of intact axons on day 36 compared to wild-type mice. Maximal significant edema was found on day 3 in TNFR2(-) mice, but also the lowest significant level at day 15 in this same group. No differences found in number of macrophages and degenerating fibers	TNFR1(-) mice did not develop heat allodynia. TNFR1(-) and TNFR2(-) mice showed decreased mechanical and cold hyperalgesia; and less changes in skin temperature
33	Immunohistochemistry, von Frey filaments and Hargreaves test	Significant increase in immunoreactivity cells of TNF- $\alpha$ and TNFR1 in ipsilateral DRG compared to sham; and increased expression of TNF- $\alpha$ and TNFR1 bilaterally in dorsal and ventral horn of the spine. Significant decrease in immunoreactivity was found in DRG and spinal cord after thalidomide injection at any time point of evaluation after surgery	Significant and long-lasting bilateral mechanical allodynia and thermal hyperalgesia in transection group. Thalidomide injection attenuated both types of allodynia when it was applied in the first 7 days post-surgery

CCI chronic constriction injury, *rhEpo* recombinant human erythropoietin, DRG dorsal root ganglion, NP nucleus pulposus, sTNFR soluble TNF receptor, TNFR1(-) deficient TNF receptor 1, TNFR2(-) deficient TNF receptor 2, TNFR1 TNF receptor 1, TNFR2 TNF receptor 2, GFAP-IR glial fibrillary acidic protein-immunoreactive

response. At the same time, its increased expression in nervous tissues upon a pain stimulus suggests that this cytokine is essential for the development of pain sensation [65–69].

Several authors published similar results with regard to TNF- $\alpha$  presence in peripheral and central nervous tissue after neural injury (Fig. 2). However, part of the main divergences remains in the involvement and interactions of

TNFR1 and TNFR2. Thus far, it seems that both receptor types play a different role in the development of allodynia and hyperalgesia depending on the site of the lesion and the moment of the cascade activation. For example, TNFR1 has shown constitutive expression in the uninjured spinal cord in modulatory centers like the dorsal horn, whereas these areas hardly express TNFR2 [44, 70]. Nevertheless, this does not imply that after peripheral nerve injury and



**Fig. 2** Mechanisms involved in pain. TNF- $\alpha$  increases hyperexcitability and consequently neuropathic pain via different peripheral and central mechanisms. At the site of injury TNF- $\alpha$  leads to activation of p38 and JNK, followed by increased TTX-R and VR1 expression, accompanied by production of other inflammatory agents and substance P. In the dorsal horn of the spinal cord, TNF- $\alpha$  induces

increased activity of NMDA and AMPA receptors, leading to depolarization. It is not completely elucidated yet the role of TNF- $\alpha$  on inhibitory neurons, but it is suggested that it increases GABA and glycine release from presynaptic terminals, producing hyperexcitability

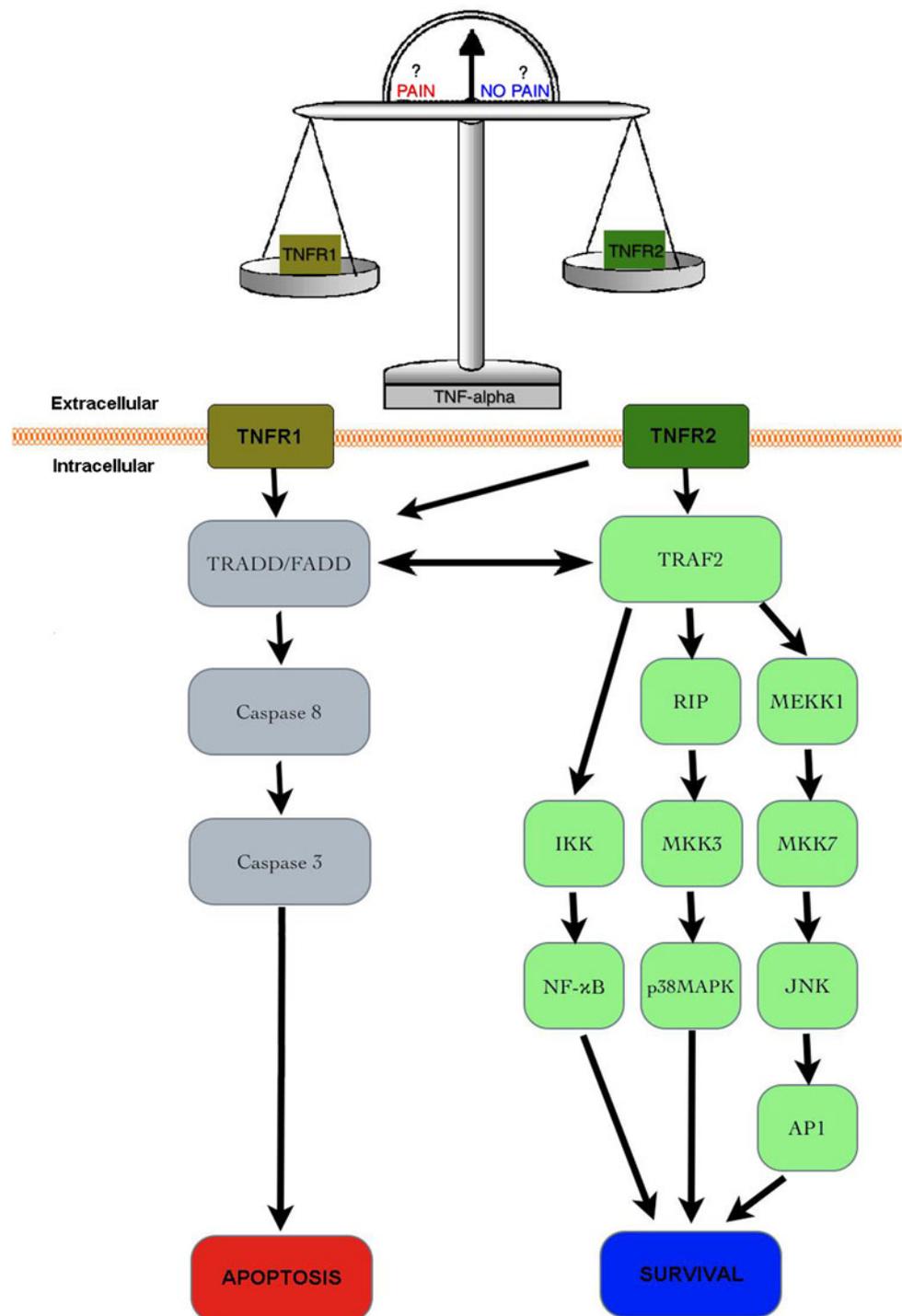
TNF- $\alpha$  release, TNFR2 could not be involved in nociceptive regulation [47]. This could also be dependant on the proximity of these lesions from the spinal cord, illustrated by earlier manifestation of pain in spinal nerve lesions (h) than in sciatic nerve lesions (days) [45, 59]. In judgment of the literature reported to our days, it is not still clear if the final nociceptive outcome is more dependant on the amount of cytokine or on the activation/inhibition of specific receptors (Fig. 3).

The increase of TNF- $\alpha$  immunoreactive cells or mRNA levels showed a similar temporal resolution, regardless of the studied tissue or injury model. As previously described, TNF- $\alpha$  production typically starts to reach maximal levels within 24 h, which are maintained for over 2 weeks and then usually drop [37, 39–41, 45, 47, 54, 56, 59, 60]. This paradigm is important for understanding the development of painful symptomatology and for the therapeutic timing approach. Behavioral data show that the clinical

presentation follows the presence of increased amounts of TNF- $\alpha$  in neural tissue [71–73]. Fluctuation in TNF- $\alpha$  levels tended to match with increased or decreased states of mechanical allodynia and thermal hyperalgesia. Progressive nociception in injured animals was established after the first few days and maintained until its highest point around the second week, followed by a gradual drop up to baseline thresholds [37, 54, 56]. This transient pain behavior may be a consequence of the continuous adaptation of pain perception that familiarizes the animal with the applied stimuli, yet in each study the cytokine levels correlated closely to the pain sensation [41, 53]. In this respect, TNF- $\alpha$  involvement in pain could also be inferred in studies where wild-type mice showed greater levels of pain when compared to genetically TNF knock-out animals [74, 75].

The pharmacological therapies proved to be successful when applied during the first 7 days post-lesion, which is

**Fig. 3** Intracellular pathways related to TNFR1 and TNFR2. On the *left*, TNFR1 is primarily related to the induction of apoptosis; though, it can interact via TRAF2 to promote cell survival under certain circumstances. On the *right*, TNFR2 function is oriented to follow parallel pathways that will lead to cell survival and proliferation. Probably the balance and interaction between these two receptors determine the final damage produced by lesions, and therefore the amount of pain



consistent with the temporal resolution of TNF- $\alpha$  expression, probably avoiding the initial inflammatory response [66, 76]. This is also an essential matter in the apparent dual intervention of TNF- $\alpha$  in neurons as toxic or protective agent. Originally, the function of this cytokine has been described as harmful, but previous evidence and data in this review suggest that protection and regeneration

could be involved in a receptor-dependant mode. It has been described that TNFR2 has a fundamental participation in remyelination of neurons after injury, probably linked to its late participation in the inflammatory cascade [44, 70, 77–79]. This suggestion also comes from delayed expression of TNFR2, which coincides with behavioral recovery in the late phases of pain sensation. Thus, it is of vital

importance to understand the temporal expression of TNF- $\alpha$  and its receptors in order to develop a successful therapeutic strategy. In these days, we can observe that the same cytokine at different moments, acting on different receptors, could bring opposite histological and clinical outcomes. These observations bring the query as to which factors are more determinant for the production of pain, the concentration of TNF- $\alpha$  or the TNFR1–TNFR2 expression ratio. In this respect, a key question that remains is why patients with chronic NP do not benefit from this self-limiting protective mechanism. We believe that a balance alteration between the TNF- $\alpha$  receptors could determine the severity of pain, not only in a temporal expression (acute or chronic) but also on the extension of the produced damage. Animal models have proven to develop and reproduce pain in a comparable manner to human observations. In summary, there is evidence from physical injury models, transgenic models, and pharmacological interference studies that TNF- $\alpha$  expression in the spinal cord modulates pain sensation. However, future perspectives for treatment still require better understanding of its receptors and intracellular pathway interactions as pain modulators. Therefore, further translational studies may be performed to address this challenging topic and design more effective therapies by regulating inflammation-induced pain sensation.

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## Appendix I

Table of excluded studies ( $n = 40$ )

Study No.	Reference	Exclusion reason
1	Carlton SM, Du J, Tan HY et al. (2009) Peripheral and central sensitization in remote spinal cord regions contribute to central neuropathic pain after spinal cord injury. <i>Pain</i> 147:265–276	I
2	Chew DJ, Leinster VH, Sakthithasan M et al. (2008) Cell death after dorsal root injury. <i>Neurosci Lett</i> 433:231–234	I
3	Czeschik JC, Hagenacker T, Schäfers M et al. (2008) TNF-alpha differentially modulates ion channels of nociceptive neurons. <i>Neurosci Lett</i> 434:293–298	I

## Appendix continued

Study No.	Reference	Exclusion reason
4	DeLeo JA, Colburn RW, Rickman AJ (1997) Cytokine and growth factor immunohistochemical spinal profiles in two animal models of mononeuropathy. <i>Brain Res</i> 759:50–57	III
5	DeLeo JA, Rutkowski MD, Stalder AK et al. (2000) Transgenic expression of TNF by astrocytes increases mechanical allodynia in a mouse neuropathy model. <i>Neuroreport</i> 11:599–602	I
6	de Novellis V, Siniscalco D, Galderisi U et al. (2004) Blockade of glutamate mGlu5 receptors in a rat model of neuropathic pain prevents early over-expression of pro-apoptotic genes and morphological changes in dorsal horn lamina II. <i>Neuropharmacology</i> 46:468–479	I
7	Deumens R, Jaken RJ, Knaepen L et al. (2009) Inverse relation between intensity of GFAP expression in the substantia gelatinosa and degree of chronic mechanical allodynia. <i>Neurosci Lett</i> 452:101–105	I
8	Gustafson-Vickers SL, Lu VB, Lai AY et al. (2008) Long-term actions of interleukin-1beta on delay and tonic firing neurons in rat superficial dorsal horn and their relevance to central sensitization. <i>Mol Pain</i> 4:63	II
9	Ignatowski TA, Covey WC, Knight PR et al. (1999) Brain-derived TNF alpha mediates neuropathic pain. <i>Brain Res</i> 841:70–77	I
10	Ignatowski TA, Sud R, Reynolds JL et al. (2005) The dissipation of neuropathic pain paradoxically involves the presence of tumor necrosis factor-alpha (TNF). <i>Neuropharmacology</i> 48:448–460	II
11	Jaken RJ, Joosten EA, Knüwer M et al. (2010) Synaptic plasticity in the substantia gelatinosa in a model of chronic neuropathic pain. <i>Neurosci Lett</i> 469:30–33	II
12	Jiang YQ, Xing GG, Wang SL et al. (2008) Axonal accumulation of hyperpolarization-activated cyclic nucleotide-gated cation channels contributes to mechanical allodynia after peripheral nerve injury in rat. <i>Pain</i> 137:495–506	II
13	Kawamura T, Akira T, Watanabe M et al. (1997) Prostaglandin E1 prevents apoptotic cell death in superficial dorsal horn of rat spinal cord. <i>Neuropharmacology</i> 36:1023–1030	III
14	Kawasaki Y, Zhang L, Cheng JK et al. (2008) Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. <i>J Neurosci</i> 28:5189–5194	I, IV

## Appendix continued

Study No.	Reference	Exclusion reason
15	Kunz S, Tegeder I, Coste O et al. (2005) Comparative proteomic analysis of the rat spinal cord in inflammatory and neuropathic pain models. <i>Neurosci Lett</i> 381: 289–293	I, II
16	Li J, Xie W, Zhang JM, Baccei ML (2009) Peripheral nerve injury sensitizes neonatal dorsal horn neurons to tumor necrosis factor-alpha. <i>Mol Pain</i> 5:10	IV
17	Lindenlaub T, Sommer C (2003) Cytokines in sural nerve biopsies from inflammatory and non-inflammatory neuropathies. <i>Acta Neuropathol</i> 105:593–602	V
18	Liu B, Li H, Brull SJ, Zhang JM (2002) Increased sensitivity of sensory neurons to tumor necrosis factor alpha in rats with chronic compression of the lumbar ganglia. <i>J Neurophysiol</i> 88:1393–1399	IV
19	Liu YL, Zhou LJ, Hu NW et al. (2007) Tumor necrosis factor-alpha induces long-term potentiation of C-fiber evoked field potentials in spinal dorsal horn in rats with nerve injury: the role of NF-kappa B, JNK and p38 MAPK. <i>Neuropharmacology</i> 52:708–715	I, IV
20	Lu VB, Biggs JE, Stebbing MJ, et al. (2009) Brain-derived neurotrophic factor drives the changes in excitatory synaptic transmission in the rat superficial dorsal horn that follow sciatic nerve injury. <i>J Physiol</i> 587:1013–1032	I, II
21	Ma W, Quirion R (2001) Increased phosphorylation of cyclic AMP response element-binding protein (CREB) in the superficial dorsal horn neurons following partial sciatic nerve ligation. <i>Pain</i> 93:295–301	I, II
22	Maione S, Siniscalco D, Galderisi U et al. (2002) Apoptotic genes expression in the lumbar dorsal horn in a model neuropathic pain in rat. <i>Neuroreport</i> 13:101–106	I, II
23	Marchand F, Tsantoulas C, Singh D et al. (2009) Effects of Etanercept and Minocycline in a rat model of spinal cord injury. <i>Eur J Pain</i> 13:673–681	VI
24	Milligan ED, Twining C, Chacur M et al. (2003) Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. <i>J Neurosci</i> 23:1026–1040	I
25	Moore KA, Kohno T, Karchewski LA et al. (2002) Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. <i>J Neurosci</i> 22:6724–6731	II
26	Murata Y, Nannmark U, Rydevik B et al. (2008) The role of tumor necrosis factor-alpha in apoptosis of dorsal root ganglion cells induced by herniated nucleus pulposus in rats. <i>Spine</i> 33:155–162	IV

## Appendix continued

Study No.	Reference	Exclusion reason
27	Murata Y, Olmarker K, Takahashi I et al. (2005) Effects of selective tumor necrosis factor-alpha inhibition to pain-behavioral changes caused by nucleus pulposus-induced damage to the spinal nerve in rats. <i>Neurosci Lett</i> 382:148–152	I
28	Murata Y, Rydevik B, Takahashi K et al. (2005) Incision of the intervertebral disc induces disintegration and increases permeability of the dorsal root ganglion capsule. <i>Spine</i> 30:1712–1716	II
29	Peng XM, Zhou ZG, Glorioso JC, Fink DJ, Mata M (2006) Tumor necrosis factor-alpha contributes to below-level neuropathic pain after spinal cord injury. <i>Ann Neurol</i> 59:843–851	VI
30	Polgár E, Hughes DI, Arham AZ et al. (2005) Loss of neurons from laminae I–III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain. <i>J Neurosci</i> 25:6658–6666	II
31	Schäfers M, Sommer C, Geis C et al. (2008) Selective stimulation of either tumor necrosis factor receptor differentially induces pain behavior in vivo and ectopic activity in sensory neurons in vitro. <i>Neuroscience</i> 157:414–423	I
32	Scholz J, Broom DC, Youn DH et al. (2005) Blocking caspase activity prevents transsynaptic neuronal apoptosis and the loss of inhibition in lamina II of the dorsal horn after peripheral nerve injury. <i>J Neurosci</i> 25:7317–7323	II
33	Singh OV, Yaster M, Xu JT, Guan Y, Guan X, Dharmarajan AM et al. (2009) Proteome of synaptosome-associated proteins in spinal cord dorsal horn after peripheral nerve injury. <i>Proteomics</i> 9:1241–1253	II
34	Siniscalco D, Fuccio C, Giordano C et al. (2007) Role of reactive oxygen species and spinal cord apoptotic genes in the development of neuropathic pain. <i>Pharmacol Res</i> 55:158–166	II
35	Sommer C, Schmidt C, George A (1998) Hyperalgesia in experimental neuropathy is dependent on the TNF receptor 1. <i>Exp Neurol</i> 151:138–142	III
36	Spengler RN, Sud R, Knight PR et al. (2007) Antinociception mediated by alpha(2)-adrenergic activation involves increasing tumor necrosis factor alpha (TNFalpha) expression and restoring TNFalpha and alpha(2)-adrenergic inhibition of norepinephrine release. <i>Neuropharmacology</i> 52:576–589	IV

## Appendix continued

Study No.	Reference	Exclusion reason
37	Sud R, Ignatowski TA, Lo CP, Spengler RN (2007) Uncovering molecular elements of brain-body communication during development and treatment of neuropathic pain. <i>Brain Behav Immun</i> 21:112–124	II
38	Yamashita M, Ohtori S, Koshi T et al. (2008) Tumor necrosis factor- $\alpha$ in the nucleus pulposus mediates radicular pain, but not increase of inflammatory peptide, associated with nerve damage in mice. <i>Spine</i> 33:1836–1842	I
39	Yang L, Zhang FX, Huang F et al. (2004) Peripheral nerve injury induces trans-synaptic modification of channels, receptors and signal pathways in rat dorsal spinal cord. <i>Eur J Neurosci</i> 19:871–883	II
40	Youn DH, Wang H, Jeong SJ (2008) Exogenous tumor necrosis factor- $\alpha$ rapidly alters synaptic and sensory transmission in the adult rat spinal cord dorsal horn. <i>J Neurosci Res</i> 86:2867–2875	IV

*I* No TNF- $\alpha$  levels measurement, *II* TNF- $\alpha$  was not involved in inflammatory response, *III* outdated for the limits of this review; older than 10 years, *IV* use of TNF- $\alpha$  as inductor of the lesion or inflammatory procedure, *V* clinical study, *VI* direct central nervous system injury

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