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The rodent tibia fracture model: A critical review and comparison with the complex regional pain syndrome literature

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Abstract

Distal limb fracture is the most common cause of complex regional pain syndrome (CRPS), thus the rodent tibia fracture model (TFM) was developed to study CRPS pathogenesis. This comprehensive review summarizes the published TFM research and compares these experimental results with the CRPS literature. The TFM generated spontaneous and evoked pain behaviors, inflammatory symptoms (edema, warmth) and trophic changes (skin thickening, osteoporosis) resembling symptoms in early CRPS. Neuropeptides, inflammatory cytokines, and nerve growth factor (NGF) have been linked to pain behaviors, inflammation, and trophic changes in the TFM model and proliferating keratinocytes were identified as the primary source of cutaneous cytokines and NGF. Tibia fracture also activated spinal glia and up regulated spinal neuropeptide, cytokine, and NGF expression, and in the brain it changed dendritic architecture. B cell expressed IgM antibodies also contributed to pain behavior, indicating a role for adaptive immunity. These results modeled many findings in early CRPS, but significant differences were also noted.

Keywords

pain; fracture; cytokine; neuropeptide; innate immunity; autoimmunity; complex regional pain syndrome

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Overview

Complex regional pain syndrome (CRPS) usually develops after limb trauma, most frequently a fracture, and presents with distal limb nociceptive, vascular, and bone changes that exceed the expected clinical course of the inciting injury in both magnitude and duration, frequently resulting in significant motor impairment and disability. CRPS symptoms gradually resolve over the first year after injury in most patients, but persistent CRPS is a serious problem, resulting in edema, pain, weakness, contractures, and bone loss. Over 80% of chronic CRPS patients are severely disabled.¹²¹ The debate regarding the underlying mechanisms of CRPS has been dynamic and controversial, and despite extensive investigation, the pathophysiology of this condition remains undefined and there is considerable disagreement on whether any treatment for CRPS is effective.^{18, 29, 67, 106} There is clearly a need for effective and safe treatments for this debilitating complication of injury. A valid CRPS animal model could advance our understanding of the post traumatic biological processes supporting the development and perpetuation of this disease and potentially identify temporally appropriate and target-specific treatments.

The spinal/supraspinal pathophysiology of CRPS can include motor dysfunction, altered body perception, sensations of numbness, anxiety, suffering and concerns about the future. It is difficult or impossible to investigate all aspects of these phenomena in animal models, thus any CRPS animal model will have experimental limitations. Furthermore, most chronic pain models are limited by the brevity of pain behaviors and the life span of rodents, thus are unlikely to entirely replicate the temporal transitions of the biologic mechanisms supporting chronic pain in the clinical setting. However, by focusing on the nociceptive, inflammatory, and trophic changes after injury, animal models provide a unique opportunity to investigate the early pathophysiology of post-traumatic CRPS.

Several animal models for CRPS have been developed in the last dozen years. One model is the chronic post-ischemia pain (CPIP) model, in which a tight fitting plastic O-ring is slipped over the distal ankle for 3 hours. After reperfusion, unilateral inflammatory symptoms (warmth and edema) are observed for several hours, while bilateral hindlimb von Frey allodynia and mechanical and cold hyperalgesia persist for 4 weeks.²⁶ There is evidence of macrophage and neutrophil infiltration into the injured hindlimb, with increased levels of cytokines and oxidative stress products in the skin and muscle, but most of these changes resolve within days or several weeks of ischemic injury.^{69, 72, 151} It has been postulated that the CPIP model mimics the pathophysiology of CRPS patients with a cold affected limb, with increased microvascular vasoconstriction, tissue hypoxia, and metabolic tissue acidosis, resulting in the generation of free radicals which further injure the vascular endothelium of the affected limb and contribute to perpetuating nociceptive sensitization. Consistent with this hypothesis, there is a decrease in the number of patent blood vessels in the CPIP hind paw muscles from 2–7 days after the ischemic injury, but not at 14 or 21 days after injury, and capillary wall thickness is increased at day 7.⁷² When vasodilators are given 2 days after the ischemic injury they reduce von Frey allodynia, but when given 7 days after injury they are mostly ineffective, suggesting that vasoconstriction or occlusion contribute to early nociceptive sensitization, but not to the perpetuation or maintenance of that sensitization.¹⁵¹ At least 25 different drugs have been tested in the CPIP model and

have been found to effectively reduce hind paw von Frey allodynia, and 3 drugs (ibuprofen, acetaminophen, and amitriptyline) were ineffective.^{69, 72, 91, 92, 151} The CRPS-like symptoms and mechanisms in CPIP are impressive, but there are several reservations regarding this model, including; 1) the pain behaviors are equal bilaterally, not unilaterally as is usually observed in CRPS, 2) the time courses of the inflammatory and ischemic changes are brief, 3) there is probably ischemic axon damage,⁶⁹ and 4) the lesion is artificial.

Another model is the needle-stick nerve injury model. The sciatic nerve is exposed surgically and pricked with a needle. The animals develop mechanical hyperalgesia on the treated and to a lesser extent also on the untreated side. The inflammatory symptoms are minor^{83, 118}.

Models which have been rarely used are the combination of spinal nerve ligation (SNL) and knee joint immobilization⁹⁸, soft tissue trauma plus an intra-arterial perfusion of muscle tissue supernatant⁴⁸ or the intra-arterial infusion of free radical donors.¹³¹

The most widely used CRPS model is a rodent tibia fracture model (TFM)⁵⁰ that duplicates most common etiology of CRPS, distal limb fractures.^{28, 110} The TFM CRPS model has been utilized in both mice and rats. After performing a closed tibia fracture under anesthesia and then casting the hindlimb for three (mice) to four (rats) weeks, most rodents develop ipsilateral hindpaw warmth, edema, mechanical allodynia, hindlimb unweighting, and periarticular osteoporosis. These symptoms occur primarily in the injured limb, but some changes are also observed in the contralateral limb. The majority of these CRPS-like symptoms spontaneously resolve over a 5-month period, with more rapid resolution observed in fracture mice.^{50, 53, 141} Whereas a review article on TFM has not been published since 2010,⁶⁸ this review provides an updated summary on how the TFM furthers understanding the neuro-immune pathogenesis of human CRPS and as a general model of acute-to-chronic post-traumatic pain transitions and mechanisms.

Strategy for article selection

A Pubmed search with the key words “*tibia*” “*fracture*” “*model*” “*complex*” “*regional*” and “*pain*” identified 28 original TFM research articles.

27, 34, 42, 44, 50–53, 57, 68, 75–81, 107, 108, 115, 123–125, 137, 140–143 These studies described molecular, cellular, and electrophysiological changes in the skin, peripheral nerve, DRG, spinal cord, brain, and bone tissues of TFM rodents, and used pharmacologic interventions and transgenic mice to examine the contribution of these neuro-immune signaling pathways to the development of CRPS-like symptoms. The current paper is a comprehensive review of these studies with comparisons to the CRPS literature and discussion of the potential experimental and clinical significance. Whenever possible the data was analyzed to differentiate between early and chronic TFM and CRPS findings.

Diagnostic criteria for CRPS

According to the current “Budapest” IASP diagnostic criteria, CRPS can be diagnosed if the following prerequisites are fulfilled:⁵⁴

1. Continuing pain, which is disproportionate to any inciting event
2. Must report at least one symptom in three of the four following categories:
 - Sensory: reports of hyperesthesia and/or allodynia
 - Vasomotor: reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry
 - Sudomotor/edema: reports of edema and/or sweating changes and/or sweating asymmetry
 - Motor/trophic: reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
3. Must display at least one sign at time of evaluation in two or more of the following categories:
 - Sensory: evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or deep somatic pressure and/or joint movement)
 - Vasomotor: evidence of temperature asymmetry and/or skin color changes and/or asymmetry
 - Sudomotor/edema: evidence of edema and/or sweating changes and/or sweating asymmetry
 - Motor/trophic: evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin)
4. There is no other diagnosis that better explains the signs and symptoms

The diagnosis of CRPS requires compliance with all 4 points. In the following sections, we cover the clinical changes that take place in CRPS versus in TFM (Table 1).

Comparison of CRPS and TFM symptoms, signs, and pathophysiology

Pain, hyperalgesia, and allodynia

Patients with CRPS suffer from a continuing pain that is disproportionate to the inciting injury. This pain is often accompanied by mechanical and thermal hyperalgesia (increased pain from a stimulus that normally provokes pain), allodynia (pain due to a stimulus that does not normally provoke pain) to light touch and/or joint movement, and joint tenderness. Prospective studies observed that improvement occurred over the first year after onset and 67–75% of patients no longer met the Budapest CRPS research criteria at the end of the first year.^{9, 11} The average numerical pain scores declined from 5.5 to 2.8 and the prevalence of allodynia declined from 67% to 25%.⁹ The prevalence of mechanical pressure hyperalgesia is 70%.⁴⁷ The prevalence of heat and cold hyperalgesia ranges from 30–53%, slightly increasing over time.^{47, 103} The primary concern for most CRPS patients at one year after onset was weakness and reduced range of motion in the injured limb.^{9, 16, 153} Unfortunately,

for CRPS patients with persistent significant pain at one-year post onset the prognosis was poor for further recovery,^{10, 39, 152} and chronic CRPS can be severely disabling.^{103, 121}

In the TFM, von Frey allodynia and mechanical unweighting of the hindpaw were present at the time of cast removal and persisted for up to 18 weeks after fracture before resolving, probably due to the frequent use of the fracture hindlimb.^{53, 125, 141} Ongoing spontaneous pain as measured by condition place preference was present at 9 weeks post fracture.¹²⁵ The allodynia, unweighting, and spontaneous pain observed in the TFM resemble the nociceptive changes observed in CRPS, but the TFM did not exhibit the heat hyperalgesia⁵⁰ that is reported in 30–53% of CRPS patients. The spontaneous resolution of nociceptive abnormalities within 5 months after fracture resembles the improvement observed in most CRPS patients over the first year after injury, but doesn't replicate more chronic CRPS.

Skin temperature and vasomotor changes

Approximately 40–56% of CRPS patients exhibited skin temperature asymmetry in the limbs of greater than 1°C.^{25, 47} In a prospective study the average temperature difference between limbs declined over the first year after the onset of CRPS, and the prevalence of skin color changes also decreased, from 95% to 45%.⁹ About half of CRPS cases showed warmth and reddening of the affected skin (inflammatory phenotype) in a large multicenter study, while the remaining half had cold, bluish skin (cold phenotype).²⁴ The inflammatory phenotype was more frequently observed in early (average duration 4.4 months) CRPS patients and the cold phenotype more frequently observed in chronic (average duration 10.9 months) CRPS patients.²⁴

In the TFM signs of inflammation predominated, resembling early CRPS. The fractured paw was usually warmer than the contralateral hindpaw at the time of cast removal and this hindpaw warmth gradually resolved within 10 weeks after fracture.⁵⁰ The development of hindpaw warmth in the TFM is dependent on calcitonin gene-related peptide (CGRP) signaling. Fracture mice deficient for the CGRP RAMP1 receptor failed to develop hindpaw warmth⁵³ and primary afferent CGRP release can induce long-term neurogenic flare responses in rodent and human skin.¹⁴⁴ Unlike early CRPS, no erythema has been described in TFM (the skin over the dorsum of the paw is obscured by fur). A primary cold phenotype has not been described in the TFM, which may be attributable to the spontaneous resolution of CRPS-like symptoms within 5 months after fracture.

Sudomotor changes and limb edema

A prospective study of CRPS patients (IASP Budapest criteria) observed a 23% prevalence of sudomotor changes (hyper- or hypohydrosis relative to the contralateral limb) in early (< 3 month duration) CRPS patients, declining to 4% prevalence at 12 months post injury.⁹ Edema in the injured limb was observed in 85% of early (<3 months duration) CRPS patients, gradually declining to 22% prevalence of limb edema at 12 months post injury.⁹ Increased spontaneous plasma extravasation⁹⁹ and facilitated extravasation responses after microdialysis electrical stimulation¹³⁹ and substance P (SP) perfusion⁷³ were observed in the CRPS limb, relative to the contralateral side. Increased spontaneous plasma extravasation

was observed in the affected limb in 59% of early (< 5 months duration) CRPS patients and was only detected in 17% of chronic CRPS patients.⁹⁹

Hyper- or hypohidrosis has not been reported in the TFM, but rodents lack sweat glands, except over the plantar aspect of the paw, so the TFM may not be ideal for detecting the sudomotor changes observed in a minority of CRPS patients. At the time of cast removal hindpaw edema is usually observed in the TFM, gradually resolving by 10 weeks post fracture,^{50, 141} similar to temporal resolution observed in most CRPS patients over the first year after injury. Spontaneous plasma protein extravasation is enhanced in the fracture paw skin at the time of cast removal, resolving within 16 weeks post fracture,^{141, 142} similar to temporal resolution observed in most CRPS patients. Facilitated extravasation responses after sciatic nerve electrical stimulation and intravenous SP perfusion were observed in the fracture limb, relative to the contralateral side, and intravenous SP perfusion induced hindpaw edema ipsilateral, but not contralateral to fracture.¹⁴² These TFM findings replicate the results of CRPS patient studies.

Motor dysfunction

A prospective study of CRPS patients (IASP Budapest criteria) observed impaired rapid motor function in 82% of early (<3 months duration) CRPS, gradually declining to a 44% prevalence of impaired motor function at 12 months post injury.⁹ Decreased range of motion in the affected joints was noted in 90% of patients^{9, 136} and chronic weakness in the affected limb was observed in most CRPS patients.¹⁵² Tremors, dystonias and muscle spasms were observed in some patients.^{103, 136}

In the TFM there is impaired motor behavior on rotarod testing,¹²⁵ consistent with the findings in reported in CRPS patients, but tremors, dystonias and muscle spasms have not been observed.

Trophic changes

Human CRPS is characterized by trophic changes in skin, hair, and nails (increased or reduced growth), connective tissue (contractures), and bones (high-turnover osteoporosis). One study performed immunohistochemical analyses of skin punch biopsies collected from the affected limb and the contralateral mirror site in 55 CRPS patients (IASP Budapest criteria).¹⁷ Immunostaining demonstrated keratinocyte and mast cell proliferation and activation in the affected skin, with increased epidermal thickness in early (< 3 months duration) CRPS and diminished keratinocyte proliferation and epidermal thinning in chronic (> 3 months duration) CRPS. Another prospective study observed that 66% of early (<3 months duration) CRPS patients (IASP Budapest criteria) self-reported abnormal (increased or decreased) growth of hair and/or nails in the affected limb, gradually declining to a 31% prevalence of hair and nail abnormalities noted at 12 months post injury.⁹ Periarticular trabecular bone loss was usually observed in CRPS affected limbs by xray and dual-energy xray absorptiometry (DEXA) and the extent of bone loss was greater and longer lasting in tibia fracture patients who developed CRPS than in patients who did not develop CRPS after tibia fracture.^{15, 65, 111} Three-phase technetium bone scanning demonstrated increased

periarticular tracer uptake in 91% of injured limbs in early CRPS, consistent with accelerated bone remodeling.¹⁵⁰

Immunostaining the hindpaw skin of the TMF rodents at the time of cast removal demonstrated keratinocyte and mast cell proliferation and activation in the fracture limb skin, with increased epidermal thickness that resolved by 16 weeks post fracture.^{78, 80, 141, 142} These findings predicted the findings of a CRPS patient skin biopsy study that replicated the TFM results.¹⁷ There are no reports on hair, nail, or connective tissue changes in the TMF. Periarticular trabecular bone loss is observed in the fracture limb by DEXA and microCT scanning, peaking at 8 weeks and persisting for at least 20 weeks post fracture,^{50, 107, 108, 137} similar to the trabecular changes reported in CRPS affected limbs. Bone histomorphometry demonstrated increased trabecular bone resorption activity in the distal femur and reduced bone formation rates at 4 weeks post fracture,¹³⁷ consistent with the CRPS bone scanning results. There are no reports of bone scanning in the TFM.

Cognitive changes

Decreased memory, global cognitive impairments, and increased anxiety and stress were frequently observed symptoms in CRPS patients.^{82, 84, 120} Similarly, the TFM mice also demonstrated memory impairments and increased anxiety behavior, with corresponding changes in dendritic architecture.¹²⁴

Neuroimmune signaling and inflammation in the periphery

Neuropeptides

Neurogenic inflammation occurs when nociceptive neurons are activated by suprathreshold stimuli, causing the co-release of the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) from distal nerve terminals. SP acts on endothelial tachykinin 1 (NK1) receptors in the microvasculature to induce protein extravasation and edema, while CGRP acts on a dimer complex of two molecules, the G-protein coupled calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein 1 (RAMP1), both of which are required for CGRP physiologic activity. CGRP induces cutaneous vasodilatation and warmth via a direct (i.e., endothelium-independent) relaxation of vascular smooth muscle.¹⁰¹ Limb edema, skin reddening, and warmth are the signs constituting the visible part of the inflammation in early post traumatic CRPS.⁹⁹

In CRPS the total number of epidermal nerve fibers might be reduced in some cases,⁹⁷ including the peptidergic nerve fibers,² or unchanged.³⁵ However, this does not exclude increased neuropeptide content in single fibers, facilitated release on stimulation, or increased sensitivity of neuropeptide receptors. Laser Doppler scanning and cutaneous microdialysis techniques have been used to demonstrate that electrically evoked protein extravasation and vasodilatation responses are facilitated in the affected skin of CRPS patients, evidence of exaggerated neurogenic inflammation occurring in the CRPS extremity.¹³⁹ Moreover, when the endogenous release of SP from C-fibers was bypassed by direct application of SP into the skin, the exaggerated protein extravasation response indicated an increased sensitivity of SP receptors.⁷³ Furthermore, serum levels of SP and CGRP are

elevated in CRPS patients.^{19, 21, 112} Collectively, these data support the hypothesis that neuropeptide signaling is enhanced in the affected skin of early CRPS patients, resulting in increased protein extravasation, edema, vasodilation, erythema, and warmth (Table 2). A subset of CRPS patients is characterized by cold and bluish skin right from the beginning of the symptoms. In these patients, endothelin 1 (ET1), a potent vasoconstrictor peptide that is also involved in various painful conditions, was increased in the fluid of skin suction blisters.⁴⁹

Neurogenic inflammatory responses were also enhanced in the TFM (Table 2). Similar to the results observed after intravenous injection of radiolabeled albumin in CRPS patients,⁹⁹ at 4 weeks post fracture spontaneous plasma extravasation was increased in the paw skin of the injured limb and this increase resolved within 16 weeks after fracture.^{141, 142}

Mirroring the SP microdialysis results observed in CRPS skin,⁷³ intravenous SP perfusion induced exaggerated extravasation responses and immediate paw edema in the injured limb but not on the contralateral side at 4 weeks post fracture.¹⁴² SP (TAC1) and CGRP (CALCA and CALCB) mRNA expression was increased in the DRGs innervating the fracture limb and corresponding DRG and sciatic nerve SP and CGRP protein levels were upregulated.^{27, 142} Furthermore, serum levels of SP and CGRP were elevated at 4 weeks post fracture.¹⁴² SP NK1 receptor mRNA and protein expression was also increased in the hindpaw skin at 4 weeks post fracture. The increase in NK1 receptor expression was localized by confocal microscopy to the endothelial cells and keratinocytes in the injured hindpaw skin.¹⁴² The expression of the CGRP co-receptors CRLR and RAMP1 were unchanged in the skin at 4 weeks post fracture, as was the SP metabolizing enzyme neutral endopeptidase (NEP).^{52, 142} At 16 weeks after fracture both sciatic nerve SP and hindpaw skin NK1 receptor protein expression had returned to normal levels.¹⁴¹

Treating fracture rats with an SP NK1 receptor antagonist attenuated the development of hindpaw edema, warmth, keratinocyte proliferation and epidermal thickening, mast cell proliferation and degranulation, and nociceptive sensitization, but had no effect on post fracture trabecular bone loss.^{50, 80, 140} Transgenic fracture mice lacking SP failed to develop hindpaw edema and warmth, and exhibited attenuated nociceptive sensitization.⁵³ Fracture mice lacking the CGRP RAMP1 receptor failed to develop hindpaw edema and also exhibited less sensitization than wildtype fracture mice.⁵³ Both in vitro experiments in keratinocyte cell cultures and in vivo experiments using intraplantar SP injections have demonstrated that SP and CGRP stimulate keratinocyte proliferation and activation, resulting in increased keratinocyte expression of NK1 and RAMP1 mRNA and protein, and increased keratinocyte expression and secretion of SP and CGRP proteins, suggesting possible autocrine or paracrine stimulatory effects and amplification of neuropeptide signaling.^{116, 140} Furthermore, SP and CGRP treatment stimulated keratinocyte mRNA expression and protein secretion of several inflammatory mediators, including tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6 (IL-6), and nerve growth factor (NGF).^{116, 117, 140} Collectively, these data indicate that post fracture facilitated SP and CGRP signaling support the epidermal proliferation, inflammation, nociceptive sensitization, and vascular changes, but not the periarticular trabecular bone loss observed in the TFM.

Inflammatory Mediators

Cytokine expression has been examined in serum, blood cells, experimental suction blister fluid, skin biopsies, and joints of CRPS patients (Table 3). In serum, TNF, IL-1, and IL-6 protein levels were normal in early and late CRPS patients, but interleukin-2 (IL-2) protein was increased and anti-inflammatory interleukin-10 (IL-10) protein decreased in early CRPS.^{58, 71, 74, 112, 129, 130} There were contradictory results for interleukin-8 (IL-8) protein levels in early CRPS and IL-8 and IL-10 protein levels were normal in chronic CRPS. In blood cells, TNF and IL-2 mRNA levels were increased, while the anti-inflammatory cytokines IL-4 and IL-10 were reduced in CRPS patients.¹²⁹ In experimental suction blister fluids TNF, IL-6, IL-8, IL-12 p40/p70, chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 4 (CCL4), and interleukin-1 receptor antagonist (IL-1RA) proteins were detectable and increased in the CRPS affected extremities.^{49, 55, 58, 61, 74, 94, 145, 146}

An immunohistochemical study evaluating bilateral skin punch biopsies collected from 55 CRPS patients observed that in early CRPS affected skin (in comparison to the contralateral side) there was upregulated keratinocyte expression of TNF and IL-6 in 40% of patients.¹⁸ Conversely, in chronic CRPS there was upregulated expression of TNF and IL-6 in only 25% of patients.¹⁸ Another study used EIA to quantify TNF levels in skin punch biopsies collected from 10 patients (average CRPS duration 9 months) and observed an increase in cutaneous TNF protein levels in 60% of the patients.¹⁷ The only clinical phenotype (with the exception of disease duration)⁷⁴ that was linked to CRPS cytokine signaling was an increased prevalence of mechanical hyperalgesia in patients with high levels of serum soluble TNF receptor 1.⁸⁷ Radiolabeled technetium anti-TNF antibody scintigraphy in CRPS patients demonstrated increased uptake in the wrist and metacarpal joints of the affected hand, compared to the contralateral side, in early (< 3 month duration) but not late CRPS.¹³

When SP or CGRP are microdialyzed in the skin of normal volunteers there is no immediate painful response, supporting the premise that SP and CGRP act as intermediate mediators in the development of inflammatory pain.¹⁴⁴ On the other hand, SP and CGRP signaling stimulated keratinocyte expression of inflammatory cytokines such as TNF, IL-1, and IL-6, and the neurotrophin NGF,^{116, 117, 140} all of which were demonstrated to induce rapid nociceptive sensitization when injected into rodent skin.^{77–79} When SP was injected into the hindpaw skin of a normal rat it evoked a long lasting nociceptive sensitization, with keratinocyte activation and sequential upregulated expression of TNF, IL-1, IL-6, and NGF skin proteins.¹⁴⁰ The time course of cytokine and NGF expression after SP injection demonstrated an inflammatory cascade with peak expression of TNF at 1 hour, peak expression of IL-1 and IL-6 between 1 and 3 hours, and peak expression of NGF at 6–24 hours post injection. The post injection time course and magnitude of NGF upregulation corresponded to the time course and magnitude of nociceptive sensitization and anti-NGF antibody blocked SP induced allodynia.¹⁴⁰ Intraplantar injection of TNF caused upregulated IL-1 expression¹⁴⁷ and IL-1 injection upregulated keratinocyte expression of NGF in hindpaw skin of normal rats.^{79, 109}

At 4 weeks post fracture there was increased ipsilateral hindpaw skin expression of TNF, IL-1, IL-6, CCL2, and NGF mRNA and protein.^{52, 53, 75, 107, 108, 137, 141, 143}

Immunohistochemistry identified keratinocytes as the primary cellular source of these inflammatory mediators in the fracture hindpaw skin.^{77–79} Furthermore, at 4 weeks post fracture there was increased TNF, IL-1, and NGF immunostaining in the tibia fracture callus⁴² and increased numbers of NGF, TrkA (the tropomyosin receptor kinase A receptor for NGF), Nav1.7 sodium channel, and TRPV1 (transient receptor potential cation-channel V1) immunostained neurons in the DRGs innervating the fracture hindlimb.²⁷ Systemic treatment with the global cytokine inhibitor pentoxifylline, the TNF inhibitor etanercept (sTNF-R1), the IL-1 inhibitor anakinra (IL-1ra), an IL-6 receptor antagonist (TB-2-081), or anti-NGF antibody (muMab 911) caused attenuated hindpaw allodynia and unweighting, but no change in warmth or edema in the 4 week fracture rats, indicating an important role for cytokine and growth factor signaling in post fracture nociceptive sensitization.

^{75, 77, 107, 108, 143} These cytokine and NGF inhibitors are large molecules that are normally unable to cross the blood-brain barrier and the intraplantar injection of each inflammatory mediator into normal hindpaw skin rapidly induced prolonged nociceptive sensitization.^{77–79} Collectively, these data suggest that these inflammatory mediators act at the nociceptor level to sensitize the hindpaw skin.

While the expression of TNF, IL-1, and IL-6 and NGF proteins in fracture hindpaw skin was elevated at 4 weeks, by 16 weeks post fracture it had returned to normal levels.¹⁴¹ Systemic administration of a peripherally restricted IL-1 receptor antagonist or of an anti-NGF antibody inhibited nociceptive behaviors at 4 but not at 16 weeks.¹⁴¹ However, spinal levels of TNF, IL-1, and NGF were elevated at 4 and 16 weeks, and intrathecal injection of an IL-1 receptor antagonist or anti-NGF antibody each reduced nociceptive behaviors at both 4 and 16 weeks.¹⁴¹ These results demonstrated that tibia fracture and immobilization caused increased peripheral inflammatory mediator production acutely, but that central spinal changes may be more important for the persistent nociceptive changes in this CRPS model.

Inflammasomes are multi-protein complexes responsible for the activation of caspase-1, a protease required for the activation of the pro-inflammatory cytokines IL-1, IL-18, and IL-33. The NALP1 (Nacht leucine-rich repeat and pyrin domain containing protein) inflammasome had been demonstrated to play a key role in several inflammatory skin conditions and was studied in the TFM. NALP1, caspase-1, and IL-1 were upregulated and co-expressed in keratinocytes after fracture.⁷⁹ In particular caspase-1 and IL-1 were important for the induction of nociceptive sensitization, since a selective caspase-1 inhibitor and an IL-1 inhibitor both attenuated post fracture pain behavior.⁷⁹

Sympathetic nervous system

Autonomic symptoms in CRPS are undisputed, but there are doubts concerning the causal involvement of the sympathetic nervous system (SNS). These doubts arise from the lack of convincing evidence from meta-analyses that blocking the SNS is effective CRPS treatment.⁹⁶ In acute CRPS, case reports even describe inhibited sympathetic activity. If an inhibition of the SNS would be present, adrenoceptors on blood vessels or nerve endings could become sensitive, which might contribute to cold extremities or sympathetically maintained pain.⁴⁰ However, changes in skin color, temperature or sweating presumably attributed to SNS hypo- or hyperactivity can also be explained by the inflammatory peptide processes

previously described, or an interaction of inflammation and the SNS. From a pathophysiological point of view, the role of the SNS in chronic CRPS is more intriguing because of evidence of central disturbances. For an extensive review of autonomic disturbances in CRPS see Schlereth et al, 2014.¹¹⁴

Using either 6-hydroxydopamine (6-OHDA) or guanethidine treatments to deplete catecholamines and block sympathetic signaling, caused a reduction in allodynia, hindlimb unweighting, warmth, and edema, and selectively blocked the increased production of IL-6 in skin at 4 weeks after fracture.⁷⁵ In addition, treatment with an IL-6 receptor antagonist reduced post fracture nociceptive sensitization.⁷⁵ In vitro experiments using a keratinocyte cell line demonstrated relatively high levels of β 2-adrenergic receptor (β 2-AR) protein expression, relative to all other ARs, and stimulation of this receptor greatly upregulated IL-6 expression in the keratinocytes, when compared to the expression of IL-1, TNF or NGF.⁷⁵ The systemic administration of a β 2-AR antagonist at 4 weeks post fracture reduced mechanical allodynia and hindlimb unweighting but had no effect on the visible inflammatory signs.⁷⁵ These data suggest that in the TFM, catecholamines released from sympathetic nerve terminals stimulate β 2-ARs expressed on epidermal keratinocytes resulting in local IL-6 production, and, ultimately, pain sensitization.

Systemic treatment with an α 1-adrenoreceptor (α 1-AR) antagonist reduced allodynia and unweighting at 4 weeks post fracture, but intraplantar injection of an α 1-AR antagonist had no effect.³⁴ At 4 and 16 weeks post fracture α 1-AR expression was increased in the bilateral sciatic nerves and at 16 weeks in the bilateral hindpaw plantar skin keratinocytes.³⁴ These results suggest that peripheral increases in α 1-ARs do not contribute directly to pain in the TFM, but that more central changes in α 1-AR signaling may contribute to nociceptive sensitization.

Autoimmunity

In early (average duration 14.5 weeks) CRPS sera, surface binding IgG serum-autoantibodies capable of functionally activating β 2-AR or M2 acetylcholine receptors were identified. These antibodies belonged to IgG1-3 subclasses and bound to the second extracellular loops of these receptors.⁷⁰ More recently, a different lab found agonistic serum IgG antibodies against α 1a-AR in a different cohort of patients with longstanding CRPS, using a similar cardiomyocyte beating assay.³⁶ A clinical CRPS phenotype, which might correlate to the presence of these autoantibodies, has not been described. However, when mice who had undergone a hindpaw skin and muscle incision were injected with a large amount of CRPS patient IgG (50 mg over a 7 day period), they developed significantly more mechanical allodynia and swelling in the injured hindpaw at 7 days post incision.¹²⁶ In addition, plasma exchange therapy was reported effective in reducing pain in 90% of chronic CRPS patients.⁶ Together, these results demonstrate that autoantibodies are present in some CRPS patients, but further research is required to confirm a pronociceptive role for autoantibodies in CRPS.

The effects of B cell depletion was investigated in the TFM, using anti-CD20 antibodies (rituximab) or B cell deficient muMT mice.⁷⁶ Three week wildtype (WT) fracture mice treated with intravenous anti-CD20 antibody (rituximab) had virtually no CD220⁺ mature B

cells and exhibited attenuated hindpaw allodynia, unweighting, warmth, and edema with a reduction in complement system membrane attack complex deposition in hindpaw skin and sciatic nerve, but there was no inhibition of post fracture increases in TNF, IL-1, IL-6, or NGF levels in the skin. The muMT mice lacking B cells and IgM had attenuated nociceptive and inflammatory changes at 3 weeks post fracture.

The temporal progression of post fracture CRPS-like changes were also evaluated in WT and muMT fracture mice.⁵¹ Post fracture pain behaviors transitioned from being initially dependent on both innate and autoimmune inflammatory mechanisms at 3 weeks post fracture to being entirely mediated by antibody responses at 12 weeks post fracture and resolving by 21 weeks post fracture. Furthermore, serum and IgM antibodies from 3 week fracture WT mice had pronociceptive effects in the fracture limb when injected into muMT fracture mice, but serum from 21 week fracture mice had no effect.⁵¹ IgM-containing immune complexes gradually increased in the fracture limb hindpaw skin, sciatic nerve, and corresponding lumbar cord, peaking at 12–18 weeks post fracture and then declining back to baseline levels by 23 weeks post fracture. Immunohistochemistry localized post fracture IgM antibody binding to antigens in the fracture hindpaw dermal cell nuclei.

Fracture WT mouse sera (but not control sera) bound to keratin 16, elongation factor1-alpha 1, peripherin, annexin A2, and beta-enolase in fracture WT mouse skin homogenate, when measured by 2-d gels and liquid chromatography-mass spectroscopy, and all these potential autoantigens were upregulated or redistributed to the cell membrane after fracture.¹²² Furthermore, fracture WT mouse and CRPS patient serum exhibited enhanced binding affinity to recombinant keratin 16 protein.¹²² Collectively, these results support the hypothesis that fracture can induce the regional expression of novel antigens and/or the translocation of nuclear and cytoplasmic antigens in the fracture limb hindpaw skin and sciatic nerve, subsequently triggering B cells to secrete autoantibodies capable of binding to those antigens and initiating a regionally restricted antibody-antigen-complement response resulting in complement sensitization of nociceptive neurons.⁶² Autoimmunity plays a crucial role in the progression of nociceptive changes in the TFM and potentially contributes to the CRPS disease process.

Neuroimmune signaling and inflammation in the CNS

Spinal Cord

One CRPS autopsy case has been reported in which the degree of astrocytic and microglial changes and neuronal loss in the spinal cord were evaluated.³⁰ The patient suffered from CRPS in the left leg for seven years followed by CRPS in the right arm. Treatment was multimodal over the years, including spinal cord stimulation. CRPS induced both glial activation and neuronal loss in the whole dorsal horn, mainly at the affected segments but extending to the entire spinal cord. Two studies have reported elevated cerebrospinal fluid levels of IL-1 and IL-6 in chronic CRPS patients,^{3, 5} but other investigators have been unable to confirm these findings.⁹⁵ Elevated cerebrospinal fluid levels of cytokines and neurotrophic factors have also been observed in other chronic pain states.²⁰

CRPS patients frequently exhibit widespread regional pain and allodynia extending beyond the initial injury site. Furthermore, the intensity of pain in the first week after wrist fracture is the best predictor of the development of CRPS in the ensuing 4 months.⁹³ Intense and sustained peripheral sensory nociceptive afferent activation can induce increased excitability in the spinal cord neurons, leading to the spread of pain sensitivity beyond the site of injury (secondary hyperalgesia) and allodynia. These changes are commonly referred to as central sensitization.^{148, 149} It is widely believed that spinal microglia and astrocytes are key contributors to the development of central nociceptive sensitization in a variety of rodent pain models, including neuropathic, inflammatory, and bone cancer.^{63, 90, 104, 138} These spinal immunocytes, when activated after injury, release proinflammatory cytokines and growth factors capable of enhancing spinal neuronal excitability. Reciprocal interactions between neurons and glia are postulated to play a crucial role in the perpetuation of injury-induced pain.

Immunohistochemistry and PCR were used to evaluate spinal cord microglia and astrocyte activation in the TFM. Lumbar cord microglia and astrocytes were activated at 4 weeks post fracture and the intrathecal injection of a microglia inhibitor (minocycline) or an astrocyte toxin (L-2-aminoadipic acid, LAA) reversed the activation of microglia and astrocytes, respectively, and inhibited hindpaw allodynia and unweighting.⁸¹ Astrocytes were chronically activated at 10 weeks post fracture in mice.¹²³ Systemic treatment with an SP NK1 receptor antagonist also reversed the post fracture activation of microglia and astrocytes and nociceptive sensitization.⁸¹ Similar experiments were performed in intact rats after brief sciatic nerve electric stimulation at C-fiber intensity.⁸¹ At 48 hours after sciatic nerve C-fiber stimulation there was lumbar cord microglial activation, increased SP, CGRP, NK1, CRLR, RAMP1, TNF, IL-1, IL-6, and NGF mRNA expression, and hind paw nociceptive sensitization. These C-fiber stimulation effects were inhibited by pretreatment with an SP NK1 receptor antagonist, a microglia inhibitor, or an astrocyte toxin.⁸¹

At 4 weeks post fracture in rats there was an increase in lumbar spinal cord SP, CGRP, and NK1 mRNA and protein levels, and TNF, IL-1, IL-6, CCL2, and NGF mRNA and protein levels were also elevated.¹¹⁵ Fracture mice exhibited similar increases in spinal inflammatory mediatory mRNA expression and upregulated spinal expression of these neuropeptides and their cognate receptors. Fracture-induced increases in spinal inflammatory mediators were attenuated in transgenic fracture mice lacking SP or the CGRP RAMP1 receptor and these mice had reduced post fracture nociceptive sensitization.¹¹⁵ Intrathecal injection of selective receptor antagonists or inhibitors for SP, CGRP, TNF, IL-1, IL-6, CCL2, or NGF each reduced pain behaviors in the 4 weeks post fracture rats.¹¹⁵ These convergent results are consistent with the hypothesis that exaggerated C-fiber afferent SP and CGRP signaling supports spinal neuroglia activation after limb fracture and that glial activation and inflammatory mediator expression contributes to the maintenance of central nociceptive sensitization in TMS.

Systemic administration of a peripherally restricted IL-1 receptor antagonist (anakinra) or an anti-NGF antibody inhibited nociceptive behaviors at 4 but not 16 weeks.¹⁴¹ However, spinal cord levels of NK1 receptor, TNF, IL-1, and NGF proteins were elevated at 4 and 16 weeks, and intrathecal injection of an NK1 receptor antagonist, an IL-1 inhibitor, or anti-

NGF antibody, each reduced nociceptive behaviors at both 4 and 16 weeks.¹⁴¹ These results demonstrate that fracture induced peripheral changes in neuropeptide signaling and inflammatory mediator production have pronociceptive effects in the first 4 weeks after fracture, but that central spinal changes may be more important for persistent nociceptive changes in the TFM. Similarly, over time keratinocyte and mast cell proliferation and inflammatory mediator expression in skin and experimental skin blister fluid return to normal levels in most CRPS patients,^{17, 74, 145} but in many patients the pain and nociceptive sensitization persist, perhaps due to ongoing glial activation and the residual over-expression of spinal inflammatory mediators.^{3, 5, 30}

The receptive fields sizes of lumbar dorsal horn wide dynamic range (WDR) neurons were dramatically enlarged at 4 weeks after fracture and recovered to normal size at 12 weeks post fracture, corresponding to the same time frame as the post fracture resolution of hindpaw mechanical allodynia and unweighting in the TFM.⁵⁷ Interestingly, WDR neurons on both the ipsi- and the contralateral sides of the lumbar cord developed enlarged receptive fields at 4 weeks after fracture, albeit to a lesser extent in the nonfracture limb, and mechanical allodynia was also observed in the contralateral limb, but again to a lesser extent than in the fracture limb^{57, 123}. Although speculative, similar bilateral changes in spinal signaling could contribute to the bilateral sensitization observed in chronic CRPS.¹²⁸

Brain

Decreased memory, global cognitive impairments, neglect,⁴³ tremors, dystonias, and increased anxiety and stress are symptoms that have been described in CRPS patients that may be attributable to changes of cortical function.^{82, 103, 120, 136} An extensive review of the central changes investigated by brain imaging in CRPS would go beyond the scope of this review. Resting state fMRI revealed changes in connectivity between different brain areas,^{8, 23} in particular between the medial prefrontal cortex (mPFC) and the insular cortex.⁸ This finding was supported by gray matter atrophy in the right insula, right ventromedial prefrontal cortex (vmPFC) and right nucleus accumbens in long standing CRPS.⁴⁵ The cortical representation of the CRPS affected hand in the contralateral primary somatosensory cortex was reduced after non-painful tactile stimulation of the hand,^{64, 86, 102} and these changes correlated with pain severity. Painful electrical stimulation of the symptomatic hand activated the posterior cingulate cortex⁴¹ and brushing an allodynic hand led to increased activation of the so-called “pain (salience) matrix”, in particular the cingulate cortex.⁸⁵

In contrast to CRPS, little is known about cortical changes in the TFM. Behaviorally, fracture mice exhibit anxiety-related behavior and impairment in novel object recognition and social memory. Linking these behavioral alterations to structural changes in relevant brain regions, increased complexity of dendrites in the contralateral amygdala and contralateral perirhinal cortex was observed, indicative of cytoarchitectural remodeling after tibia fracture. A biochemical analysis of synaptophysin, a proxy for synaptic abundance, revealed decreased levels of hippocampal synaptophysin in the absence of significant changes in areas such as the amygdala and perirhinal cortex.¹²⁴ Finally, there was a significant decrease of BDNF protein in the perirhinal cortex and hippocampus,¹²⁴ These data are in agreement with other social stress rodent studies where depression and anxiety or

stress are associated with signs of memory impairment¹⁰⁰ and decreased levels of BDNF in the hippocampus.^{22, 37} It is noteworthy that, despite the previous studies linking increased amygdala BDNF levels to stress and trauma,¹² there was no change of BDNF in the amygdala in the TFM.¹²⁴

Treatment efficacy in the TFM

There are no published reports examining the analgesic efficacy of SP (aprepitant) or CGRP (erenumab) receptor antagonists in CRPS. Aprepitant is used clinically as an antiemetic, but clinical trials with various SP receptor antagonists in chronic pain conditions (low back pain and arthritis) were unsuccessful,⁵⁶ although one study did demonstrate modest analgesic efficacy after third molar extraction.³² Various CGRP receptor antagonists have successfully reduced migraine headache frequency in clinical trials.⁴⁶ Anecdotal reports suggest that anti-TNF antibody injections might alleviate pain in CRPS,^{14, 33, 38, 60} but a phase II randomized clinical trial examining the efficacy of the anti-TNF drug lenalidomide in chronic CRPS was negative.⁸⁹

As reviewed in section 3.2.1, there is extensive evidence from the TFM that within the first 4 weeks after fracture SP and CGRP signaling was upregulated in hindpaw skin, sciatic nerve, and spinal cord and that these neuropeptides further stimulated expression of SP, NK1, CGRP, CRLR, RAMP1, TNF, IL-1, IL-6, CCL2, and NGF proteins in hindpaw skin keratinocytes, sciatic nerve, and spinal cord. Systemic and intrathecal injection of an SP NK1 receptor antagonist (LY303870) reduced pain behaviors and systemic NK1 receptor antagonist treatment reversed the post fracture upregulation of inflammatory mediators in skin and spinal cord. Systemic treatment with the global cytokine inhibitor pentoxifylline, the TNF inhibitor etanercept (sTNF-R1), the IL-1 inhibitor anakinra (IL-1ra), an IL-6 receptor antagonist (TB-2-081), or anti-NGF antibody (muMab 911, mouse antigen version of tanezumab) caused attenuated hindpaw allodynia and unweighting, but no change in warmth or edema in the 4 week fracture rats, indicating an important role for cytokine and growth factor signaling in post fracture nociceptive sensitization.^{75, 77, 107, 108, 143} While the expression of TNF, IL-1, and IL-6 and NGF proteins in fracture hindpaw skin was elevated at 4 weeks, by 16 weeks post fracture it had returned to normal levels.¹⁴¹ Systemic administration of a peripherally restricted IL-1 receptor antagonist or of an anti-NGF antibody inhibited nociceptive behaviors at 4 weeks, but not at 16 weeks.¹⁴¹ However, spinal levels of TNF, IL-1, and NGF were elevated at 4 and 16 weeks, and intrathecal injection of an IL-1 receptor antagonist or anti-NGF antibody each reduced nociceptive behaviors at both 4 and 16 weeks.¹⁴¹ These results demonstrate that tibia fracture caused increased peripheral inflammatory mediator production acutely, but that increases in spinal inflammatory mediators are required for persistent nociceptive sensitization in the TFM.

Promising data from five small randomized controlled trials suggest that bisphosphonates may be an effective CRPS treatment, especially early in the course of the disease.^{1, 88, 105, 134, 135} Treatment with either alendronate or zoledronate, when administered for 28 days starting at the time of fracture, inhibited the development of hindpaw allodynia and reduced hindpaw unweighting in the TFM.¹³⁷ Orally administered zoledronate treatment also reversed established allodynia and unweighting when started at 4 weeks post fracture

and continued for 21 days, but these nociceptive behaviors returned after discontinuing treatment. Histomorphometric and uCT analysis demonstrated that alendronate treatment reversed trabecular bone loss and blocked the increase in osteoclast numbers and erosion surface observed in bilateral distal femurs and in L5 vertebra of fracture rats. Alendronate treatment inhibited fracture-induced increases in hindpaw inflammatory mediators, reducing post-fracture levels of TNF, IL-1, IL-6 and NGF, but had no effect on skin and sciatic nerve IgM deposition.¹³⁷ Collectively, these results indicate that bisphosphonate therapy inhibits pain, osteoclast activation, trabecular bone loss, and cutaneous inflammation in the TFM, data supporting the hypothesis that bisphosphonate therapy can provide effective multimodal treatment for CRPS.

Ketamine, a centrally acting anesthetic agent believed to work through the blockade of N-methyl-D-aspartate (NMDA) receptors, is being used for the treatment of refractory chronic CRPS. Several day infusions are a common method of administration,^{7, 119} with anti-hyperalgesic effects lasting for 6 months or more in some patients.⁶⁶ However, neither the mechanism for the drug's analgesic effects in CRPS or its efficacy at different stages of the syndrome are clearly defined at this point. Low dose (2 mg/kg/day) ketamine infusion for 7 days had no effect in 3 weeks post fracture mice but in 7 weeks post fracture mice ketamine infusion caused a gradual resolution of mechanical allodynia over several weeks that was curative.¹²³ A single intraperitoneal injection of low dose ketamine transiently reduced allodynia within 30 min in 6 weeks post fracture mice.²⁷ Ketamine infusion at 7 weeks post fracture also reversed spinal astrocyte activation measured at 10 weeks post fracture and inhibited latent capsaicin induced nociceptive sensitization and improved motor function at 18 weeks post fracture.¹²³ Collectively, these results demonstrate that ketamine is efficacious in the more chronic, but not acute stage of TFM, data suggesting that the centrally acting drug would be relatively ineffective in early CRPS when peripheral mechanisms are more critical for supporting nociceptive sensitization.¹⁴¹

Prolonged cast immobilization of the arm resulted in the development of CRPS-like symptoms in human volunteers, including increased skin warmth and pain with joint movement and skin pinch, and these symptoms gradually resolved over several weeks after cast removal.¹²⁷ Early mobilization of the injured limb is a widely recommended treatment for CRPS.³¹ After tibia fracture with 4 weeks cast immobilization in rats there was allodynia, unweighting, warmth, edema, increased sciatic nerve SP and CGRP protein, increased skin NK1 receptors and keratinocyte proliferation, increased in inflammatory mediator protein expression in the hindpaw skin (TNF, IL-1, IL-6, NGF) and cord (IL-1, NGF), and increased spinal c-Fos activation.⁵² These same changes were observed after 4 weeks of cast immobilization alone (no fracture), except spinal IL-1 levels were not increased.⁵² Treating cast only rats with an NK1 receptor antagonist inhibited development of nociceptive and inflammatory changes, similar to the NK1 receptor antagonist effects observed in the fracture cast rats.⁵² CRPS-like symptoms such as warmth and mechanical allodynia resolved much earlier in the cast immobilized (no fracture) rats than in the fracture casted rats.^{50, 141} When tibia fracture rats were treated with intramedullary pinning instead of hindlimb casting, they began weight bearing within days and by 4 weeks post fracture all nociceptive and vascular changes had resolved and there were no increases in neuropeptide signaling or inflammatory mediator expression.⁵² Collectively, these data indicate that

immobilization alone increased neuropeptide signaling and caused nociceptive and inflammatory changes similar to those observed after tibia fracture and casting, and that early mobilization after fracture with pinning inhibited these changes. Early limb mobilization after fracture may prevent the development of CRPS.

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Highlights

- This review compared a rodent tibia fracture model (TFM) to the CRPS literature
- The TFM generated nociceptive and inflammatory symptoms resembling early CRPS
- Neuropeptide signaling and cytokine expression are up-regulated after fracture
- Fracture activated spinal glia and induced changes in brain dendritic architecture
- B cell production of IgM autoantibodies also contributed to post fracture pain

Perspective

Multiple neuroimmune signaling mechanisms contribute to the pain, inflammation, and trophic changes observed in the injured limb of the rodent tibia fracture model (TFM). This model replicates many of the symptoms, signs, and pathophysiology of early CRPS, but most post fracture changes resolve within 5 months and may not contribute to perpetuating chronic CRPS.

Summary

The TFM appears to be a valid model for CRPS, but perhaps only for the initiating acute phase. While just 7% of patients develop CRPS (“Budapest” IASP diagnostic criteria) within 3 months after a distal limb fracture,¹¹ almost all TFM animals develop CRPS-like symptoms that usually resolve within 5 months after fracture. Therefore the TFM could be regarded primarily as a model of post traumatic inflammation, which nevertheless may be a prerequisite for the development of CRPS. The TFM allows characterization of the functional contribution of neuropeptides, cytokines, trophins, catecholamines, and autoantibodies in the periphery and the spinal cord to post fracture pain behaviors, and many similar changes are also observed in early CRPS. The TFM also gives insights into morphologic and neuroplastic changes in the spinal cord after fracture, and to a lesser extent in the brain. The latter is not astonishing because human chronic pain lasting for years with all its consequences (physically, psychologically, and socially) cannot be realistically modeled in rodents. Undoubtedly, the TFM establishes the paramount contribution of the immune system in the pathophysiology of acute CRPS. It is now the task of clinicians, to re-translate step by step the TFM findings in human CRPS by performing targeted pharmacologic therapeutic interventions. The most promising approaches include inhibiting NGF (tanezumab) signaling, bisphosphonates, B cell depletion (rituximab), and plasma exchange therapy, preferably in early CRPS patients with dysregulated neuroimmune signaling. The results of such studies will support or refute the clinical significance of the TFM results and will help clarify whether “acute” and “chronic” post traumatic CRPS describes the chronology of the same disorder, or describe very different disease processes that are initiated almost immediately at the time of injury. It should also be mentioned that the TFM is a more chronic animal pain model than most inflammatory or neuropathic animal pain models and thus provides a unique opportunity to study acute-to-chronic pain transitions and neuroimmune mechanisms that may be applicable to a variety of orthopedic, post-surgical, and degenerative joint chronic pain syndromes.

Figure 1.

This cartoon illustrates the anatomic distribution of the neuropeptide signaling changes observed in the first several weeks after fracture in the tibia fracture model (TFM). CALCA: the CGRP calcitonin related polypeptide alpha gene, CALCB: the CGRP calcitonin related polypeptide beta gene, CGRP: calcitonin gene-related peptide, SP: substance P, TAC1: the SP tachykinin precursor 1 gene, NK1: the SP neurokinin 1 receptor, TACR1: the SP tachykinin 1 receptor gene.

Figure 2.

Overview of innate and adaptive immune changes in skin and spinal cord after fracture in the TFM. The neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) are released from sensory neurons and activate their cognate receptors (NK1, CRLR, and RAMP1) on cutaneous venules, causing plasma extravasation and vasodilatation. SP also stimulates NK1 receptors on dermal mast cells, causing proliferation and degranulation. Furthermore SP and CGRP activate NK1, CRLR, and RAMP1 receptors on keratinocytes and microglia, causing these innate immune cells to proliferate and express/secret inflammatory mediators (TNF, IL-1, NGF) that sensitize sensory afferent neurons. Likewise, norepinephrine (NE) is released from sympathetic neurons and stimulates β_2 adrenoceptors (β_2 -AR) on keratinocytes to induce IL-6 expression/secretion that further sensitizes sensory afferents. Antigen presenting cells (APCs, including Langerhans cells, macrophages, and microglia) are also activated by SP and CGRP released from sensory neurons. Apoptotic cells release autoantigens that are phagocytized by activated APCs and then attached to membrane bound MHC II molecules. Activated APCs migrate to lymph nodes, where they present autoantigens to T cell receptors (TCRs) and polarize T cells to the Th1/2-type immune responses associated with autoimmunity. The Th1/2 polarized T cells present autoantigens to B cells that then express IgM immunoglobulin. Norepinephrine (NE)

released by sympathetic neurons activates beta 2 adrenoceptors on B cells, further stimulating immunoglobulin expression. IgM antibodies form autoantigen-antibody complexes that bind to C1 complement, initiating the classical complement activation pathway. This results in the expression of C3a and C5a complement, thus causing the attraction and activation of keratinocytes and microglial cells that express inflammatory mediators including TNF, IL-1, IL-6, and NGF, all of which contribute to nociceptive sensitization in the fracture model of CRPS. APC: antigen presenting cell, β 2-AR: beta 2 adrenoceptor, CGRP: calcitonin gene-related peptide, CRLR: the CGRP calcitonin receptor-like receptor, IL-1: interleukin 1 β , IL-6: interleukin 6, MHC II: major histocompatibility complex class II, NGF: nerve growth factor, NE: norepinephrine, NK1: the tachykinin 1 receptor, RAMP1: the CGRP receptor activity modifying protein 1 co-receptor, SP: substance P, TAC1: the SP tachykinin precursor 1 gene, TACR1: the SP tachykinin 1 receptor gene, TCR: T cell receptor, TNF: tumor necrosis factor α .

Table 1

Temporal evolution of symptoms, signs, and pathophysiology in CRPS vs TFM

	Human CRPS	TFM
Pain, hyperalgesia, allodynia	Ongoing pain and pain with movement, partially resolving within 12 months in many patients. ^{9, 11} Prevalence of allodynia declines from 67% to 25% over first year. ⁹ Mechanical allodynia in chronic CRPS is negative predictor for treatment outcome. ^{132, 133} Prevalence of mechanical pressure hyperalgesia 70%. Prevalence of heat and cold hyperalgesia 30–53%, slightly increasing over time. ^{47, 103}	Ongoing pain as measured by condition place preference at 10 weeks. ¹²⁵ Mechanical allodynia to von Frey testing in fracture limb, usually resolving within 18 weeks. ¹⁴¹ Pain with movement as measured by hindlimb unweighting in fracture limb, usually resolving within 18 weeks. ¹⁴¹ Thermal hyperalgesia not observed. ⁵⁰
Skin, temperature, and vasomotor changes	Skin temperature asymmetry (>1°C) in 40–56% of CRPS limbs. ^{25, 47} In a prospective study the average temperature difference between limbs declined over the first year after the onset of CRPS and the prevalence of skin color changes also decreased, from 95% to 45%. ⁹	Warmth in fracture limb, usually resolving within 10 weeks. ⁵⁰ No report on skin discoloration. No report on cold limbs.
Sudomotor changes and limb edema	Prevalence of edema in the injured limb declines from 85% to 22% over first year. ⁹ Increased plasma extravasation and vasodilation (spontaneous ⁹⁹ and after electrical stimulation ¹³⁹ or substance P perfusion ⁷³ in the CRPS limb, spontaneous extravasation often resolving within 5 months. ⁹⁹ Prevalence of hypo- or hyperhidrosis in CRPS skin declines from 23% to 4% over the first year. ⁹	Edema in fracture limb, usually resolving by 10 weeks. ⁵⁰ Enhanced plasma extravasation (spontaneous, and after electrical stimulation or substance P perfusion) in fracture limb, ¹⁴² with increased spontaneous extravasation usually resolving within 16 wks. ¹⁴¹ No report on sweating (rodents lack sweat glands except over plantar skin of their feet).
Motor dysfunction	Prevalence of impaired rapid motor function declines from 82% to 44% over first year. ⁹ Decreased range of motion in 90% of patients ^{9, 136} and chronic weakness in the affected limb in most CRPS patients. ¹⁵² Tremors, dystonias and muscle spasms observed in some patients. ^{103, 136}	Impaired motor behavior on rotarod testing. ¹²⁵
Trophic changes	Keratinocyte and mast cell proliferation and epidermal thickening in early (<3 months) CRPS skin, then diminished keratinocyte proliferation and epidermal thinning in chronic CRPS. ¹⁷ Prevalence of abnormal hair and/or nail growth declines from 66% to 31% in CRPS affected limbs over the first 12 months. ⁹ Trabecular bone loss in CRPS affected limbs, extent of bone loss greater and longer lasting in tibia fracture patients with CRPS vs tibia fracture patients without CRPS. ^{15, 65, 111} Increased periarticular tracer uptake on bone scan in 91% of affected limbs in early CRPS, consistent with rapid bone remodeling. ¹⁵⁰	Keratinocyte and mast cell proliferation and epidermal thickening in fracture limb, resolving within 16 weeks. ^{78, 80, 141, 142} No reports on hair or nail growth changes. Trabecular bone loss in fracture limb progressing for 8 weeks and persisting at least 20 weeks. ^{78, 80, 141, 142} Increased trabecular bone resorption and decreased bone formation in fracture limb at 4 weeks. ¹³⁷ No reports on bone scanning.
Cognitive changes	Decreased memory, cognitive impairment, increased anxiety and stress. ^{82, 84, 120}	Decreased memory and increased anxiety behavior. ¹²⁴

Table 2

Neuropeptide signaling in CRPS vs TFM

	CRPS		TFM	
	Early <16 weeks	Chronic >16 weeks	Early 3–4 weeks	Chronic 16 weeks
Blood Plasma	Plasma protein levels side-to-side differences: ↑ SP, no change CGRP. ¹¹² Plasma protein levels vs controls: No change SP, CGRP, ¹¹³ ↑CGRP. ²¹	Plasma protein levels side-to-side differences: No change CGRP. ^{19, 58} Plasma protein levels vs controls: No change SP, ↓CGRP, ¹¹³ ↑CGRP. ¹⁹	Plasma protein levels vs controls: ↑ SP, ↑CGRP. ¹⁴²	Plasma protein levels vs controls: N/A
Skin	Extravasation and vasodilatation: ↑Spontaneous plasma extravasation. ⁹⁹	Extravasation and vasodilatation: Spontaneous plasma extravasation returns to normal. ⁹⁹ ↑Electrically evoked plasma extravasation and vasodilation response. ¹³⁹ ↑Substance P perfusion evoked plasma extravasation. ⁷³	Extravasation and vasodilatation: ↑Plasma extravasation (spontaneous, and after substance P perfusion) in fracture limb. ^{141, 142} Skin protein/mRNA levels: ↑NK1/ <i>TACR1</i> , no change CRLR/ <i>CALCRL</i> , RAMP1/ <i>RAMP1</i> , NEP/ <i>NEP</i> . ^{52, 142}	Extravasation and vasodilatation: Spontaneous plasma extravasation returns to normal within 16 weeks. ¹⁴¹ Skin protein levels: NK1 returns to normal within 16 weeks. ¹⁴¹
Peripheral nerve	Sciatic nerve protein levels: N/A	Sciatic nerve protein levels: N/A	Sciatic nerve protein levels: ↑ SP, ↑CGRP. ^{141, 142}	Sciatic nerve protein levels: SP returns to normal within 16 wks. ¹⁴¹
DRG	DRG protein/mRNA levels: N/A	DRG protein/mRNA levels: N/A	DRG protein/mRNA levels: ↑ SP/ <i>TAC1</i> , ↑CGRP/ <i>CALCA</i> . ^{27, 142}	DRG protein/mRNA levels: N/A
Spinal Cord	Cord protein/mRNA levels: N/A	Cord protein/mRNA levels: N/A	Cord protein/mRNA levels: ↑ SP/ <i>TAC1</i> , ↑CGRP/ <i>CALCA</i> and <i>CALCB</i> , ↑NK1/ <i>TACR1</i> , no change <i>CALCRL</i> or <i>RAMP1</i> mRNA. ^{115, 141}	Cord protein levels: ↑NK1, SP returns to normal within 16 weeks. ¹⁴¹

CALCA: the CGRP calcitonin related polypeptide alpha gene, CALCB: the CGRP calcitonin related polypeptide beta gene, CALCRL: the CRLR calcitonin gene-related peptide type 1 receptor precursor gene, CGRP: calcitonin gene-related peptide, CRLR: the CGRP calcitonin receptor-like receptor, N/A: not available, NEP: neutral endopeptidase, NK1: the tachykinin 1 receptor, RAMP1: the CGRP receptor activity modifying protein 1 co-receptor, SP: substance P, TAC1: the SP tachykinin precursor 1 gene, TACR1: the tachykinin 1 receptor gene

Table 3

Cytokine and NGF signaling in CRPS vs TFM

	CRPS		TFM	
	Early <3 months	Chronic >3 months	Early 3–4 weeks	Chronic 16 weeks
Blood	Serum protein levels: ↑IL-2, ¹²⁹ ↑IL-8, ¹¹² ↓IL-10, ¹²⁹ ↓TGF-β1. ¹²⁹ No change in TNF, ^{74, 129} IL-8, ¹²⁹ IL-4, ¹²⁹ IL-6, ¹¹² CCL2, CCL4. ⁷⁴ Blood cell mRNA levels: ↑TNF, ↑IL-2, ↓IL-8, ↓IL-4, ↓IL-10, and no change TGF-β1. ¹²⁹	Serum protein levels: TNF, IL-1, IL-6, IL-8, IL-10 are normal, ^{58, 71, 112, 130} but see Alexander et al. ⁴	Serum protein levels: N/A	Serum protein levels: N/A
Keratinocytes, Skin, Blister fluid	Keratinocyte protein expression: ↑TNF and ↑IL-6 in 40% of patients. ¹⁷	Keratinocyte protein expression: TNF and IL-6 are increased in 25% of patients. ¹⁷ Skin protein levels: ↑TNF. ⁷¹ Blister fluid protein levels: ↑TNF, ↑IL-6. ^{49, 58, 59, 94, 145} ↑TNF, ↑IL-6, ↑IL-8, ↑IL-12p40/p70, ↑CCL2, ↑CCL4, ↑IL-1 RA, ↓IL-10, ↓Eotaxin. ⁵⁵ NGF N/A	Keratinocyte protein expression: ↑TNF-α, ↑IL-1, ↑IL-6, ↑NGF protein. ^{53, 78, 79} Skin protein and mRNA levels: ↑TNF, ^{52, 53, 75, 137, 141, 143} ↑IL-1, ^{52, 53, 75, 137, 141, 143} ↑IL-6, ^{52, 53, 75, 137, 141, 143} ↑NGF, ^{52, 53, 75, 108, 137, 141, 143} and no change in IL-10 protein levels. ¹⁰⁸	Skin protein levels: TNF, IL-1, IL-6, and NGF are normal. ¹⁴¹
Peripheral nerve and DRG	Nerve and DRG protein levels: N/A	Nerve and DRG protein levels: N/A	Nerve and DRG protein levels: ↑TNF, ¹⁰⁷ ↑NGF, TrkA. ²⁷	Nerve and DRG protein levels: N/A
Spinal cord	CSF protein levels: N/A	CSF protein levels: ↑IL-1, and no change TNF, IL-8, IL-10, and CCL2; contradicting results for IL-6. ^{3, 5, 95}	Cord protein and mRNA levels: ↑TNF, ^{81, 141} ↑IL-1, ^{81, 141} ↑IL-6, ^{81, 141} ↑CCL2, ^{44, 81} ↑NGF. ^{81, 141}	Cord protein levels: ↑TNF-α, ↑IL-1, ↑NGF, and IL-6 is normal. ¹⁴¹
Bone	Bone protein and mRNA levels: N/A Scintigraphy: ↑ TNF binding in affected joints. ¹³	Bone protein and mRNA levels: N/A Scintigraphy: No change in TNF binding. ¹³	Bone protein and mRNA levels: ↑ NGF, ¹⁰⁸ and no change in TNF protein levels. ¹⁰⁷ ↑ TNF, IL-1, NGF on bone callus IHC. ⁴²	Bone protein and mRNA levels: N/A

CCL2: C-C motif chemokine 2 (MCP 1), CCL4: C-C motif chemokine 4 (MIP 1β), IHC: immunohistochemistry, IL-1: interleukin 1β, IL-1RA: interleukin 1 receptor antagonist, IL-2: interleukin 2, IL-4: interleukin 4, IL-6: interleukin 6, IL-8: interleukin 8, IL-10: interleukin 10, IL-12p40/p70: p40 and p70 subunits of IL-12, NGF: nerve growth factor, TGF-β1: transforming growth factor beta 1, TNF: tumor necrosis factor α