

## A new hypothesis for the pathophysiology of complex regional pain syndrome



Marc Russo<sup>a,\*</sup>, Peter Georgius<sup>b</sup>, Danielle M Santarelli<sup>a</sup>

<sup>a</sup> Hunter Pain Clinic, 91 Chatham Street, Broadmeadow, NSW 2292, Australia

<sup>b</sup> Pain Rehab, Suite 4 Noosa Central, 6 Bottlebrush Avenue, Noosa Heads, QLD 4567, Australia

### ARTICLE INFO

#### Keywords:

Complex regional pain syndrome  
CRPS  
Reflex sympathetic dystrophy  
Dendritic cell  
Immune system

### ABSTRACT

Complex Regional Pain Syndrome (CRPS) has defied a clear unified pathological explanation to date. Not surprisingly, treatments for the condition are limited in number, efficacy and their ability to enact a cure. Whilst many observations have been made of physiological abnormalities, how these explain the condition and who does and doesn't develop CRPS remains unclear. We propose a new overarching hypothesis to explain the condition that invokes four dynamically changing and interacting components of tissue trauma, pathological pain processing, autonomic dysfunction (both peripheral and central) and immune dysfunction, primarily involving excessive and pathological activation of dendritic cells following trauma or atrophy. We outline pathophysiological changes that may initiate a cascade of events involving dendritic cells and the cholinergic anti-inflammatory pathway resulting in the condition, and the changes that maintain the condition into its chronic phase. This hypothesis should provide fertile ground for further investigations and development of new treatments that holistically address the nature of the disorder along its developmental continuum.

### Introduction

The clinical presentation of Complex Regional Pain Syndrome (CRPS) was first clearly and eloquently described by Silas Weir Mitchell in 1872. Many observations have been made on CRPS yet it defies complete understanding. Its variable presentations at onset and its protean presentation over time have made explanation difficult. Progress has been made in codifying the diagnostic criteria [1], which continue to be refined [2–5], however, many of the current hypotheses for explaining the condition are more a description of associated findings for which causality has not clearly been established. No single explanation has adequately explained the condition and this has led to authors even denying its existence [6–8]. The current diagnostic criteria include regional pain that is disproportionate to the initial trauma, skin colour and temperature changes, edema, vasomotor and sudomotor changes, motor dysfunction and trophic changes.

Any model for the condition should ideally explain the following:

1. Typically, only 0.5–2% of injury/trauma patients develop CRPS [9].
2. Some patients with CRPS have no history of trauma [10].
3. CRPS can have protean presentations, with each patient displaying different symptoms and signs such as warm or cold limb, oedema, allodynia, hyperalgesia, abnormal sweating and skin and nail tissue

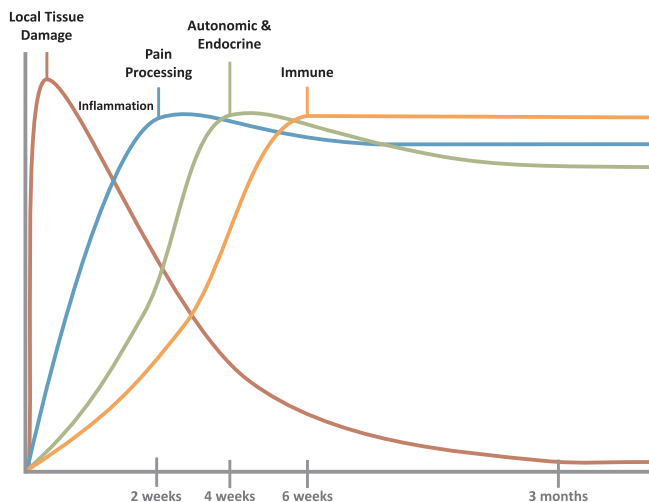
changes [11].

4. CRPS is almost universally restricted to the limb (upper or lower) and, often with chronicity, the symptoms (especially pain) progress proximally up the limb, often to the shoulder/hip but not beyond [12].
5. Whilst appearing to be a neuropathic pain condition, CRPS is mostly unresponsive to standard neuropathic pain treatments [13].
6. Florid dystonia can be a feature of CRPS in a subset of patients and is often treatment unresponsive [14].
7. The peripheral signs of CRPS mostly fade and disappear in the chronic phase of the condition, although the pain mostly remains [15].

The rat tibia fracture model of CRPS developed by Wade Kingery and colleagues has yielded tantalising insights into keratinocyte activation, spinal cord transcriptional changes, autoimmunity development and acute versus chronic changes [16–22], and we believe reframing CRPS into a dynamic multicomponent disease best explains the condition. We present a four-component model of tissue trauma, pathological pain processing, autonomic dysregulation and immune dysfunction to explain the condition and its cardinal features (Fig. 1). These abnormalities interact and reinforce each other to produce the chronic phase of the condition.

\* Corresponding author.

E-mail address: [marcr@hunterpainclinic.com.au](mailto:marcr@hunterpainclinic.com.au) (M. Russo).



**Fig. 1.** The proposed four component model of CRPS depicted as a time course of homeostatic disturbances: tissue trauma (red line), pathological pain processing (blue line), autonomic dysregulation (green line) and immune dysfunction (orange line). Note: In any individual, these four components can vary in degree in terms of homeostatic disturbance and relative time course of activation.

It is the immune component of our model that has been least clarified to date. We propose that a class of immunosurveillance cells known as dendritic cells are the primary drivers of immune dysfunction [23,24]. Dendritic cells reside predominantly in the skin and subcutaneous tissue. Following antigen-capture, dendritic cells migrate to the draining regional lymph nodes where they present antigen to T-cells and initiate an adaptive immune response [24]. Furthermore, dendritic cell activation and migration into the systemic circulation has effects on both peripheral and central neurogenic signalling [25]. These processes and further downstream effects of dendritic cell activation that may lead to the generation of neuropathic in CRPS are discussed in detail in the second half of this article.

#### Components of the model

The reader should note that what is being presented here is a hypothesis (which is constructed before any applicable research has been done) and not a theory (which is a construct that explains the observable data generated in a series of investigations or experiments). Furthermore, we are not suggesting that each and every factor that is discussed within this article has the ability to activate, exacerbate or maintain the disease in each and every CRPS patient, rather, that these are examples, whether validated by prior investigations in subsets of CRPS patients, or supported by animal models, that would each provide some level of support for one (or more) of the four components in our hypothesis and model. In any individual, these four components can vary in degree in terms of homeostatic disturbance and relative time course of activation.

#### Component 1: tissue trauma

Most cases of CRPS can be linked to some form of trauma. Tissue trauma may include bone fractures, soft tissue injuries, chemical or thermal burns, and may be complicated by factors such as limb immobilisation or disuse [26], tissue hypoxia [27], neurogenic inflammation [28,29], stress [30], and so forth. Tissue trauma brings about a well understood course of inflammation, peripheral nociceptor activation and upregulated neural processing. This is later followed by an anti-inflammatory response that dampens the aforementioned changes and restores the tissue and organism to homeostasis. Under certain circumstances, however, protective mechanisms can become

overwhelmed by various compounding, initiating factors, leading to deleterious and profound downstream effects.

Two major activators of immune cells, particularly dendritic cells, involved in the genesis of neuropathic pain include damage associated molecular products (DAMPs), which are endogenous products released after trauma, surgery or sepsis that drive an inflammatory response, such as heme, extracellular hemoglobin, interleukins (i.e. IL-1 $\alpha$ , IL-33), heat shock proteins, and high mobility group box protein HMGB1 [31–33], and pathogen associated molecular products (PAMPs), which are exogenous molecules released from infiltrating microorganisms [34–41].

Tissue hypoxia has been identified as a dendritic cell activator [42,43] and has direct relevance to CRPS, with multiple studies indicating impaired tissue oxygenation in the affected limb of CRPS patients [27,44–46]. In addition, sympathetic nervous system hyperactivity and whole-body stress response producing high circulating catecholamine levels have also been implicated in the activation or modulation of dendritic cells [47–50].

The component of tissue trauma directly contributing to CRPS symptoms and signs will wane over time but may be complicated by direct injury to peripheral nerves (in the case of CRPS-II), which will prolong neuropathic pain presentation.

#### Component 2: pathological pain processing

Pain pathogenesis has been well described by Gold and Gebhart [51]. Pathological pain processing is associated with symptoms/signs of allodynia and hyperalgesia and can consist of excessive activation of the peripheral nerve(s), dorsal root ganglion (DRG), dorsal horn of the spinal cord, thalamus, cortex and basal ganglia. Typically, such activity starts peripherally, with activation of more proximal structures occurring over time, however that time course is variable and central nervous system (CNS) changes can occur quickly [52]. Due to the interactions between the autonomic and immune systems with the pain processing system [25,53,54], disturbances in their function can perpetuate abnormal pain processing and produce a state of chronic neuropathic pain [55,56].

Peripheral nerve involvement (i.e. nerve injury, degeneration) can be profound in CRPS [57,58]. An important consequence of nerve involvement is upregulation of microglia in the spinal cord, leading to a state of gliosis, which contributes to central sensitisation and neuropathic pain [59,60]. The first documentation of gliosis in CRPS was in a case report of autopsy findings in the spinal cord of a chronic case of CRPS (Fig. 2) [61].

There is also evidence of neuroinflammation and altered resting functional connectivity of the brain in CRPS [62]. For example, basal ganglia, whilst best known for their role in coordinating motor function, are involved in nociception and CRPS [14,63,64]. An imaging study of the brain of CRPS patients has shown neuroinflammation of basal ganglia, which was attributed to microglial activation, and found a significant correlation between the inflammatory score of the caudate nucleus and pain score as well as the affective dimensions of pain [65]. Basal ganglia outputs to the subthalamic nuclei, thalamus and the spinal cord have also been implicated in CRPS [14]. The dystonia and proprioceptive deficits that are sometimes seen in CRPS have been best explained by dysfunction in the basal ganglia and the basal-thalamic-frontal cortex circuit [66–69]. Additionally, we hypothesise that muscle disuse behaviour, casting or unloading produces both distal peripheral axon degeneration and altered motor cortical maps, with subsequent alteration in basal ganglia output to motor efferents to the spinal cord, and to efferents to the subthalamic nuclei, with subsequent reductions in motor function and “neglect-like” limb behaviour. For a comprehensive review on this subject, we refer the reader to a recent publication by Azqueta-Gavaldon et al. [14].

One common mechanism underlying this aberrant neural activation is the dendritic cell. Activated dendritic cells interact with glial cells on

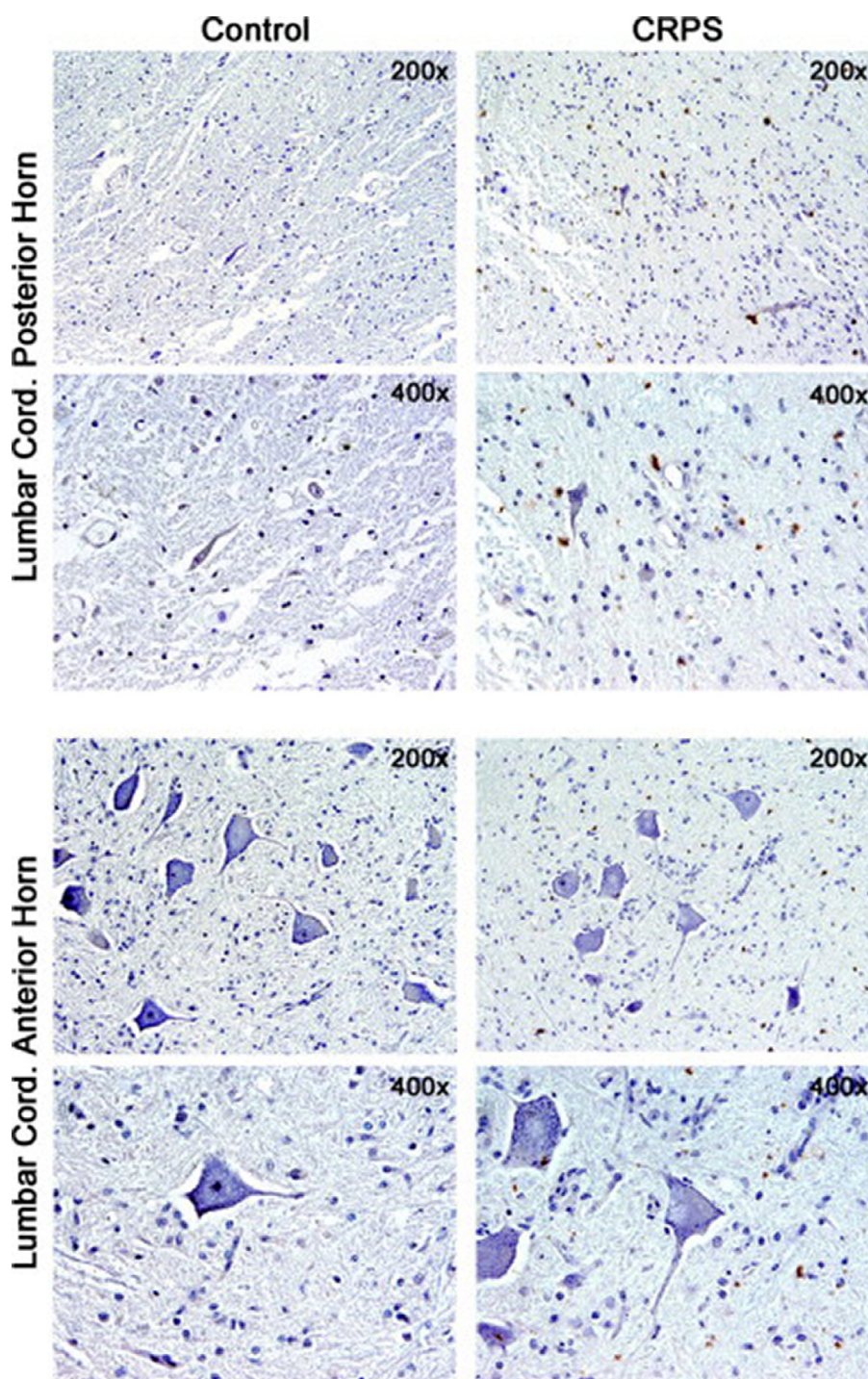


Fig. 2. Microglial cell activation in CRPS. Immunohistochemistry for CD-68. Reproduced from Del Valle et al. [61] with permission from the publisher.

the local peripheral nerve, the DRG, the spinal cord and the cerebral cortex to produce glial cell activation and thus pathoanatomical changes in neural function leading to hyperalgesia and allodynia [70–72]. Dendritic cell interaction with the spinal DRG produces central sensitisation and regionally limited neuropathic pain [56,73,74]. Activated dendritic cells migrate systemically and intrathecally through pathological access to the blood brain barrier at DRG level to affect cortical function and, in particular, basal ganglia function [74–78]. These downstream effects are further discussed later.

### Component 3: autonomic dysregulation

The autonomic nervous system is one of the primary homeostatic mechanisms of the body, maintaining organism integrity through a dynamic balancing of sympathetic and parasympathetic activity. Its involvement in the pathophysiology of CRPS has been well researched [58,79,80]. The clinical signs of sympathetic dysfunction in CRPS include distal limb sweating, altered temperature (hot/cold) of the limb, and altered skin color and oedema. Over time these signs typically fade as the disease moves into a more advanced phase dominated by glial and cortical changes [52,81].



The vagus nerve is one of the major controllers of autonomic balance and is known to mediate anti-inflammatory pathways via acetylcholine – the “cholinergic anti-inflammatory pathway” [82–85]. “The inflammatory reflex” proposed by Tracey and colleagues describes inhibition of pro-inflammatory cytokine release (i.e. TNF $\alpha$ , IL-1, IL-18) from tissue macrophages and monocyte-derived dendritic cells via acetylcholine binding to the  $\alpha 7$  subunit of the nicotinic acetylcholine receptor ( $\alpha 7nAChR$ ) [82,86,87]. The anti-inflammatory action of the vagus nerve extends to the spleen via acetylcholine activation of splenic neurons, which release norepinephrine and stimulate acetylcholine-producing T-cells, which inhibits splenic pro-inflammatory cytokine release [88,89]. In our model, we focus on the implications of the inflammatory reflex in the lymph node as this results in a regional lymph distribution unique to the CRPS limb, whereas spleen, liver and thymus involvement may have a lesser role in such a regionally restricted condition as CRPS. Support for lymph node involvement comes from work by Wülfing and Günther [90], who showed that lymph nodes are intensely innervated by the autonomic nervous system of which forms a neural meshwork that encapsulates dendritic cells and macrophages and modulates their function (“neurally hard-wired”).

Other research has shown that acetylcholine activity determines the balance of self-tissue recognition versus antigen detection response of dendritic and other immune cells [91,92]. Salamone and colleagues [91] showed that human dendritic cells express multiple acetylcholine receptors and acetylcholine synthesizing enzymes, and that acetylcholine modulation of dendritic cells depends on their maturation state and the receptor involved; In immature dendritic cells, antigen processing and pro-inflammatory cytokine release is stimulated by acetylcholine, whereas the opposite occurs in mature dendritic cells. Similarly, the sympathetic nervous system via norepinephrine release modulates dendritic cell activity depending on the receptor involved –  $\alpha 1$ -adrenoreceptors ( $\alpha 1$ -ARs) are stimulator while  $\beta$ - adrenoreceptors ( $\beta$ -ARs) are inhibitory in terms of dendritic cell migration and inflammatory cytokine release [48,49]. Excess sympathetic activity and/or loss of vagal efferent activity will alter this balance and lead to activation of dendritic cells. This has deleterious effects on immune function and pain processing.

An excess of sympathetic activity relative to parasympathetic activity in CRPS has been observed using heart rate variability analysis [93–96]. This imbalance is likely to be both peripheral and central in origin and can involve immune dysfunction. This may cause autoantibody production to autonomic neurons and lead to autonomic disturbances, as has been observed in subsets of CRPS patients [97]. Thus, no system acts in isolation from the other. The relative sympathetic overactivity likely explains the largely positive response traditionally associated with use of sympathetic nerve blocks as a treatment for CRPS of less than 12 months duration [98–100]. The associated interplay of tissue damage, abnormal pain processing, spinal cord/central sensitisation and immune dysfunction may explain the significant negative response rate of this therapy and its inability to alter symptoms in established chronic cases of CRPS [58].

We hypothesise that autonomic imbalance and disturbance to the cholinergic anti-inflammatory pathway is one of the four major pathophysiological elements contributing to CRPS. Support for this has been reviewed by Walker and Drummond [101], with more recent support coming from animal models [102]. Note should be made of the high rates of smoking in CRPS [103,104], which may be an attempt on the part of the patient to initially activate this pathway, albeit at the expense of subsequently altering cortical functional connectivity in the direction of nociceptive transmission facilitation [105–107].

#### Component 4: immune dysfunction

We hypothesise that the primary immune activator in CRPS is the dendritic cell (Fig. 3). The direct evidence for this was first shown in 1998 in a histopathology study in which abnormal levels of dendritic

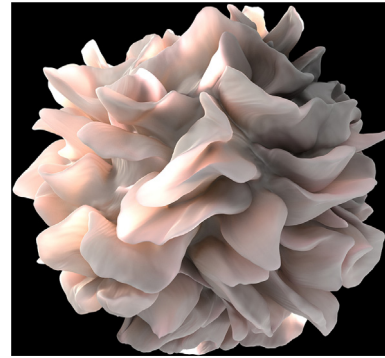


Fig. 3. High-resolution 3D rendition of a dendritic cell (immature) by Bliss and Subramaniam (related reference: [182]). Image from National Institutes of Health (NIH) [Public domain], via Wikimedia Commons.

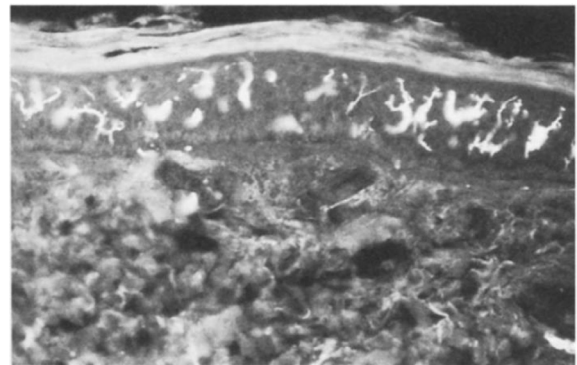


Fig. 4. Immunostaining of skin biopsy from a patient with CRPS showing an abundance of Langerhans cells in the epidermis. Figure reproduced from Calder et al. [108] with permission from the publisher.

cells in the epidermis (Langerhans cells) were observed in the skin biopsies of CRPS patients with severe pain, but not in non-CRPS nerve injury/repair patients (Fig. 4) [108]. The significance of this was not appreciated at the time.

In essence, our hypothesis predicates activation and maturation of dendritic cells by specific initiator mechanisms such as those aforementioned (Fig. 5). The next section provides a comprehensive overview of dendritic cell activation and migration and discusses how their overactivity may contribute to the generation of neuropathic pain in CRPS.

#### Dendritic cells in detail

Dendritic cells are a class of antigen presenting cells tasked with performing immunosurveillance and coordination of the host response [109]. They are the most effective antigen presenting cells compared to macrophages and B-cells [92]. Dendritic cell precursors originate from hematopoietic stem cells in bone marrow via an intermediate progenitor (myeloid or lymphoid); these include common dendritic cell precursors, plasmacytoid dendritic cell precursors and monocytes [110]. Dendritic cell precursors, monocyte-derived dendritic cells and plasmacytoid dendritic cells circulate in the bloodstream and migrate to peripheral tissue under inflammatory conditions [24]. Conventional dendritic cells typically populate non-lymphoid tissue while plasmacytoid dendritic cells predominantly reside in lymphoid tissue and lymph nodes [111].

Dermal dendritic cells may arise from common dendritic cell precursors or circulating monocytes [110]. Capture of an antigen and/or activation by noxious signals (i.e. during trauma or other inflammatory conditions), such as DAMPS, PAMPS, interleukins (i.e. IL-1 $\beta$ , IL-6, IL-18), TNF $\alpha$  and interferons (i.e. IFN $\alpha$  and IFN $\gamma$ ), promotes



markers and neuropeptide receptors [25,53,118–120]. Another key finding was that IL-6 (and other neurotrophins) produced by Langerhans cells are able to stimulate nerve differentiation [121].

#### *Dendritic cells and neuropathic pain*

An in depth review of the involvement of the immune system in the generation of neuropathic pain has been presented by Calvo, Dawes and Bennet [56]. We discuss how dendritic cell activation and recruitment may lead to neuropathic pain.

Various myeloid cells, including monocytes, dendritic cells and neutrophils in the periphery, and microglia in the CNS express “triggering receptors expressed by myeloid cells” 1 and 2 (TREM-1 and TREM-2) and a transmembrane adaptor protein “DNAX-activating protein 12 kDa” (DAP12) [122–124]. TREM-1 is a known amplifier of inflammation; activation and signalling via DAP12 leads to the production of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-2, IL-6, IL-8, TNF $\alpha$ , monocyte chemoattractant protein 1 (MCP1) / chemokine (C-C motif) ligand 2 (CCL2), and decreased expression of anti-inflammatory cytokines such as IL-10 [123]. It is of interest that periodontitis (which is associated with an increased oral load of both commensal and pathogenic bacteria) is a potent activator of the dendritic cell TREM-1 pathway, leading to a more florid inflammatory cytokine release [125]. TREM-2 has more of a myeloid cell regulatory function. For example, TREM-2 signalling via DAP12 in dendritic cells leads to upregulation of CC chemokine receptor 7 (CCR7), partial dendritic cell maturation and dendritic cell survival, leading to systemic dendritic cell activation [124,126]. TREM-2/DAP12 signalling has also been implicated in inflammatory microglial activation and neuroinflammation [127,128]. Importantly, it has been shown that TREM-2/DAP12 signalling in microglia in the dorsal horn after spinal nerve injury leads to inflammatory cytokine release and the generation and exacerbation of neuropathic pain [127,129].

Recent studies have highlighted the balance between TREM-1, TREM-2 and DAP12 expression in the CNS as an important factor in the development of neuroinflammation and neuropathic pain [128]. In essence, TREM-DAP12 activation and signalling can be seen as producing a widespread inflammatory response, priming the milieu for neuropathic pain activation [130]. We hypothesise that this is part of the mechanism whereby dendritic cell and microglia activation can cause neuropathic pain in CRPS cases.

#### *Dendritic cell migration*

Maturation and migration of dendritic cells are tightly linked processes (Fig. 6). Dendritic cell migration appears to be dependent on maturation, which is predominantly initiated by antigen capture, DAMPs, stress (circulating catecholamines) or inflammatory signals/cytokines [112]. TNF- $\alpha$  and IL-1 $\beta$  are said to be important initiators of migration in Langerhans cells, specifically by modulation of cell adhesion protein expression, namely E-cadherin, thus mobilising and priming the cells for migration [131–134].

Matrix metalloproteinases (MMPs) are crucial drivers of skin dendritic cell migration to lymph nodes as they “clear a path” by remodelling the extracellular matrix [135]. A detailed series of experiments in vitro (cultured mouse and human skin explants) and in vivo (mouse ear) have been conducted by Ratzinger et al. to characterise MMP driven dendritic cell migration. In cultured tissue, treatment with a non-specific MMP inhibitor significantly reduced dermal dendritic cell and epidermal Langerhans cell migration but did not prevent their maturation [136]. A reduction in the number of migrating dendritic cells was observed in the lymphatic vessels. When cultured without the inhibitor, spontaneous dermal and epidermal dendritic cell migration was observed. In vivo experimentation showed that injection of inflammatory cytokines (i.e. TNF $\alpha$ ) significantly boosted Langerhans cell migration and activation, while co-injection with an MMP inhibitor

completely blocked migration, though cells were still activated. MMP-9 and MMP-2 have been identified as mediators of Langerhans cell passage through the basement membrane, and are partially involved in mediating dendritic cell migration through the dermis [136–139].

Tissue inhibitors of metalloproteinases (TIMPs), especially TIMP-1 and TIMP-2, are endogenous regulators of MMP function [140]. Imbalance in the MMP/TIMP ratio may lead to increased dendritic cell migration, excessive tissue remodelling/degradation and upregulation of the immune response, and has been associated with numerous chronic inflammatory diseases [135]. For example, *P. gingivalis*, a pathogen associated with chronic periodontitis, has been found to stimulate dendritic cells and induce a high MMP-9/TIMP-1 ratio in vitro [141].

#### *Lymph node response*

It has been well documented in the rat tibial bone fracture model of CRPS that after trauma there is an increase in the weight and total cell number of the regional draining lymph nodes, including a high concentration of migrating dendritic cells [142]. More recent work has shown that the glucocorticoid-induced TNF receptor-related protein (GITR) and its ligand (GITRL) promote epidermal inflammatory cytokine production by keratinocytes and epidermal dendritic cells, which results in migration of cutaneous dendritic cells to the draining lymph nodes [143]. In a partial sciatic nerve ligation model, this resulted in infiltrating macrophages and T-cells, with an increase in GITR on T-cells and an increase in GITRL on macrophages [144]. Blocking the GITR-GITRL pathway with a neutralising antibody blocked the development of neuropathic pain. Taken together, these findings indicate that after trauma or nerve injury there is activation and migration of dendritic cells to the regional lymph nodes which are involved in the production of neuropathic pain.

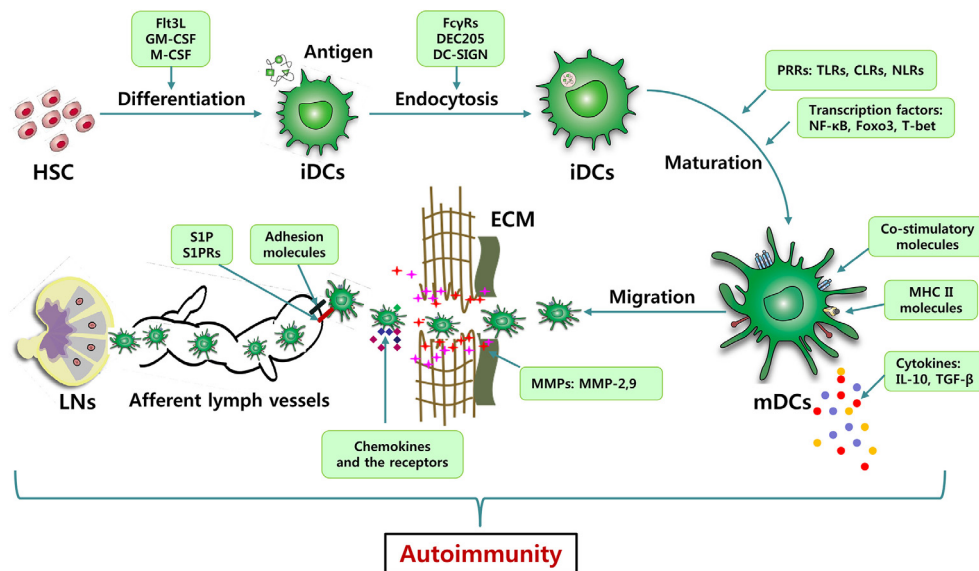
The lymph node response is under the control of the autonomic nervous system. Under normal conditions, dendritic cells are held in a state of immune tolerance by parasympathetic innervation of lymphoid tissue and acetylcholine release - the “cholinergic anti-inflammatory pathway” [91,92], and by sympathetic innervation and norepinephrine release [48,49]. In CRPS, loss of tolerance and maintenance of dendritic cells due to autonomic imbalance, which may be caused by altered CNS output and/or whole-body stress response causing an increase in catecholamines (norepinephrine), leads to initiation of an adaptive immune response and an inflammatory cascade [78,92,145,146]. This results in a regional response with the activation of keratinocytes (hyperplasia), osteoclasts (bone resorption), and monocytes, with further colony expansion of dendritic cells within the bloodstream and bone marrow [147]. The abundant circulating dendritic cells migrate back to the target tissue and potentiate the cascade. Adding to this is the release of neuropeptides from sensory neurons (i.e. A $\delta$  and C fibers), which adds to the immune response and neurogenic inflammation [148]. Activated dendritic cells also promote antibody production, leading to further tissue injury [149]. The net effect is worsening nociception and nerve injury with neuropathic pain, autonomic instability and vascular changes [146,147].

We hypothesise that this explains the regional truncation of pain in CRPS to the limb and its persistence. Fig. 7 illustrates the lymph node response and downstream effects in the context of acute and subacute CRPS.

#### *Downstream effects*

There are several downstream effects of dendritic cell activation/migration that we propose may be responsible for the maintenance of the CRPS pain in any given individual. These changes are likely to be differentially expressed in individual patients. These are briefly discussed below and graphically summarised in Fig. 8.





**Fig. 6.** An illustrated overview of dendritic cell maturation and migration. Figure reproduced from Li et al. [112] with permission from the publisher. HSC: haematopoietic stem cell; iDC: immature dendritic cell; mDC: mature dendritic cell; ECM: extracellular matrix; LNs: lymph nodes.

### Endothelial dysfunction

Endothelial dysfunction in CRPS has been extensively investigated and reviewed [150]. Impairments in vascular regulation systems leading to imbalance between vasoconstriction and vasodilation have also been observed in CRPS-affected limbs compared to unaffected limbs [151]. We hypothesise that dendritic cell activation is the primary mechanism of the endothelial dysfunction seen in CRPS.

### Microglial activation

Whilst dendritic cells are not usually present within the CNS, high concentrations are found in close proximity within the meninges, choroid plexus and perivascular space, and they may infiltrate the CNS under inflammatory conditions [152]. It has been shown that dendritic cell adhesion to cerebral endothelial cells depends on dendritic cell maturation and activation of the endothelium, and that this is regulated by specific receptor-ligand interactions, thus allowing systemic dendritic cells to penetrate the blood brain barrier [76]. Within the CNS, dendritic cells are intimately intertwined with microglia and astrocytes [77]. Research has shown that during CNS inflammation, microglia may differentiate into brain dendritic cells, or acquire a dendritic cell phenotype, and it has been indicated that mature inflammatory dendritic cells remain in the CNS even after the inflammation process has been terminated [153]. We therefore hypothesise that dendritic cells are one of the primary mediators of neuroinflammation in the CNS in CRPS patients.

### Spinal cord central sensitisation

Sensitisation of the DRG and a role for epigenetic modification of DRG neurons in CRPS has been reviewed [154,155]. Briefly, studies in rodent nerve injury/neuropathic pain models have shown that following nerve injury, T-cells, monocytes, macrophages and migrating dendritic cells infiltrate the site of injury/injured nerve and the DRG, while activation of microglia and astrocytes is seen in the spinal cord [70–72]. Nerve injury promotes increased expression of BDNF, which data suggests may activate dendritic cells [156], and this has been implicated in the development of neuropathic pain [157]. Activation of TRPV1 at the DRG leads to maturation and activation of dendritic cells and the dual transmission of pain and inflammatory signals. This

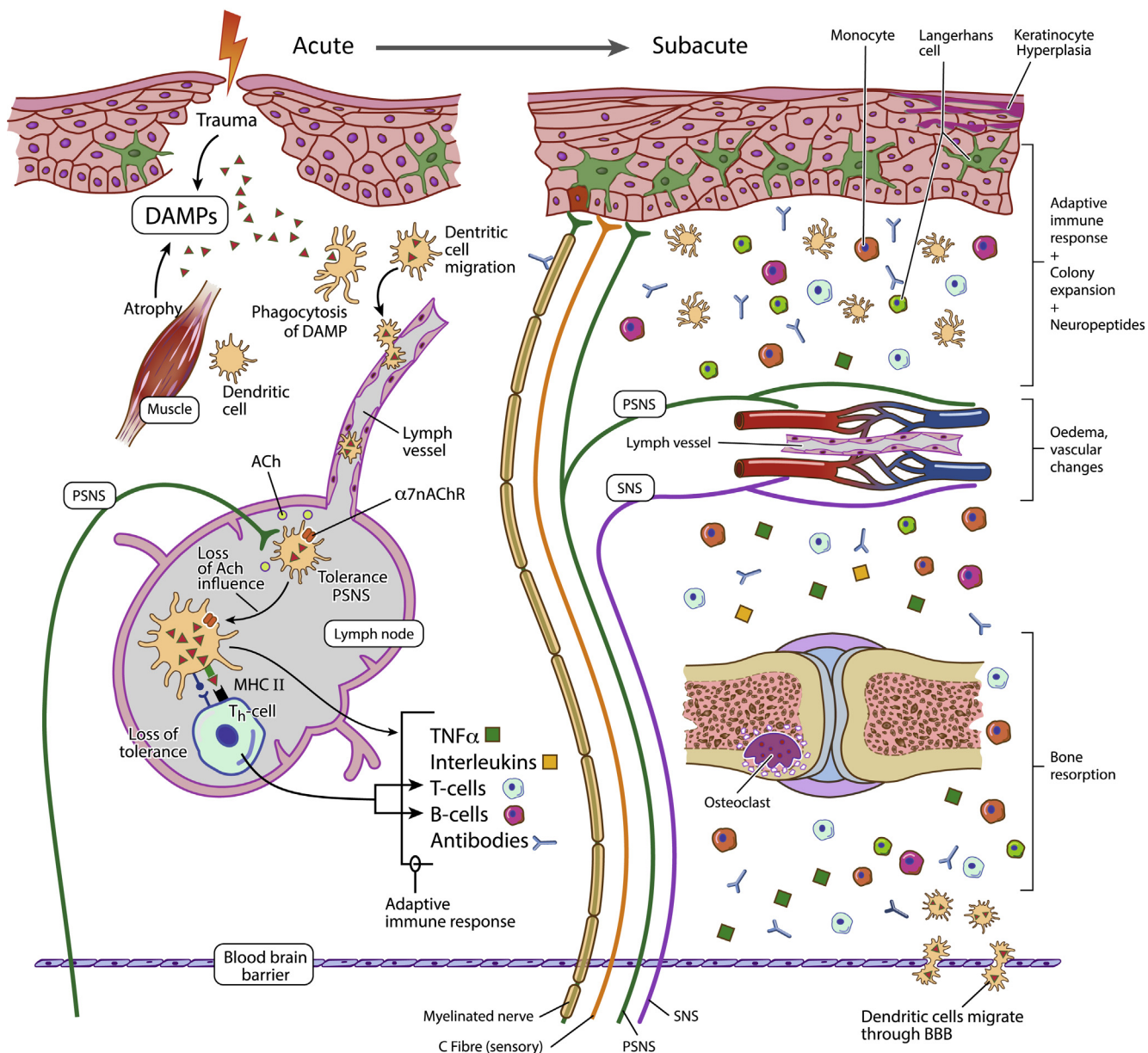
demonstrates a commonality between neural and immune mechanisms and functional pathways from the peripheral to the central nervous system [158].

### Basal ganglia and cortex activation

It is known that local inflammation may activate brain regions via the recruitment of peripheral immune cells to the CNS and the generation of an inflammatory cytokine cascade [62,78]. Basal ganglia function (particularly dopamine function) has been identified as a primary target of these inflammatory cytokines, particularly IFN $\alpha$  [159,160]. IFN $\alpha$ , which is produced by microglia, astrocytes and plasmacytoid dendritic cells during immune activation, is a potent inducer of various other inflammatory cytokines, namely TNF $\alpha$ , IL-1, IL-6 and MCP-1, which further potentiate neuroinflammation [78]. This leads to further recruitment of monocytes to the brain which may differentiate into dendritic cells and contribute to this inflammatory cascade [161].

### Autoimmunity

Extensive research has provided evidence for autoimmunity against the autonomic nervous system in the pathophysiology of subsets of CRPS patients. Pathogenic autoantibodies against neuronal structures, particularly autonomic neurons, have been discovered in CRPS patients, and the  $\beta$ 2-AR and the muscarinic acetylcholine receptor M2 have been identified as autoantigens of these autoantibodies [97,162]. In a mouse IgG-transfer-trauma model for CRPS, serum IgG from chronic CRPS patients induced key clinical indicators of the human disease, namely swelling and mechanical hyperalgesia, which supports the autoimmunity concept [163]. Antibodies to the  $\alpha$ 1a-AR have also been implicated in an autoimmunity mechanism in chronic CRPS [164]. Further supportive of an autoimmunity component in CRPS involving dendritic cells is an investigation in which IgG immune complexes were shown to stimulate migration of dendritic cells from peripheral tissue to draining lymph nodes in vitro [165]. The role dendritic cells play in autoimmunity is yet to be comprehensively explored, although it is known that they are capable of facilitating autoimmunity via various mechanisms, such as by priming autoreactive T-cells and promoting B-cell autoreactive responses [149,166].



**Fig. 7.** The immune response in acute and subacute CRPS. Antigens and/or DAMPs released due to tissue trauma or atrophy are engulfed by and/or activate dendritic cells, which then migrate to the regional lymph node. A loss of immune tolerance due to altered parasympathetic nervous system (PSNS) control (i.e. lack of acetylcholine and hence loss of suppression via  $\alpha 7nAChR$ ) leads to activation of dendritic cells and antigen presentation to T-helper ( $T_H$ ) cells via MHC class II molecules. This results in an adaptive immune response and inflammatory cascade, with release of inflammatory cytokines (i.e.  $TNF\alpha$ , IL-1, IL-18) and activation of T-cells and B-cells with production of antibodies. This inflammatory response activates cells within the regional lymphatic system and results in colony expansion of monocytes, circulating dendritic cells, migrating Langerhans cells, keratinocytes and osteoclasts, as well as release of neuropeptides from sensory neurons. Downstream effects include oedema, vascular and trophic changes such as bone resorption. Dendritic cells may also migrate through the blood brain barrier (BBB) and have pathological effects on cortical function, particularly basal ganglia.

**Evidence against the hypothesis**

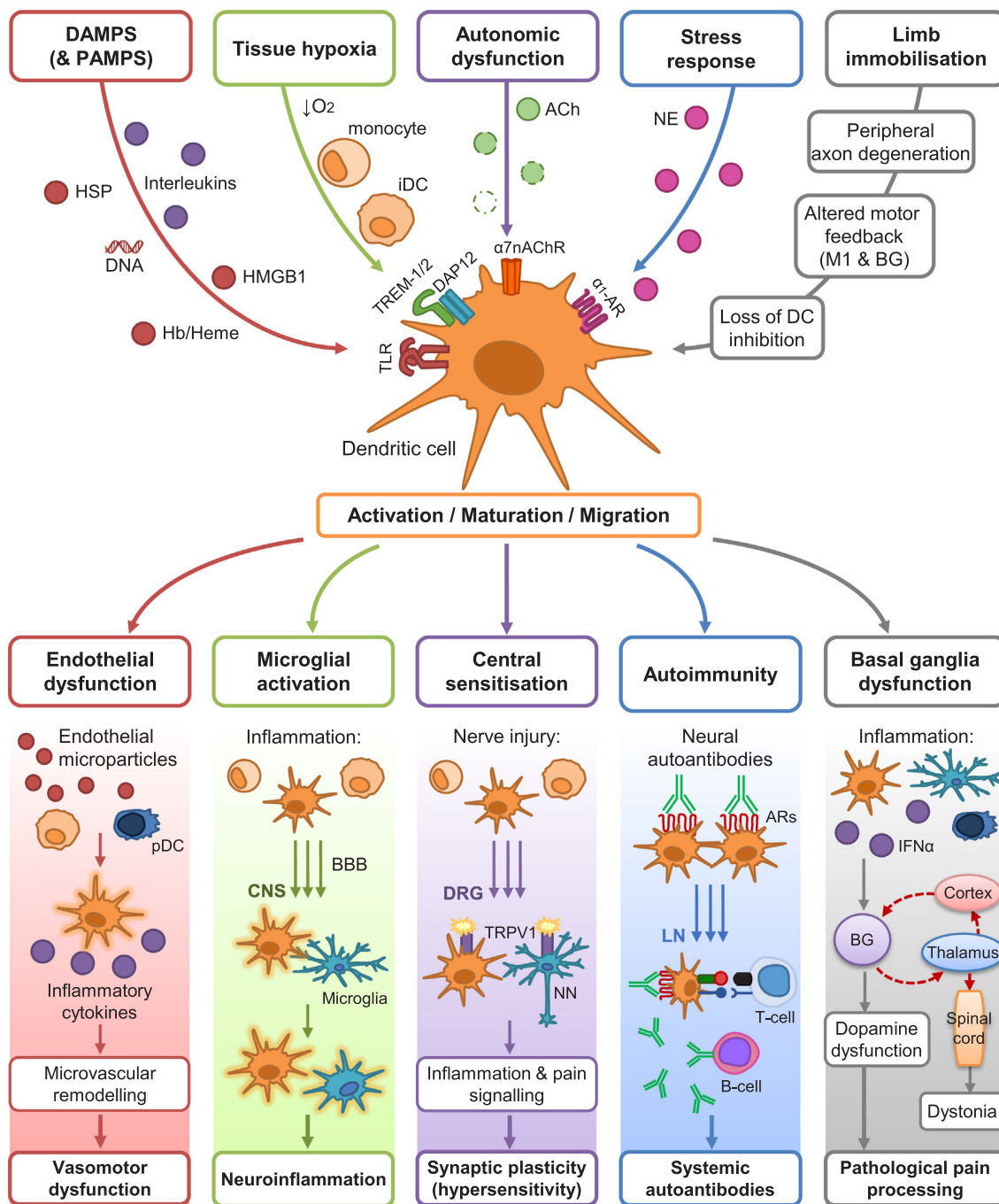
The hypothesis has a number of disparate strands of supporting evidence, but currently lacks a detailed immunological signature examination across serum, cerebrospinal fluid, dorsal root ganglion lysate, skin biopsy and skin blister fluid analysis. This would be the comprehensive investigation to show support or refutation of the dendritic cell activation hypothesis.

One study by Osborne and colleagues observed no significant difference in overall immune cell population or mast cell density in skin punch biopsies between chronic CRPS-affected and non-affected limbs [167]. There were, however, significantly less Langerhans cells in the epidermis of CRPS-affected limbs compared to non-affected limbs, and

no significant difference when compared to healthy controls or non-CRPS pain controls. This particular finding in their experimental set up is not supportive of the hypothesis presented here, but analyses one particular aspect of possible immune activation. The results of this study were in contrast to the Calder study, in which CRPS-affected upper limbs (as opposed to predominantly lower limb CRPS patients in the Osborne study) displayed a higher density of Langerhans cells than non-CRPS repaired nerve injury limbs [108].

Our clinical research group is currently collaborating with expert basic science researchers to investigate the immune signature present in the serum of CRPS patients and control subjects utilising a mass cytometry approach. This method allows for up to 40 immunological markers, including those for specific dendritic cell populations, to be





**Fig. 8.** Activation of tissue dendritic cells by five major initiating factors (top) and the major secondary effects implicated in the development of CRPS according to our model (bottom).  $\alpha1$ -AR: alpha-1 adrenergic receptor;  $\alpha7nAChR$ : alpha-7 nicotinic acetylcholine receptor; ACh: acetylcholine; ARs: adrenergic receptors; BBB: blood brain barrier; BG: basal ganglia; CNS: central nervous system; DAP12: DNAX-activating protein 12 kDa; DRG: dorsal root ganglion; Hb: hemoglobin; HMGB1: high mobility group box protein 1; HSP: heat shock protein; iDC: immature dendritic cell; IFN $\alpha$ : interferon alpha; LN: lymph node; M1: primary motor cortex; NE: norepinephrine; NN: nociceptive neuron; pDC: plasmacytoid dendritic cell; TLR: toll-like receptor; TREM-1/2: triggering receptor expressed on myeloid cells 1 or 2; TRPV1: transient receptor potential cation channel subfamily V member 1.

analysed, and, along with quantification of serum cytokine levels, would represent a comprehensive analysis of the serum immune status. Further work, however, would need to be performed to analyse the other tissue sites referred to above.

Critically, there is likely to be a difference between the immune signature in early CRPS (presumptive strong signal) and the signature in well-established chronic cases of many years duration in which the immune signature may be muted by immune activation exhaustion, and where centrally driven cortical changes may predominate [167].

*Therapeutic implications*

Typically, several treatments are added to baseline physical therapy in CRPS management. As each individual treatment fails, another is added to the process. Historically, response rates are poor. Considering the model presented here, a multitargeted approach could be considered. Inhibition of dendritic cell activation, maturation and migration may be a new area to explore in CRPS research. We would counsel against specific monoclonal antibodies as being a likely useful approach as their target is too specific to likely work. Instead, we believe that

immunomodulatory drugs with wide and varied mechanisms of action that inhibit dendritic cell function are more likely to be found effective for this condition.

Several effective treatments for CRPS can now be better understood as functioning through their immune modulation of the condition. Zoledronic acid is actually a potent inhibitor of dendritic cell function [168–171] and this action may be more important than its osteoclast action [172]. Oral corticosteroids also inhibit dendritic cell function [173–175]. Their role may be time sensitive during the earlier phase of CRPS. Other means of arresting dendritic cell expansion could now be considered.

Inhibition of systemic inflammatory dendritic cell activity may be addressed by restoring or enhancing the cholinergic anti-inflammatory pathway. Vagus nerve stimulation is a well-studied method of achieving this and has been applied to other inflammatory diseases [176–178]. Implanted vagus nerve stimulators are approved for seizure and headache disorders and could be trialled in CRPS patients. Selective agonism of  $\alpha 7nAChR$  to inhibit pro-inflammatory cytokine release from monocyte-derived dendritic cells and tissue macrophages in lymph tissue also represents a potential therapeutic avenue to pursue [179–181].

## Conclusion

We propose that the pathophysiology of CRPS may be better understood as four components of altered function in terms of tissue trauma, abnormal pain processing, autonomic imbalance and immune system alteration, with a cascade of dendritic cell activation, recruitment, maturation and migration initially to the draining lymph nodes and subsequently to the DRG and cortical networks where basal ganglia output is altered. Also on an immune basis, there may be partial loss of recognition of self as regards dendritic cell function and this can include autoantibody production. The condition may best be considered as an immune-neurological disorder, with a combination of adaptive immune response and pathological pain processing. Whilst the multiple activation pathways in this condition have precluded a successful single “magic bullet” approach, this new understanding might allow a multimodal treatment strategy targeting specific activation points to better restore homeostasis and resolution of the condition. This hypothesis will only be useful to the extent that specific experiments validate components of the model and provide robust experimental evidence to underpin this approach, for example, investigations of dendritic cell subpopulations and activation levels in acute CRPS presentations. We encourage the research community to consider testing this hypothesis in animal and human studies.

## Sources of support

None.

## Funding

None.

## Conflict of interest

The authors report no conflicts of interest.

## Acknowledgements

This manuscript is dedicated both John Sinclair Calder, an iconoclast clinician and researcher whose work gave us a pivotal insight into peripheral nerve damage and the involvement of the immune system in complex regional pain syndrome some thirty years ago, and Wade Kingery, who has worked tirelessly for twenty years on unravelling the mechanisms of this disease.

## References

- [1] Galer BS, Bruhl S, Harden RN, IASP. diagnostic criteria for complex regional pain syndrome: a preliminary empirical validation study. *International Association for the Study of Pain. Clin J Pain* 1998;14:48–54.
- [2] Bruhl S, Harden RN, Galer BS, et al. External validation of IASP diagnostic criteria for Complex Regional Pain Syndrome and proposed research diagnostic criteria. *International Association for the Study of Pain. Pain* 1999;81:147–54.
- [3] Harden RN, Bruhl S, Galer BS, et al. Complex regional pain syndrome: are the IASP diagnostic criteria valid and sufficiently comprehensive? *Pain* 1999;83:211–9.
- [4] Harden RN, Bruhl S, Stanton-Hicks M, Wilson PR. Proposed new diagnostic criteria for complex regional pain syndrome. *Pain Med* 2007;8:326–31.
- [5] Harden RN, Bruhl S, Perez RS, et al. Validation of proposed diagnostic criteria (the “Budapest Criteria”) for Complex Regional Pain Syndrome. *Pain* 2010;150:268–74.
- [6] Pearce JM. Chronic regional pain and chronic pain syndromes. *Spinal Cord* 2005;43:263–8.
- [7] Del Pinal F, Editorial. I have a dream reflex sympathetic dystrophy (RSD or Complex Regional Pain Syndrome – CRPS I) does not exist. *J Hand Surg Eur* 2013;38:595–7.
- [8] Bass C. Complex regional pain syndrome medicalises limb pain. *BMJ* 2014;348:g2631.
- [9] Ratti C, Nordio A, Resmini G, Murena L. Post-traumatic complex regional pain syndrome: clinical features and epidemiology. *Clin Cases Miner Bone Metab* 2015;12:11–6.
- [10] de Rooij AM, Perez RS, Huygen FJ, et al. Spontaneous onset of complex regional pain syndrome. *Eur J Pain* 2010;14:510–3.
- [11] Birklein F, Dimova V. Complex regional pain syndrome-up-to-date. *Pain Rep* 2017;2:e624.
- [12] GalveVilla M, Rittig-Rasmussen B, Moeller L, Mikkelsen Schear, Groendahl Poulsen A. Complex regional pain syndrome. *Man. Ther.* 2016;26:223–30.
- [13] Gierthmuhlen J, Binder A, Baron R. Mechanism-based treatment in complex regional pain syndromes. *Nat Rev Neurol* 2014;10:518–28.
- [14] Azqueta-Gavaldon M, Schulte-Gocking H, Storz C, et al. Basal ganglia dysfunction in complex regional pain syndrome – a valid hypothesis? *Eur J Pain* 2017;21:415–24.
- [15] Bean DJ, Johnson MH, Kydd RR. The outcome of complex regional pain syndrome type 1: a systematic review. *J Pain* 2014;15:677–90.
- [16] Li WW, Guo TZ, Li XQ, Kingery WS, Clark JD. Fracture induces keratinocyte activation, proliferation, and expression of pro-nociceptive inflammatory mediators. *Pain* 2010;151:843–52.
- [17] Wei T, Guo TZ, Li WW, Hou S, Kingery WS, Clark JD. Keratinocyte expression of inflammatory mediators plays a crucial role in substance P-induced acute and chronic pain. *J Neuroinflamm* 2012;9:181.
- [18] Gallagher JJ, Tajerian M, Guo T, et al. Acute and chronic phases of complex regional pain syndrome in mice are accompanied by distinct transcriptional changes in the spinal cord. *Mol Pain* 2013;9:40.
- [19] Birklein F, Drummond PD, Li W, et al. Activation of cutaneous immune responses in complex regional pain syndrome. *J Pain* 2014;15:485–95.
- [20] Li WW, Guo TZ, Shi X, et al. Autoimmunity contributes to nociceptive sensitization in a mouse model of complex regional pain syndrome. *Pain* 2014;155:2377–89.
- [21] Wei T, Guo TZ, Li WW, Kingery WS, Clark JD. Acute versus chronic phase mechanisms in a rat model of CRPS. *J Neuroinflamm* 2016;13:14.
- [22] Tajerian M, Hung V, Khan H, et al. Identification of KRT16 as a target of an autoantibody response in complex regional pain syndrome. *Exp Neurol* 2017;287:14–20.
- [23] Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nat Rev Immunol* 2009;9:679–91.
- [24] Haniffa M, Gunawan M, Jardine L. Human skin dendritic cells in health and disease. *J Dermatol Sci* 2015;77:85–92.
- [25] Scholzen T, Armstrong CA, Bunnett NW, Luger TA, Olerud JE, Ansel JC. Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. *Exp Dermatol* 1998;7:81–96.
- [26] Pepper A, Li W, Kingery WS, Angst MS, Curtin CM, Clark JD. Changes resembling complex regional pain syndrome following surgery and immobilization. *J Pain* 2013;14:516–24.
- [27] Koban M, Leis S, Schultze-Mosgau S, Birklein F. Tissue hypoxia in complex regional pain syndrome. *Pain* 2003;104:149–57.
- [28] Leis S, Weber M, Schmelz M, Birklein F. Facilitated neurogenic inflammation in unaffected limbs of patients with complex regional pain syndrome. *Neurosci Lett* 2004;359:163–6.
- [29] Birklein F, Schmelz M. Neuropeptides, neurogenic inflammation and complex regional pain syndrome (CRPS). *Neurosci Lett* 2008;437:199–202.
- [30] Speck V, Schlereth T, Birklein F, Maihofner C. Increased prevalence of posttraumatic stress disorder in CRPS. *Eur J Pain* 2017;21:466–73.
- [31] Frank MG, Watkins LR, Maier SF. The permissive role of glucocorticoids in neuroinflammatory priming: mechanisms and insights. *Curr Opin Endocrinol Diabetes Obes* 2015;22:300–5.
- [32] Frank MG, Weber MD, Watkins LR, Maier SF. Stress sounds the alarm: The role of the danger-associated molecular pattern HMGB1 in stress-induced neuroinflammatory priming. *Brain Behav Immun* 2015;48:1–7.
- [33] Frank MG, Weber MD, Fonken LK, Hershman SA, Watkins LR, Maier SF. The redox state of the alarmin HMGB1 is a pivotal factor in neuroinflammatory and microglial priming: a role for the NLRP3 inflammasome. *Brain Behav Immun*

- 2016;55:215–24.
- [34] Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunol Rev* 2007;220:35–46.
- [35] Hirsiger S, Simmen HP, Werner CM, Wanner GA, Rittirsch D. Danger signals activating the immune response after trauma. *Mediat Inflamm*. 2012;2012:315941.
- [36] Kato J, Svensson CI. Role of extracellular damage-associated molecular pattern molecules (DAMPs) as mediators of persistent pain. *Prog Mol Biol Transl Sci* 2015;131:251–79.
- [37] Nace G, Evankovich J, Eid R, Tsung A. Dendritic cells and damage-associated molecular patterns: endogenous danger signals linking innate and adaptive immunity. *J Innate Immun* 2012;4:6–15.
- [38] Stoecklein VM, Osuka A, Lederer JA. Trauma equals danger–damage control by the immune system. *J Leukoc Biol* 2012;92:539–51.
- [39] Maslanik T, Mahaffey L, Tannura K, Beninson L, Greenwood BN, Fleshner M. The inflammasome and danger associated molecular patterns (DAMPs) are implicated in cytokine and chemokine responses following stressor exposure. *Brain Behav Immun* 2013;28:54–62.
- [40] Ferreira I, Liberal J, Martins J, Silva A, Neves BM, Cruz MT. Inflammasome in dendritic cells immunobiology: implications to diseases and therapeutic strategies. *Curr Drug Targets* 2016.
- [41] Kato J, Agalave NM, Svensson CI. Pattern recognition receptors in chronic pain: Mechanisms and therapeutic implications. *Eur J Pharmacol* 2016;788:261–73.
- [42] Bosco MC, Pierobon D, Blengio F, et al. Hypoxia modulates the gene expression profile of immunoregulatory receptors in human mature dendritic cells: identification of TREM-1 as a novel hypoxic marker in vitro and in vivo. *Blood* 2011;117:2625–39.
- [43] Bosco MC, Varesio L. Dendritic cell reprogramming by the hypoxic environment. *Immunobiology* 2012;217:1241–9.
- [44] Heerschap A, den Hollander JA, Reynen H, Goris RJ. Metabolic changes in reflex sympathetic dystrophy: a 31P NMR spectroscopy study. *Muscle Nerve* 1993;16:367–73.
- [45] Birklein F, Weber M, Neundorfer B. Increased skin lactate in complex regional pain syndrome: evidence for tissue hypoxia? *Neurology* 2000;55:1213–5.
- [46] Bellingham GA, Smith RS, Morley-Forster P, Murkin JM. Use of near infrared spectroscopy to detect impaired tissue oxygen saturation in patients with complex regional pain syndrome type 1. *Can J Anaesth* 2014;61:563–70.
- [47] Saint-Mezard P, Chavagnac C, Bosset S, et al. Psychological stress exerts an adjuvant effect on skin dendritic cell functions in vivo. *J Immunol* 2003;171:4073–80.
- [48] Maestroni GJ. Adrenergic modulation of dendritic cells function: relevance for the immune homeostasis. *Curr Neurovasc Res* 2005;2:169–73.
- [49] Maestroni GJ. Sympathetic nervous system influence on the innate immune response. *Ann NY Acad Sci* 2006;1069:195–207.
- [50] Seiffert K, Granstein RD. Neuroendocrine regulation of skin dendritic cells. *Ann NY Acad Sci* 2006;1088:195–206.
- [51] Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. *Nat Med* 2010;16:1248–57.
- [52] Bruehl S. An update on the pathophysiology of complex regional pain syndrome. *Anesthesiology* 2010;113:713–25.
- [53] Egan CL, Viglione-Schneck MJ, Walsh LJ, et al. Characterization of unmyelinated axons uniting epidermal and dermal immune cells in primate and murine skin. *J Cutan Pathol* 1998;25:20–9.
- [54] Misery L. Langerhans cells in the neuro-immuno-cutaneous system. *J Neuroimmunol* 1998;89:83–7.
- [55] Richardson JD, Vasko MR. Cellular mechanisms of neurogenic inflammation. *J Pharmacol Exp Ther* 2002;302:839–45.
- [56] Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. *Lancet Neurol* 2012;11:629–42.
- [57] Geertzen JH, Bodde MI, van den Dungen JJ, Dijkstra PU, den Dunnen WF. Peripheral nerve pathology in patients with severely affected complex regional pain syndrome type I. *Int J Rehabil Res* 2015;38:121–30.
- [58] Chen SS, Zhang JM. Progress in sympathetically mediated pathological pain. *J Anesth Perioper Med* 2015;2:216–25.
- [59] Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. *Prog Neurobiol* 1999;57:563–81.
- [60] Calvo M, Zhu N, Grist J, Ma Z, Loeb JA, Bennett DL. Following nerve injury neuregulin-1 drives microglial proliferation and neuropathic pain via the MEK/ERK pathway. *Glia* 2011;59:554–68.
- [61] Del Valle L, Schwartzman RJ, Alexander G. Spinal cord histopathological alterations in a patient with longstanding complex regional pain syndrome. *Brain Behav Immun* 2009;23:85–91.
- [62] Linnman C, Becerra L, Borsook D. Inflaming the brain: CRPS a model disease to understand neuroimmune interactions in chronic pain. *J Neuroimmune Pharmacol* 2013;8:547–63.
- [63] Barker RA. The basal ganglia and pain. *Int J Neurosci* 1988;41:29–34.
- [64] Chudler EH, Dong WK. The role of the basal ganglia in nociception and pain. *Pain* 1995;60:3–38.
- [65] Jeon SY, Seo S, Lee JS, et al. [11C]-(R)-PK11195 positron emission tomography in patients with complex regional pain syndrome: A pilot study. *Medicine (Baltimore)* 2017;96:e5735.
- [66] Schott GD. Peripherally-triggered CRPS and dystonia. *Pain* 2007;130:203–7.
- [67] van Rijn MA, Marinus J, Putter H, van Hilten JJ. Onset and progression of dystonia in complex regional pain syndrome. *Pain* 2007;130:287–93.
- [68] Reinersmann A, Maier C, Schwenkreis P, Lenz M. Complex regional pain syndrome: more than a peripheral disease.  *Pain Manage* 2013;3:495–502.
- [69] Avanzino L, Fiorio M. Proprioceptive dysfunction in focal dystonia: from experimental evidence to rehabilitation strategies. *Front Hum Neurosci* 2014;8:1000.
- [70] Braun JS, Kaissling B, Le Hir M, Zenker W. Cellular components of the immune barrier in the spinal meninges and dorsal root ganglia of the normal rat: immunohistochemical (MHC class II) and electron-microscopic observations. *Cell Tissue Res* 1993;273:209–17.
- [71] Kim CF, Moalem-Taylor G. Detailed characterization of neuro-immune responses following neuropathic injury in mice. *Brain Res* 2011;1405:95–108.
- [72] Austin PJ, Kim CF, Perera CJ, Moalem-Taylor G. Regulatory T cells attenuate neuropathic pain following peripheral nerve injury and experimental autoimmune neuritis. *Pain* 2012;153:1916–31.
- [73] Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. *Nat Rev Drug Discov* 2014;13:533–48.
- [74] Reimer M, Remppe T, Diedrichs C, Baron R, Gierthmuhlen J. Sensitization of the nociceptive system in complex regional pain syndrome. *PLoS One* 2016;11:e0154553.
- [75] Curtin JF, King GD, Barcia C, et al. Fms-like tyrosine kinase 3 ligand recruits plasmacytoid dendritic cells to the brain. *J Immunol* 2006;176:3566–77.
- [76] Arjmandi A, Liu K, Dorovini-Zis K. Dendritic cell adhesion to cerebral endothelium: role of endothelial cell adhesion molecules and their ligands. *J Neuropathol Exp Neurol* 2009;68:300–13.
- [77] Sagar D, Foss C, El Baz R, Pomper MG, Khan ZK, Jain P. Mechanisms of dendritic cell trafficking across the blood-brain barrier. *J Neuroimmun Pharmacol* 2012;7:74–94.
- [78] Felger JC, Miller AH. Cytokine effects on the basal ganglia and dopamine function: the subcortical source of inflammatory malaise. *Front Neuroendocrinol* 2012;33:315–27.
- [79] Drummond PD, Drummond ES, Dawson LF, et al. Upregulation of alpha1-adrenoceptors on cutaneous nerve fibres after partial sciatic nerve ligation and in complex regional pain syndrome type II. *Pain* 2014;155:606–16.
- [80] Finch PM, Drummond ES, Dawson LF, Phillips JK, Drummond PD. Up-regulation of cutaneous alpha1-adrenoceptors in complex regional pain syndrome type I. *Pain Med* 2014;15:1945–56.
- [81] Schattschneider J, Binder A, Siebrecht D, Wasner G, Baron R. Complex regional pain syndromes: the influence of cutaneous and deep somatic sympathetic innervation on pain. *Clin J Pain* 2006;22:240–4.
- [82] Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. *Brain Behav Immun* 2005;19:493–9.
- [83] Tracey KJ. Physiology and immunology of the cholinergic anti-inflammatory pathway. *J Clin Invest* 2007;117:289–96.
- [84] Pereira MR, Leite PE. The involvement of parasympathetic and sympathetic nerve in the inflammatory reflex. *J Cell Physiol* 2016;231:1862–9.
- [85] Hoover DB. Cholinergic modulation of the immune system presents new approaches for treating inflammation. *Pharmacol Ther* 2017.
- [86] Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000;405:458–62.
- [87] Tracey KJ. The inflammatory reflex. *Nature* 2002;420:853–9.
- [88] Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 2011;334:98–101.
- [89] Andersson U, Tracey KJ. Neural reflexes in inflammation and immunity. *J Exp Med* 2012;209:1057–68.
- [90] Wulfig C, Gunther HS. Dendritic cells and macrophages neurally hard-wired in the lymph node. *Sci Rep* 2015;5:16866.
- [91] Salamone G, Lombardi G, Gori S, et al. Cholinergic modulation of dendritic cell function. *J Neuroimmunol* 2011;236:47–56.
- [92] Fujii T, Mashimo M, Moriawaki Y, et al. Physiological functions of the cholinergic system in immune cells. *J Pharmacol Sci* 2017;134:1–21.
- [93] Schulze J, Troeger C. Increased sympathetic activity assessed by spectral analysis of heart rate variability in patients with CRPS I. *Handchir Mikrochir Plast Chir* 2010;42:44–8.
- [94] Terkelsen AJ, Molgaard H, Hansen J, Finnerup NB, Kroner K, Jensen TS. Heart rate variability in complex regional pain syndrome during rest and mental and orthostatic stress. *Anesthesiology* 2012;116:133–46.
- [95] Taneyama C, Yokota S, Goto H. Patients with complex regional pain syndrome type I: fractal dynamics of heart rate variability and baroreflex evaluations. *Clin J Pain* 2013;29:962–6.
- [96] Bartur G, Vatine JJ, Raphaely-Beer N, Peleg S, Katz-Leurer M. Heart rate autonomic regulation system at rest and during paced breathing among patients with CRPS as compared to age-matched healthy controls. *Pain Med* 2014;15:1569–74.
- [97] Kohr D, Tschernatsch M, Schmitz K, et al. Autoantibodies in complex regional pain syndrome bind to a differentiation-dependent neuronal surface autoantigen. *Pain* 2009;143:246–51.
- [98] Schwartzman RJ, Liu JE, Smullens SN, Hyslop T, Tahmouh AJ. Long-term outcome following sympathectomy for complex regional pain syndrome type 1 (RSD). *J Neurol Sci* 1997;150:149–52.
- [99] Singh B, Moodley J, Shaik AS, Robbs JV. Sympathectomy for complex regional pain syndrome. *J Vasc Surg* 2003;37:508–11.
- [100] Alkoshha HM, Elkiran YM. Predictors of long-term outcome of thoracic sympathectomy in patients with complex regional pain syndrome type 2. *World Neurosurg*. 2016;92:74–82.
- [101] Walker S, Drummond PD. Implications of a local overproduction of tumor necrosis factor-alpha in complex regional pain syndrome. *Pain Med* 2011;12:1784–807.
- [102] Terrando N, Yang T, Ryu JK, et al. Stimulation of the alpha7 nicotinic acetylcholine receptor protects against neuroinflammation after tibia fracture and endotoxemia in mice. *Mol Med* 2015;20:667–75.
- [103] An HS, Hawthorne KB, Jackson WT. Reflex sympathetic dystrophy and cigarette



- smoking. *J Hand Surg Am* 1988;13:458–60.
- [104] Hsu C, Harden RN, Houle T. Nicotine and caffeine intake in complex regional pain syndrome. *J Back Musculoskelet Rehabil* 2002;16:33–8.
- [105] Weiland BJ, Sabbineni A, Calhoun VD, Welsh RC, Hutchison KE. Reduced executive and default network functional connectivity in cigarette smokers. *Hum Brain Mapp* 2015;36:872–82.
- [106] Bi Y, Yuan K, Guan Y, et al. Altered resting state functional connectivity of anterior insula in young smokers. *Brain Imaging Behav* 2017;11:155–65.
- [107] Wang C, Bai J, Wang C, von Deneen KM, Yuan K, Cheng J. Altered thalamo-cortical resting state functional connectivity in smokers. *Neurosci Lett* 2017;653:120–5.
- [108] Calder JS, Holten I, McAllister RM. Evidence for immune system involvement in reflex sympathetic dystrophy. *J Hand Surg Br* 1998;23:147–50.
- [109] Miyan JA, Broome CS, Afan AM. Coordinated host defense through an integration of the neural, immune and haemopoietic systems. *Domest Anim Endocrinol* 1998;15:297–304.
- [110] Qu C, Brinck-Jensen NS, Zang M, Chen K. Monocyte-derived dendritic cells: targets as potent antigen-presenting cells for the design of vaccines against infectious diseases. *Int J Infect Dis* 2014;19:1–5.
- [111] McKenna K, Beignon AS, Bhardwaj N. Plasmacytoid dendritic cells: linking innate and adaptive immunity. *J Virol* 2005;79:17–27.
- [112] Li X, Han Y, Sun E. Sniping the scout: Targeting the key molecules in dendritic cell functions for treatment of autoimmune diseases. *Pharmacol Res* 2016;107:27–41.
- [113] Alnaeeli M, Park J, Mahamed D, Penninger JM, Teng YT. Dendritic cells at the osteo-immune interface: implications for inflammation-induced bone loss. *J Bone Miner Res* 2007;22:775–80.
- [114] Alnaeeli M, Teng YT. Dendritic cells: a new player in osteoimmunology. *Curr Mol Med* 2009;9:893–910.
- [115] Cumberbatch M, Dearman RJ, Griffiths CE, Kimber I. Langerhans cell migration. *Clin Exp Dermatol* 2000;25:413–8.
- [116] Villablanca EJ, Mora JR. A two-step model for Langerhans cell migration to skin-draining LN. *Eur J Immunol* 2008;38:2975–80.
- [117] Hosoi J, Murphy GF, Egan CL, et al. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature* 1993;363:159–63.
- [118] Asahina A, Hosoi J, Grabbe S, Granstein RD. Modulation of Langerhans cell function by epidermal nerves. *J Allergy Clin Immunol* 1995;96:1178–82.
- [119] Staniek V, Misery L, Dezutter-Dambuyant C, Claudy A, Schmitt D. Expression of neuropeptides on human epidermal Langerhans cells. *Adv Exp Med Biol* 1995;378:147–50.
- [120] Lambert RW, Granstein RD. Neuropeptides and Langerhans cells. *Exp Dermatol* 1998;7:73–80.
- [121] Torii H, Yan Z, Hosoi J, Granstein RD. Expression of neurotrophic factors and neuropeptide receptors by Langerhans cells and the Langerhans cell-like cell line XS52: further support for a functional relationship between Langerhans cells and epidermal nerves. *J Invest Dermatol* 1997;109:586–91.
- [122] Colonna M. TREMs in the immune system and beyond. *Nat Rev Immunol* 2003;3:445–53.
- [123] Tessarz AS, Cerwenka A. The TREM-1/DAP12 pathway. *Immunol Lett* 2008;116:111–6.
- [124] Molloy EJ. Triggering Receptor Expressed on Myeloid Cells (TREM) family and the application of its antagonists. *Recent Pat Antiinfect Drug Discov* 2009;4:51–6.
- [125] Hyder LA, Gonzalez J, Harden JL, et al. TREM-1 as a potential therapeutic target in psoriasis. *J Invest Dermatol* 2013;133:1742–51.
- [126] Bouchon A, Hernandez-Munain C, Cella M, Colonna M. A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. *J Exp Med* 2001;194:1111–22.
- [127] Kobayashi M, Konishi H, Takai T, Kiyama H. A DAP12-dependent signal promotes pro-inflammatory polarization in microglia following nerve injury and exacerbates degeneration of injured neurons. *Glia* 2015;63:1073–82.
- [128] Owens R, Grabert K, Davies CL, et al. Divergent Neuroinflammatory regulation of microglial TREM expression and Involvement of NF-kappaB. *Front Cell Neurosci* 2017;11:56.
- [129] Kobayashi M, Konishi H, Sayo A, Takai T, Kiyama H. TREM2/DAP12 signal elicits proinflammatory response in microglia and exacerbates neuropathic pain. *J Neurosci* 2016;36:11138–50.
- [130] Ji RR, Chamesian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. *Science* 2016;354:572–7.
- [131] Enk AH, Angeloni VL, Udey MC, Katz SI. An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune responses in skin. *J Immunol* 1993;150:3698–704.
- [132] Schwarzenberger K, Udey MC. Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression in situ. *J Invest Dermatol* 1996;106:553–8.
- [133] Cumberbatch M, Dearman RJ, Kimber I. Langerhans cells require signals from both tumour necrosis factor-alpha and interleukin-1 beta for migration. *Immunology* 1997;92:388–95.
- [134] Stoitzner P, Zanella M, Ortner U, et al. Migration of langerhans cells and dermal dendritic cells in skin organ cultures: augmentation by TNF-alpha and IL-1beta. *J Leukoc Biol* 1999;66:462–70.
- [135] Osman M, Tortorella M, Londei M, Quarantino S. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases define the migratory characteristics of human monocyte-derived dendritic cells. *Immunology* 2002;105:73–82.
- [136] Ratzinger G, Stoitzner P, Ebner S, et al. Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. *J Immunol* 2002;168:4361–71.
- [137] Uchi H, Imayama S, Kobayashi Y, Furue M. Langerhans cells express matrix metalloproteinase-9 in the human epidermis. *J Invest Dermatol* 1998;111:1232–3.
- [138] Kobayashi Y, Matsumoto M, Kotani M, Makino T. Possible involvement of matrix metalloproteinase-9 in Langerhans cell migration and maturation. *J Immunol* 1999;163:5989–93.
- [139] Murphy G, Gavrilovic J. Proteolysis and cell migration: creating a path? *Curr Opin Cell Biol* 1999;11:614–21.
- [140] Bode W, Fernandez-Catalan C, Grams F, et al. Insights into MMP-TIMP interactions. *Ann NY Acad Sci* 1999;878:73–91.
- [141] Jotwani R, Eswaran SV, Moonga S, Cutler CW. MMP-9/TIMP-1 imbalance induced in human dendritic cells by *Porphyromonas gingivalis*. *FEMS Immunol Med Microbiol* 2010;58:314–21.
- [142] Szczesny G, Olszewski WL, Zaleska M. Limb lymph node response to bone fracture. *Lymphat Res Biol* 2004;2:155–64.
- [143] Kamimura Y, Iwai H, Piao J, Hashiguchi M, Azuma M. The glucocorticoid-induced TNF receptor-related protein (GITR)-GITR ligand pathway acts as a mediator of cutaneous dendritic cell migration and promotes T cell-mediated acquired immunity. *J Immunol* 2009;182:2708–16.
- [144] Kobayashi Y, Kiguchi N, Fukazawa Y, Saika F, Maeda T, Kishioka S. Macrophage-T cell interactions mediate neuropathic pain through the glucocorticoid-induced tumor necrosis factor ligand system. *J Biol Chem* 2015;290:12603–13.
- [145] Pavlov VA, Tracey KJ. Neural regulators of innate immune responses and inflammation. *Cell Mol Life Sci* 2004;61:2322–31.
- [146] Schlereth T, Drummond PD, Birklein F. Inflammation in CRPS: role of the sympathetic supply. *Auton Neurosci* 2014;182:102–7.
- [147] König S, Schlereth T, Birklein F. Molecular signature of complex regional pain syndrome (CRPS) and its analysis. *Expert Rev Proteom* 2017:1–11.
- [148] Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat Neurosci* 2012;15:1063–7.
- [149] Sozzani S, Del Prete A, Bosio D. Dendritic cell recruitment and activation in autoimmunity. *J Autoimmun* 2017.
- [150] Kortekaas MC, Niehof SP, Stolker RJ, Huygen FJ. Pathophysiological mechanisms involved in vasomotor disturbances in complex regional pain syndrome and implications for therapy: a review. *Pain Pract* 2016;16:905–14.
- [151] Dayan L, Salman S, Norman D, Vatine JJ, Calif E, Jacob G. Exaggerated vasoconstriction in complex regional pain syndrome-1 is associated with impaired resistance artery endothelial function and local vascular reflexes. *J Rheumatol* 2008;35:1339–45.
- [152] Almolda B, Gonzalez B, Castellano B. Are microglial cells the regulators of lymphocyte responses in the CNS? *Front Cell Neurosci* 2015;9:440.
- [153] Fischer HG, Reichmann G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol* 2001;166:2717–26.
- [154] Wang F, Stefano GB, Kream RM. Epigenetic modification of DRG neuronal gene expression subsequent to nerve injury: etiological contribution to complex regional pain syndromes (Part II). *Med Sci Monit* 2014;20:1188–200.
- [155] Wang F, Stefano GB, Kream RM. Epigenetic modification of DRG neuronal gene expression subsequent to nerve injury: etiological contribution to complex regional pain syndromes (Part I). *Med Sci Monit* 2014;20:1067–77.
- [156] Noga O, Peiser M, Altenahr M, et al. Differential activation of dendritic cells by nerve growth factor and brain-derived neurotrophic factor. *Clin Exp Allergy* 2007;37:1701–8.
- [157] Uchida H, Matsushita Y, Ueda H. Epigenetic regulation of BDNF expression in the primary sensory neurons after peripheral nerve injury: implications in the development of neuropathic pain. *Neuroscience* 2013;240:147–54.
- [158] Basu S, Srivastava P. Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. *Proc Natl Acad Sci USA* 2005;102:5120–5.
- [159] Capuron L, Pagnoni G, Drake DF, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiat* 2012;69:1044–53.
- [160] Haroon E, Raisin CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. *Neuropsychopharmacology* 2012;37:137–62.
- [161] D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factor-alpha signaling during peripheral organ inflammation. *J Neurosci* 2009;29:2089–102.
- [162] Kohr D, Singh P, Tschernatsch M, et al. Autoimmunity against the beta2 adrenergic receptor and muscarinic-2 receptor in complex regional pain syndrome. *Pain* 2011;152:2690–700.
- [163] Tekus V, Hajna Z, Borbely E, et al. A CRPS-IgG-transfer-trauma model reproducing inflammatory and positive sensory signs associated with complex regional pain syndrome. *Pain* 2014;155:299–308.
- [164] Dubuis E, Thompson V, Leite MI, et al. Longstanding complex regional pain syndrome is associated with activating autoantibodies against alpha-1a adrenoceptors. *Pain* 2014;155:2408–17.
- [165] Clatworthy MR, Aronin CE, Mathews RJ, Morgan NY, Smith KG, Germain RN. Immune complexes stimulate CCR7-dependent dendritic cell migration to lymph nodes. *Nat Med* 2014;20:1458–63.
- [166] Ganguly D, Haak S, Sisirak V, Reizis B. The role of dendritic cells in autoimmunity. *Nat Rev Immunol* 2013;13:566–77.
- [167] Osborne S, Farrell J, Dearman RJ, et al. Cutaneous immunopathology of longstanding complex regional pain syndrome. *Eur J Pain* 2015;19:1516–26.
- [168] Bringmann A, Schmidt SM, Weck MM, et al. Zoledronic acid inhibits the function of Toll-like receptor 4 ligand activated monocyte-derived dendritic cells. *Leukemia* 2007;21:732–8.
- [169] Wolf AM, Rumpold H, Tilg H, Gastl G, Günsilius E, Wolf D. The effect of zoledronic

- acid on the function and differentiation of myeloid cells. *Haematologica* 2006;91:1165–71.
- [170] Chen YJ, Chao KS, Yang YC, Hsu ML, Lin CP, Chen YY. Zoledronic acid, an aminobisphosphonate, modulates differentiation and maturation of human dendritic cells. *Immunopharmacol Immunotoxicol* 2009;31:499–508.
- [171] Orsini G, Failli A, Legitimo A, Adinolfi B, Romanini A, Consolini R. Zoledronic acid modulates maturation of human monocyte-derived dendritic cells. *Exp Biol Med (Maywood)* 2011;236:1420–6.
- [172] Wang L, Guo TZ, Wei T, et al. Bisphosphonates inhibit pain, bone loss, and inflammation in a rat tibia fracture model of complex regional pain syndrome. *Anesth Analg* 2016;123:1033–45.
- [173] Shodell M, Shah K, Siegal FP. Circulating human plasmacytoid dendritic cells are highly sensitive to corticosteroid administration. *Lupus* 2003;12:222–30.
- [174] Boor PP, Metselaar HJ, Mancham S, Tilanus HW, Kusters JG, Kwekkeboom J. Prednisolone suppresses the function and promotes apoptosis of plasmacytoid dendritic cells. *Am J Transplant* 2006;6:2332–41.
- [175] Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of Toll-like receptors. *Clin Immunol* 2006;120:260–71.
- [176] Zhao M, He X, Bi XY, Yu XJ, Gil Wier W, Zang WJ. Vagal stimulation triggers peripheral vascular protection through the cholinergic anti-inflammatory pathway in a rat model of myocardial ischemia/reperfusion. *Basic Res Cardiol* 2013;108:345.
- [177] Koopman FA, Schuurman PR, Vervoordeldonk MJ, Tak PP. Vagus nerve stimulation: a new bioelectronics approach to treat rheumatoid arthritis? *Best Pract Res Clin Rheumatol* 2014;28:625–35.
- [178] Koopman FA, Chavan SS, Miljko S, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2016;113:8284–9.
- [179] Hone AJ, McIntosh JM. Nicotinic acetylcholine receptors in neuropathic and inflammatory pain. *FEBS Lett* 2018;592:1045–62.
- [180] Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384–8.
- [181] de Jonge WJ, Ulloa L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *Br J Pharmacol* 2007;151:915–29.
- [182] Felts RL, Narayan K, Estes JD, et al. 3D visualization of HIV transfer at the virological synapse between dendritic cells and T cells. *Proc Natl Acad Sci USA* 2010;107:13336–41.