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The role of T-lymphocytes in neuropathic pain initiation, development of chronicity and treatment

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ABSTRACT

Ongoing research has strongly suggested the role the immune system plays in the pathogenesis of neuropathic pain. T cells appear to be one of the main regulators of the immune system with many mediators appearing to promote or suppress pain resolution. Limited effective therapies are available for treatment of neuropathic pain. Treatments available appear to modulate specific T cell with altered ratios present 3 months post treatment and parallels clinical improvement. This further supports the neuro-immune basis for neuropathic pain chronicity. Identification of novel immune mediators involved in pain development may suggest new target areas in treatment.

1. Introduction

The internationally accepted definition of neuropathic pain is an abnormal pain sensation due to a primary lesion or disease of the somatosensory system. Chronic neuropathic pain (CNP) is a huge burden on healthcare systems globally due to poor therapeutic outcomes. CNP pain affects 7–8% of the European population with more than half of these patients having severe pain resulting in inability to work or function normally. Disability, loss of earnings, depression, anxiety and forced early retirement is frequently the devastating result (Global et al., 2016; Didiera et al., 2008; Attal et al., 2011). A lack of international consensus regarding diagnostic criteria in addition to a paucity of knowledge regarding the pathophysiology of neuropathic pain in vivo limits both the effectiveness of current therapeutic options and has resulted in a lack of direction for development of novel more effective therapies. The development of quantitative sensory testing and punch skin biopsy has been welcomed however it is time consuming, expensive and not available for routine use. While radicular pain can be confirmed with MR imaging demonstrating nerve root compression, this does not exclude axonal disease or central effects. Most patients treated pharmacologically experience significant deleterious side effects resulting in poor therapeutic compliance. Most drugs have high NNT/NNH ratios (number needed to treat and number needed to harm). This ratio describes two statistical concepts measuring the effect of a given medical therapy on one person. Contraindications and side-effects exclude many patients from effective medical therapy. Lack of effectiveness of current pharmacological options has led to the management of CNP being predominantly interventional with associated costs (Attal et al., 2010; Finnerup et al., 2015). Interventional treatment such as pulsed radiofrequency to the dorsal root ganglion (DRG PRF) and spinal cord stimulation (SCS) are the only therapies to demonstrate effective and long-term relief but access to this therapy is limited. Ongoing research has demonstrated these therapies do have at least in part an immunomodulatory mechanism of action (Das et al., 2018; Royds et al., 2020a, 2020b; Moore et al., 2020; Laumet et al., 2019a). It has previously been shown that neuroimmune modulation and activation of the central nervous system occurs in response to stressors producing cytokines, interleukins and other mediators involved in pain processing (Deleo et al., 2004; DeLeo, 2006).

CNP is associated with a several other symptoms including sleep disturbance, anxiety, lethargy and depression. This is described as sickness behavior and is extremely common in patients with CNP. Pain is one of the four cardinal features of inflammation, and it was these observations that led to the realization that pathophysiology of CNP had an immune component to it and the magnitude of this is now being identified by clinical research (Konsman et al., 2002).

The immune system consists of a complex array of interactions in both innate and adaptive immunity. T cells are one of the main regulators of the adaptive immune system depending on their secreted inflammatory products which determine whether these T cells have a pro- or anti-nociceptive role. Recent evidence suggests that patients with chronic

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neuropathic pain have a different T cell phenotypic profile versus a control population (Luchting et al., 2014, 2015).

As CNP is a common and debilitating clinical problem with limited therapeutic options it is imperative that we develop our understanding of the pathophysiology of this disease to improve outcomes for patients. Whilst there is significant pre-clinical evidence supporting the role of immune activation and in particular T cell activity in nerve injury this article will explore the in vivo human evidence regarding the role of T cells in CNP. This article will describe the clinical evidence supporting the hypothesis that T cells play a major role in human CNP in vivo by examining both the T lymphocyte phenotype in a number of clinical conditions and the effect of successful interventional techniques on T lymphocyte phenotype.

1.1. Phenotypes of T-Lymphocytes

Maturation of naïve T lymphocytes (Tn) occurs early but activation only occurs upon naïve T lymphocytes being presented with specific antigens. Tn lymphocytes circulate through lymphoid organs and if they encounter an antigen, activation and proliferation to effector T lymphocytes (Teff) occurs. These cells recognise types of Major Histocompatibility Complex (MHC-1, MHC-2) which are presented by antigen presenting cells (APCs) for example $CD4^+$ T cells recognise MHC-2 on specific APC's (macrophages and microglia). When activated, these $CD4^+$ T cells become T-helper (Th) cells which function to help cells from both innate and adaptive immune systems to optimise their immune response. $CD4^+$ cells further differentiate into Th1, Th2, T17 and T regulatory cells (Tregs). (Luchting et al., 2014, 2015).

1.1.1. $CD4^+$ T cells

Th1 and Th 17 cells act largely as proinflammatory whilst Th2 and Treg cells act as anti-inflammatory cells. Each of these subsets differ in their pattern of cytokine production and selection of transcription factors when activated:

- Th1 expresses T-bet, Signal transducer and activator of transcription 4 (STAT 4), interferon Gamma (IFN gamma), interleukin-2 (IL-2)
- Th2 expresses STAT 5, IL-4, IL-10, IL-13
- Th17 express ROR-gamma T, IL-17
- Treg express Forkhead box P3 (FOXP3), tumour growth factor Beta (TGF beta), IL-10

Th1 cells lead to a **cell-mediated response** with activation triggering the release of IL-2 and IFN-gamma and consequent activation of macrophages (pro-inflammatory response)Th2 cell activation leads to a **humoral response** triggered by cytokine release and increased activity of B cells, eosinophils and mast cells (anti-inflammatory response).Th17 cell activation produces IL-17 with Treg cells producing IL-10, having opposite effects.

1.1.2. T regulatory cells

Treg cells have an interesting role as they act to suppress other cells involved in immunity including other T cell subsets. Their cytokines have a regulatory role in co-activation and suppression of other T-cells and their respective cytokines. High concentrations of IL-12 and IFN-gamma instruct naïve T cells to differentiate into a Th1 profile, while IL-4 and IL-2 promote Th2, IL-6 and IL-21. TGFb instruct toward Th17 subset differentiation. The anti-inflammatory cytokine TGFb turns the cells toward the Treg maturation line.

1.1.3. $CD8^+$ T cells

The $CD8^+$ T cells differentiate into cytotoxic T cells and suppressor/regulatory T cells. Typically, the role of the $CD8^+$ T cells is to kill viruses and tumour cells. Many phenotypes of $CD8^+$ T cell are known including activating, suppressing and regulatory $CD8^+$ T cells as well as effector and memory CD8+T cells but their effects and function in neuropathic pain chronicity have not been fully investigated to date.

Tn cells respond to antigen activation by proliferating to T cells most of which die after antigen removal. However, some will become memory T lymphocytes (Tm) that may reside centrally or in peripheral tissue. Small subsets of Tm cells circulate in the blood and will be become effector cells Teff (Teff) if activated by further antigen or inflammation (Totsch and Sorge, 2017).

1.1.4. Natural Killer cells

Natural killer cells (NK cells) are a distinct lineage of immune cells, which make up 10–20% of the circulating lymphocytes in the body. They play a large role in the innate and adaptive immune system. Direct cytotoxicity (without receptor requirement) and secretion of immunoregulatory cytokines and chemokines are the two main mechanisms of NK cell function. NK cells do not express CD3 or CD4 (surface markers of T-cells) but they express levels of CD56 and CD16 depending on the subset type (Zhu and Paul, 2008).

1.2. Role of T lymphocytes in development of neuropathic pain

1.2.1. Acute

In naïve dorsal horn, spinal cord and dorsal root ganglia, there are small numbers of surveillance glial cells, major histocompatibility complex (MHC)-associated markers and T cells present for macrophage activation. When a peripheral nerve injury occurs, endothelial activation results in release of many mediators including bradykinin; histamine, adenosine triphosphate, cytokines (C3a, C5a, PGE2, IL-1b, IL-6 IL-17, IFN-gamma and TNF-alpha and others) and neurotrophins. This results in neuronal release of an injury-activated cytokine called fractalkine or CX3C. Fractalkine receptor (CX3CR1) -expressing monocytes and macrophages are activated and facilitate neuronal “cross talk” producing nociceptive sensitisation with release of free radicals, activation of transient receptor potential ankyrin 1 (TRPA1) channels producing an acute pain response. An increased cell density of MHC is observed which likely represents increased glial cell and macrophage number and activity and increased active T-cells (both pro- and anti-inflammatory) in the DRG (Gadani and Walsh, 2015; Yu et al., 2020). This is the maintenance phase which can occur as soon as one week post injury. Th-1 lymphocytes congregate at the injured peripheral nerve driving acute inflammation.

1.2.2. Chronic

Higher numbers of activated T cells and macrophages cells are present in DRG and the dorsal horn compared to controls three months after nerve transection (Du et al., 2018). In the DRG support for cross talk between the immune system and the injured neuron following nerve injury leads to a release of extracellular vesicles by neurons which are then engulfed by macrophages. This increased activity is in response to peripheral sensitisation mechanisms from peripheral nerve injury (Kwon et al., 2013).

Luchting investigated levels of T lymphocyte subsets and cytokines in peripheral blood of patients with neuropathic pain compared to healthy controls. The study found that levels of T-cell mediated pro-inflammatory cytokines were elevated in patients with neuropathic pain. Additionally, the ratio of cytokine and $CD4^+$ T lymphocyte profiles were largely proinflammatory (compared to controls). A spontaneous increase in activity of central neuronal pain pathways was observed also (Luchting et al., 2015).

1.3. Clinical evidence for T lymphocyte activation in chronic neuropathic pain

There is a paucity of clinical studies investigating the role of T cells in CNP due to ethical restricions and technical challenges. A modest amount of observational in vivo experience is available and this is hugely relevant

from a translational medicine point of view. There is evidence in vivo for T-lymphocyte activity in HIV neuropathy, Diabetic peripheral neuropathy, lumbar back pain, complex regional pain syndrome (CRPS), Herpes Zoster Neuralgia, Polyneuropathy (PAIN article) and Chemotherapy induced peripheral neuropathy (CIPN).

1.3.1. HIV neuropathy

Examination of CSF and blood in matched patients with HIV peripheral neuropathy vs. HIV patients without peripheral neuropathy (PN), identified activated $CD8^+$ T-lymphocytes in greater quantities in the CSF of HIV patients with PN versus without PN. Similar levels of $CD4^+$ T lymphocytes were found in both groups in both CSF and blood. This further suggests that activated $CD8^+$ lymphocytes accumulate in CSF of HIV patients with neuropathy states (Wang et al., 1097).

1.3.2. Diabetic peripheral neuropathy

Approximatively 25 times more $CD3^+$ T cells were counted per section in sural nerve biopsies compared to control patients. The infiltrated T cells were mostly $CD8^+$ T cells and $CD25^+$ cells, an indication of either $CD4^+$ or $CD8^+$ Treg cells (Agarwal et al., 2018).

1.3.3. Radicular lumbar back pain

Peripheral blood samples of patients with radiologically confirmed lumbar disc herniation versus healthy controls demonstrated elevated $CD4^+$ T lymphocytes and CD4+/CD8+ lymphocytes counts in the herniated disc group compared to the control group, ($P < 0.05$). There were also statistically significant differences between both groups in the counts of $CD3^+$ and $CD8^+$ T lymphocytes ($P < 0.05$). (Tian et al., 2009).

Investigation of peripheral blood levels of Th17 and IL 17 in two groups (non-ruptured lumbar disc and ruptured lumbar disc) compared results to healthy controls demonstrated a significantly increased Th17 and IL 17 in both disc groups. Levels were greater in patients with ruptured discs. Higher VAS scores directly correlated with higher levels of Th17 and IL 17 in the ruptured disc group (McLachlan and Hu, 2014; Cheng et al., 2013).

This suggests that T lymphocyte alterations occur with painful disc herniation and rupture and may contribute to pain experienced by patients.

1.3.4. Complex regional pain syndrome

Examination of serum in patients with CRPS versus health controls demonstrated an expansion of central memory $CD8^+$ and $CD4^+$ T lymphocytes without changes to naïve and effector subsets and increased number of Th1 and Treg cells - 4.98-fold and 2.18-fold respectively. The presence of increased numbers of long-lived central memory $CD4^+$ and $CD8^+$ T lymphocytes may indicate a chronic inflammatory process with ongoing cellular damage in CRPS (Russo et al., 2019).

In patients with Herpes Zoster neuralgia, examination of CSF demonstarted high $CD8^+$ levels, which were assocaited with pronounced central sensitisation as compared to patients with polyneuropathy where less central sensitisation was noted. $CD8^+$ cells have been identified as infiltrating ganglia in patienst with herpes zoster and Varicella antigens induce T cell activation (Lassen et al., 2021).

1.3.5. Chemotherapy-induced peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) is an unpleasant and persistent side-effect resulting from platinum-based chemotherapy. This is mediated at least in part by Toll like receptor 4 (TLR4) activation and increased monocyte chemoattractant protein-1 (MCP-1) in patients with CIPN, both of which are T-cell mediated proinflammatory mediators. Intrathecal injection of MCP-1 neutralising antibodies reduced platinum-induced macrophage recruitment into the DRG and also blocked the behavioural signs of CIPN (allodynia) (Li et al., 2014). Intrathecal treatment with the TLR4 antagonist lipopolysaccharide-RS (LPS-RS) blocked mechanical hypersensitivity, reduced MCP-1 expression, and blocked the infiltration of macrophages into the DRG in platinum rats (Laumet et al., 2019b).

Intrathecal (IT) injection of $CD8^+$ cells worsened hypersensitivity in rodents with CINP but IT Treg and anti $CD8^+$ injection transiently reduced mechanical allodynia.(Liu). $CD8^+$ cells with prior exposure to platinum chemotherapy appeared capable of improving symptoms of CIPN, suggesting that "educated" $CD8^+$ T cell may be a therapeutic target for treatment in the future (Krukowski et al., 2016).

1.4. Central neuroimmune effects of recognized pain treatments

Examination of the effect of recognized therapies in cerebrospinal fluid in patients with CNP will provide insights in mechanisms of CNP and chronicity. Three pain treatments to date have shown to be clinically effective in the treatment of neuropathic pain – Tricyclic antidepressant treatment, Pulsed radiofrequency to the dorsal root ganglion (DRG PRF) and Spinal Cord Stimulation (SCS).

1.4.1. Tricyclic antidepressants

Our group has shown that treatment with Amitriptyline and nortriptyline significantly lowers frequencies of both naïve and mature $CD8^+$ T-cells after treatment with amitriptyline or nortriptyline or a combination of both compared to control. Similarly, the frequencies of naïve and mature $CD4^+$ T-cells were also significantly lower following combination drug treatment. Significantly lower frequencies of IFN-γ-producing $CD8^+$ T-cells were observed with all treatment combinations ($p < 0.05$) and frequencies of IL-17-producing $CD4^+$ and $CD8^+$ T-cells were significantly lower following amitriptyline treatment ($p < 0.05$). Frequencies of Natural Killer T-cells were significantly higher following treatment with nortriptyline ($p < 0.05$). Amitriptyline therapy for 8 weeks resulted in a >30% reduction in pain after treatment. This data indicates that both amitriptyline and nortriptyline modulate the phenotype and function of T-cells (Royds et al., 2020b).

1.4.2. Pulsed radiofrequency of dorsal root ganglion

DRG PRF is a minimally invasive, xray-guided pain procedure, performed on an day-care basis for patients with radicular pain. It consists of a high-frequency alternating current applied to a dorsal root ganglion in short high voltage bursts (20 msec) followed by silent periods (480msec) to allow for heat dissipation, keeping the surrounding tissues less than 42 °C. Van Zundert demonstrated a better outcome compared to placebo for patients with cervical radicular pain treated with DRG PRF (Van Zundert et al., 2007). A similar study investigating thoracic DRG vs paravertebral nerve DRG in post-mastectomy pain syndrome showed greater effectiveness with thoracic DRG PRF (Hetta et al., 2020).

We examined the proteomic CSF profiles both pre- and post DRG PRF therapy in patients with chronic neuropathic pain (CNP). Quantification of CSF lymphocytes, levels of particular cytokines, chemokines and growth factors were analysed pre and post treatment. Data revealed significant reductions in CD56, CD3, NK cell frequencies and IFN-γ levels in treatment responders, while CD8 T cell frequencies and IL-6 levels were increased. IL-17 inversely correlated with post-treatment pain severity score and pre and post-treatment pain scores. These results further support the concept that chronic radicular pain is a centrally mediated neuroimmune phenomenon and the mechanism of action of DRG PRF treatment is immunomodulatory (Das et al., 2018).

A triple-blinded randomised controlled trial compared patients receiving PRF to DRG vs sham therapy. CSF cellular and peptide constituents were investigated. Overall, the results showed PRF therapy resulted in a significant reduction in pain score (Numerical Rating Scale) at 3 months (6.8–2.6, $p < 0.05$). PRF reduced the TNF-α concentration and $CD3^+$ count in CSF with a reduced CD4/CD8 ratio in patients with CNP (compared to historical controls 1.4 versus 3.0–4.2). The majority of $CD3^+$ cells in the NP patients were activated effector memory cells (80%) versus the surveillance central memory cells (85%) seen in healthy controls (Moore et al., 2020).

1.4.3. Spinal cord stimulation

Spinal cord stimulation (SCS) is an implantable, non-pharmacological method of treating neuropathic pain. Despite multiple mechanisms of action proposed, the exact mechanism remains unknown. Activation of spinothalamic and dorsal column tracts play a role as well as modulation of spinal interneurons and neurotransmitter activation. The role of neuroimmune system, specifically glial cell activation, in neuropathic pain and SCS modulation is now becoming clear. Our group investigated patients with neuropathic pain who had an SCS placed as treatment. CSF was sampled prior to implant of SCS and following 8 weeks of continuous Burst-SCS. Results showed a reduction in pain of >50% following 8 weeks of Burst-SCS. Analysis of the CSF proteome indicated a significant alteration in protein expression most related to synapse assembly and immune regulators. There was significantly lower expression of the proteins: growth hormone A1 (PRL), somatostatin (SST), nucleobindin-2 (NUCB2), Calbindin (CALB1), acyl-CoA binding protein (DBI), proSAAS (PCSK1N), endothelin-3 (END3) and cholecystokinin (CCK) after Burst-SCS.

This suggests reduction in central excitability most likely related to neuroimmune regulators and synapse assembly due to the alteration in the protein expression levels of CSF proteins post implantation of a SCS device (Royds et al., 2020a; Sato et al., 2014).

1.5. T lymphocyte quantification

Since recognition of the neuroimmune interaction in the pathogenesis of neuropathic, trials have been performed in both animal and clinical models investigating the effects of pharmacologically altered T lymphocyte levels utilising the method of flow cytometry which allows detailed cellular quantification of CSF.

1.5.1. Flow cytometry

Flow cytometry is a laboratory technique which allows rapid analysis of single cells or particles as they pass multiple lasers whilst suspended in a buffering solution. Each particle or cell allows for analysis of visible light scatter and one or multiple fluorescence parameters. Samples are prepared for fluorescence measurement by staining with fluorescent dyes or staining with fluorescently conjugated antibodies (McKinnon, 2018).

Flow cytometry analysis of healthy cerebrospinal fluid has been performed by de Graaf in 2011. This study showed a predominance of $CD4^+$ T lymphocytes, mostly with a central memory phenotype with very low frequencies of B lymphocytes, NK cells, and NK T lymphocytes (de Graaf et al., 2011). A seminal paper by Svenningsson et al. analysed CSF of 18 healthy individuals using two and three-colour flow cytometry to investigate the distribution of lymphocyte subpopulations in CSF and their phenotypic characteristics were investigated. This paper also found $CD4^+$ T lymphocytes constituted the vast majority of CSF lymphocytes while the number of B lymphocytes and NK cells were low. Most T lymphocytes exhibited the phenotype of memory cells in both the $CD4^+$ and $CD8^+$ subpopulations. Two markers for recent activation, HLA-DR and interleukin-2 receptor were not upregulated when compared with peripheral blood in the majority of CSF T lymphocytes. However, a fraction of T lymphocytes co-expressing the NK cells markers CD56 and/or CD16 showed a pronounced upregulation of HLA-DR in CSF as compared with peripheral blood. This study documents that the cellular composition of the normal CSF differs profoundly from peripheral blood regarding all major lymphocyte subpopulations (Svenningsson et al., 1995). This is taken into account in studies addressing questions regarding cellular immune reactions in the central nervous system under pathological conditions. In comparison to peripheral venous blood, which has a predominance of $CD3^+$ T lymphocytes and a low ratio of CD4/CD8 ratio, CSF lymphocytes constitute a dominance of $CD4^+$ T lymphocytes versus B lymphocytes and NK cells and an increased CD4/CD8 ratio.

1.5.2. Mass cytometry - CytoF

A newer technique for CSF cellular characterisation is a combination of Mass spectroscopy and flow cytometry. Mass cytometry is related to flow cytometry but uses metal ion labels instead of fluorochromes. With virtually no overlap between mass spectra, multidimensional data acquisition of more than 100 parameters per cell is possible (usually in the range of 30–60) allowing a throughput of up to 500 cells per second (Yao et al., 2014). This is attractive for interrogation of immune responses when sample sizes are limited (<10,000 cells). This is a practical concern when using flow cytometry for CSF as a limited amount of CSF is available for analysis and can make data acquisition and reproducibility difficult.

1.6. Future areas of investigation

1.6.1. Checkpoint inhibitors

1.6.1.1. PD-1/PD-L1 role in pain chronicity. Progressive death T-cell receptor (PD-1) is a receptor produced by T-cells in response to a particular ligand found on the surface of certain cells, mostly tumour cells. PD-L1 has been found in both normal neuronal tissue of the DGR and in tissue from melanoma. When administered to mouse models, it has inhibited both acute and chronic pain. Similarly, when PD-1 blockers were administered to mice, mechanical allodynia was experienced. PD-1/PD-L1 complexes form, which deactivates T-cell activity and inhibits innate human immunity mediated by T-cells. This allows proliferation and a pro-inflammatory state in the body. PD-1/PD-L1 complex is mostly seen in certain tumours eg small cell lung cancers, certain melanomas. Presence and deactivation of T-cell mediated PD-1 by binding PD-L1 allows early tumours to proliferate and expand without innate immunity activation. Clinically, this is seen as patients presenting later with initially painless advanced cancers that become painful only when metastasis are present. This raises the question of what role PD-1/PD-L1 complex plays in pain signaling pathways. The mechanism of endogenous PD-1-mediated pain signaling may suggest a novel pathway in the pathogenesis of pain (Chen et al., 2017; Hirth et al., 2017). This finding identifies an area for further investigation with the potential for a new treatment target. Furthermore, identification of PD-1 in patients with neuropathic pain may act a biomarker. This would provide an additional diagnostic tool for neuropathic pain, in addition to history, clinical examination and positive MRI findings.

1.6.2. Induction of educated $CD8^+$ cells

Many pathways exist which influence the fate of T cells. Potential "refate" of these pathways may result in an increase in the ratio of anti-inflammatory T cells and their mediators. In vitro "educated" autologous $CD8^+$ cells may theoretically function in patients more susceptible to development of neuropathic pain eg patients at risk of CIPN (Laumet et al., 2019b).

Treg response can also be amplified with the use of a B7 receptor agonist causing co-stimulation of $CD28^+$ cells. This has shown to reduce the numbers of macrophages at the peripheral site of nerve injury with decreased activation of astrocytes and microglia in the dorsal horn of the spinal cord (in chronic constriction sciatic nerve injury of mouse model). (Sorge et al., 2015).

T cell pathway modulating vaccination mediates recruitment of T cells to the site of peripheral injury and modulates production of $CD4^+$ producing IL-10. Theoretically this may reduce development of neuropathic pain but these have not been tested in neuropathic pain models (Austin et al., 2012).

1.6.3. Fractalkine

Fractalkine is an essential T cell-mediated chemokine which may be the link allowing cross-talk between peripheral injured neurons which activate T-cells peripherally and result in activation of satellite glial cells

centrally. Further investigation regarding the role fractalkine plays in patients with neuropathic pain should be studied (Montague-Cardoso et al., 2020).

1.6.4. Kyneurine pathway

The kyneurine pathway (KP) is a key pathway involved in tryptophan catabolism. It now has a recognized pro-inflammatory role in certain neuroinflammatory disorders (Alzheimers, Multiple sclerosis, amylotropic lateral sclerosis) and appears to be another link between the immune system, inflammation and neurological conditions. Activation of the KP pathway appears to be linked to pro-inflammatory mediators with IFN-gamma being the most extensively investigated to date. Raised levels of a key enzyme indole-2-3-dioxygenase (IDO) were found in the spinal cords of rat models after day 7 of a chronic constriction injury, consistent with KP pathway. The same study demonstrated that the repeated intraperitoneal administration of minocycline not only attenuated tactile and thermal hypersensitivity but also diminished the levels of ID0, suggesting a possible role for KP blockers or IDO blockers (mediated by cytokine-producing T-cells). (Rojewska et al., 2018).

1.6.5. Micro RNA

The molecular mechanisms underlying T cell alterations in neuropathic pain have been investigated. It is known that SIRT1 controls Treg differentiation and function (i) by promoting Foxp3 gene expression and (ii) by preventing Foxp-3 degradation. A recent study showed that targeting of SIRT1 by specific miRNAs (miR-124a and miR-155, expressed in pain and inflammatory syndrome) promoted Treg differentiation of human $CD4^{+}$ T cells in patients with chronic pain (Li et al., 1016). Further investigation into identification and interaction of miRNA and their target pathways in inflammation may represent further understanding of neuropathic pain development.

2. Conclusion

There is emerging clinical evidence supporting the hypothesis that the pathogenesis of chronic neuropathic pain is a centrally-mediated neuroimmune phenomenon. Clinical research has shown T lymphocyte activity appears to have a significant role in this pathogenesis. Identification of a T lymphocyte signature in chronic neuropathic pain may allow us to develop an easy to use biomarker and facilitate the development of new therapeutic options and optimisation of current interventional therapy. This will shift treatment focus to a disease-modifying approach and improve outcomes for patients. There is growing clinical evidence demonstrating that the mechanism of action of Pulsed RF to the DRG and SCS involves central immunomodulation in the responder population. Larger scale clinical research is urgently required in this field.

Declaration of competing interest

I declare no conflict of interest with this paper.

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