

Inside the pain lab

Research led by **Dr Ji Zhang** into the immune aetiology of chronic pain is leading to the discovery of a new treatment strategy and drug targets for chronic pain patients



Could you begin by summarising the central aims of your research programme?

The overall aim is to investigate the immune factors that contribute to the aetiology of neuropathic pain by exploring the interactions between injured neurons and their surrounding non-neuronal cells, and the impact of glial/immune cell activation on pain behaviour. Better understanding of how other cells in the environment dynamically influence neuronal signal transmission will advance mechanistic studies of chronic pain and open new avenues of effective treatment.

How might reducing inflammation be a strategy for relieving chronic pain?

Neuropathic pain represents heterogeneous conditions, which can neither be explained by a single cause nor by a specific anatomical lesion. Until now, most of our results have been generated using animal models of nerve injury-associated pain, where a robust inflammatory response peaks shortly after injury, spreading from the periphery to the centre. We suggest considering an anti-inflammation treatment as early as possible after the trauma or post-surgery, which can prevent or lessen secondary neuronal damage associated with this inflammatory reaction and reduce the chances of developing neuropathic pain. At the chronic stage, inflammatory response is

reduced to low levels; activated glial/immune cells are fewer than at the acute phase, but these cells are always involved in the abnormal pain behaviour. Whether these non-neuronal cells continue to elicit neuropathic pain through inflammatory mechanisms, and to what extent their involvement is critical in its persistence, are not fully established. Thus, it is a little early to make predictions about the efficacy of anti-inflammation in long-term chronic pain patients; and for other types of pain, the role of inflammation might be different. In one of our pilot animal studies, we observed the potential of a drug targeting inflammation for relieving neuropathic pain associated with diabetic neuropathy at both early and late stages.

What are the implications of identifying the origins of activated spinal microglia?

Our discovery that macrophages infiltrate and differentiate into fully functional microglia in the CNS parenchyma opens a door to new therapeutic strategies. Bone marrow-derived macrophages, innately attracted to the spinal cord dorsal horn, ipsilateral to the nerve injury, can be used as cellular vehicles to deliver anti-inflammatory agents limiting microgliosis and neuronal hypersensitivity. Targeting glial cells in treating pain is not only a new concept – it is also an exciting new drug-delivery technology, and a similar approach might be used to treat neurodegenerative diseases in which bone marrow-derived cells massively infiltrate the affected regions.

To what extent can your finding on CCR2 – that expression in either resident- or bone marrow derived-microglia is sufficient for the development of neuropathic pain in mice – be translated to human models?

In mice deficient in the MCP-1 receptor, CCR2, microglial activation did not occur post-injury, and hypersensitivity to mechanical stimuli was attenuated compared with controls. Hypersensitivity was not decreased by selective knockout of CCR2 on either CNS-resident or bone marrow-derived microglia alone, leading us to conclude that both CNS-resident and bone marrow-derived microglia should be targeted to relieve pain. This would require

the development of a compound which can easily reach CNS to inhibit both peripheral and central inflammation, and normalise neuronal and non-neuronal cell functions. Some pharmaceutical companies are already validating CCR2 antagonists, while other new technologies, such as using stem cells or nano-carriers as non-invasive delivery systems, are also in development.

Could you explain the genetic and pharmacological approaches you used to inhibit either central or peripheral inflammation associated with nerve injury?

We have tested the development of pain in mice where some genes relating to inflammation are deleted:

- In TLR2 KO mice, nerve injury-induced thermal hyperalgesia is abolished, whereas mechanical allodynia is partially reduced. The role of TLR2 in nerve injury-induced neuropathic pain is essentially mediated through macrophages in peripheral inflammatory response, as the infiltration of macrophages into the injured nerve is severely impaired in TLR2 KO mice
- Mice lacking both IL-1 β and TNF or both IL-1 type 1 receptor and TNF type 1 receptor show reduced nociceptive sensitivity compared with wild-type littermates after injury
- Neither CCR2 KO nor CCR5 KO mice develop neuropathic pain following nerve injury. Spinal microglial activation in these mice is almost completely abolished

We have also used several pharmacological approaches such as oral administration of a CCR2/CCR5 dual antagonist (RAP-103), peripheral (intraneural) or central (intrathecal infusion) injection of anti-inflammatory cytokine TGF- β 1, and intraperitoneal or oral administration of statins – a cholesterol-lowering drug with powerful anti-inflammatory properties. Pain attenuation using this treatment is associated with reduced inflammatory response in either the periphery or spinal cord. All methods reveal that anti-inflammation is a promising strategy in the fight against debilitating neuropathic pain.

Going to great pains

The Pain Immunology Lab in Montreal is investigating whether targeting one or more inflammatory pathways involved in the development of neuropathic pain could offer a route to new therapeutic strategies

NEUROPATHIC PAIN AFFECTS 2 to 3 per cent of the adult population and results from injuries or disorders of the peripheral or central nervous system. It is problematic because of its severity and chronicity – that is, persistent pain underlies the patient's disability after an injury has healed. Such pain is costly to the healthcare system and devastating to individual sufferers. Furthermore, many forms of neuropathic pain cannot be adequately treated with conventional analgesics.

Neurons exist in a dynamic environment, surrounded by non-neuronal cells including glial, immune and endothelial varieties. For decades, pain and its modulation were thought to be mediated solely by neurons, a view now being challenged by findings highlighting the active participation of adjacent glial cells in the initiation or maintenance of pain. Either within damaged nerves or remotely in the central nervous system, injury causes immune cells to be recruited, spinal glial cells to be activated and changes in vascular structures; thus creating an inflammatory reaction involved in the pathogenesis of chronic pain.

MECHANISMS OF NEUROPATHIC PAIN

Pain research on the cellular mechanisms of the pathological hypersensitivity – such as ectopic or spontaneous nerve activity, peripheral and central hyperexcitability, neuronal phenotypic changes in pain-conducting pathways, secondary neurodegeneration, and morphological reorganisation – has led to major successes.

Dr Ji Zhang is an Assistant Professor at the Alan Edwards Center for Research on Pain at McGill University in Montreal. Her current research at the Pain Immunology Lab focuses on the pathogenesis, behaviour and relief of chronic pain.

"Injuries which damage the neuronal pain transmission pathway also disturb their

neighbours," outlines Zhang, "and following an injury to the nerve, the integrity of the neuronal pathway microenvironment is altered. Studying only neuronal transmission from the periphery to the centre or from one synapse to another is incomplete, as the influence of non-neuronal cells on neuronal transmission can be significant."

The shift in understanding provides an incredible opportunity for a new therapeutic approach to neuropathic pain

Indeed, improving our understanding of how non-neuronal cells influence neuronal signal transmission should advance mechanistic studies of chronic pain. Inflammation mediated by non-neuronal cells can directly enhance the excitability of neurons and contribute to axonal damage, some changes on the neuronal pathway being secondary to the inflammation. Exploring the dialogue between neurons and surrounding non-neuronal cells, and the consequence of this interaction in pain development, provides a holistic view of the mechanisms of abnormal pain. Zhang's findings are leading to increased awareness of the contribution of immune and inflammatory systems; in fact, she believes that non-neuronal cells are important, not negligible players in the pathogenesis of neuropathic pain. They actively modulate neuronal pain transmission.

"Neurons live with glia; they must be together," she explains. "We have sufficient evidence from preclinical studies to show

that nerve injury triggers an inflammatory reaction involving non-neuronal cells from the periphery to the centre, and the inhibition of inflammation reduces abnormal pain."

THE ROLE OF MICROGLIA AND MACROPHAGES

The Pain Immunology Lab has made some groundbreaking discoveries in recent years. Zhang's team was the first to demonstrate that, following peripheral nerve injury, blood-borne macrophages with the ability to cross the blood-spinal cord barrier infiltrate spinal parenchyma, proliferate, and differentiate into functional microglia. Either resident microglia or bone marrow-derived microglia is sufficient for the development of neuropathic pain, the effective relief of which requires the targeting of both CNS-resident microglia and blood-borne macrophages.

More recent work has shown that an injury to the peripheral nerve alters the integrity of the blood spinal cord barrier, the breakdown of which allows the penetration of systemic inflammatory molecules and immune cells into the spinal cord, enhancing central inflammatory reaction. In addition, the blood nerve barrier – an important component in nerve homeostasis – is also disrupted, exposing the nerve to the general circulation. The group has also provided a deeper insight into the role of macrophages, which are crucial in the inflammatory cascade during the process of degeneration and regeneration following an injury to the peripheral nerve.

"Our results reveal that within injured nerves, only small, round-shaped macrophages are able to secrete cytokines and chemokines, while large, foamy phagocytic macrophages lack this function," Zhang reveals. "Selectively reducing cytokine- or chemokine-secreting macrophages can attenuate neuropathic pain, without delaying the

INTELLIGENCE

PAIN IMMUNOLOGY LAB

OBJECTIVES

- To characterise the glial phenotypes in the circumstance of inflammation, nerve injury and chronic neurodegenerative diseases, and the correlation with pain behaviour
- To examine the impact of neuron-glia, neuron-immune interaction in the pathogenesis of chronic pain
- To develop a new therapeutic strategy by targeting both spinal glial cells and bone marrow-derived macrophages for an effective pain relief

KEY COLLABORATORS

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processes of nerve regeneration and scar-removal undertaken by the phagocytic macrophages.”

INCREDIBLE OPPORTUNITY

Current, frequently-used treatments for neuropathic pain include tricyclic antidepressants, gabapentin, pregabalin, serotonin-norepinephrine reuptake inhibitors, tramadol, opioids and topical lidocaine. Yet, despite the arrival of newer drugs and the rationale for combination therapies, the general response among patients to most treatments is modest at best. While the role of immune and glial cells in the development and persistence of pain post-injury challenges the conventional theory that neural activity is solely responsible for the changes behind neuropathic pain, the translation of such knowledge into clinical use for human beings remains largely underexplored.

“This shift in our understanding provides an incredible opportunity to progress to a new therapeutic approach beneficial for millions of people suffering from neuropathic pain,” reflects Zhang. “Limited evidence is currently available on the role of inflammation in persistent neuropathic pain states in humans, and very few clinical studies have tested immunosuppressive drugs, or drugs interfering with glial functions, on neuropathic pain.”

The efficacy of some anti-inflammatory compounds, such as Anakinra, Etanercept and Infliximab, is limited to pain associated with rheumatoid arthritis and other inflammatory conditions, and considering that current treatments offer only moderate relief alongside the undesirable and sometimes severe side effects, there is a pressing need to develop new therapeutics. Targeting one or more inflammatory pathways involved in the development of neuropathic pain is an exciting strategy, but large, high-quality, randomised clinical trials on various anti-inflammatory molecules are needed to determine whether glial and immune cell-mediated inflammation can be leveraged to help treat this type of pain.

TEAM ZHANG

Zhang’s group comprises several PhD students whom she credits for much of the lab’s success. These include Stefania Echeverry, who has contributed significantly to an understanding of spinal microglia activation, the integrity of the blood-spinal cord barrier and spinal inflammatory response; Seung Hwan Lee, who is focusing on the roles of macrophages in peripheral inflammation within the nerve; and Tony Lim, who is characterising changes in vascular structures following nerve injury. Zhang is also grateful to her Research Assistant Xiang Qun Shi, an expert in animal models and behavioural testing, and YiChen Wu and Hao Huang – both more recent additions to the lab.

“I should thank all members of the lab, without whose intelligence and diligence we could never have generated such a huge amount of data in only five years,” Zhang enthuses. “I feel very privileged to be working with this excellent team.”

FUTURE BENEFITS

The Pain Immunology Lab will continue at the forefront of neuroinflammation and chronic pain. The increased awareness of the contribution of immune and inflammatory systems in neuropathic pain is significant, and Zhang is particularly proud of their discovery of the importance of MCP-1/CCR2 signalling in the interaction between neurons and their surrounding non-neuronal cells: “The impact of this signalling pathway in mediating inflammation in chronic pain is a subject of ongoing research at the lab, and I believe that CCR2 antagonists are promising candidates for tackling neuropathic pain”.

In addition to furthering understanding on the crosstalk between neurons and non-neuronal cells at different stages of nerve injury-associated neuropathic pain, the team will now dedicate itself to promoting the translation of knowledge into clinical settings, so that patients will see real benefits in the near future.

