

The role of microglia *versus* peripheral macrophages in maladaptive plasticity after nerve injury

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Microglia and macrophages in the development of maladaptive plastic changes after peripheral nerve injury: Microglia and macrophages encompass the innate immune response to injury in the central and peripheral nervous systems, respectively, and are intimately involved in the pathogenesis of maladaptive changes (Tsuda, 2019). These dynamic cells can influence neuronal activity in active and quiescent states. Conflicting findings argue that peripheral macrophages facilitate the development of nerve injury-induced neuropathic pain, as opposed to central microglia (Lopes et al., 2017; Yu et al., 2020). It is imperative to discern their spatiotemporal contributions to the development and maintenance of maladaptive conditions, such as neuropathic pain (Inoue and Tsuda, 2018). The individual role of these cell types is difficult to parse out because both microglia and macrophages exhibit a keen ability to react quickly to injury and remain reactive after injury-induced changes. Appropriate methods to isolate and characterize these cells in downstream applications is necessary to uncover key findings (Agalave et al., 2020).

While the location and time points these cells mediate maladaptive changes remain unclear, it is obvious that certain neuroimmune activation pathways, such as Fractalkine (CX₃CL1)-Fractalkine receptor (CX₃CR1), high mobility group box 1 (HMGB1)-toll-like receptor 4 (TLR4), ATP-Purinergic channels, and HMGB1-protease activated receptor 2 (PAR2), are important in the neuroimmune signaling process. Interestingly, along with cell-type etiology of maladaptive plasticity, evidence points to an underlying sexual dimorphic mechanism in immune cell signaling in response to injury. This sex difference can be attributed to “biased signaling” processing in similar cell types or different cell types altogether.

Contribution of macrophages and microglia in nerve injury-induced neuropathic pain models: Several experimental nerve injury models are commonly used to study neuropathic pain, such as spared nerve injury, spinal nerve ligation, and chronic constriction injury (Figure 1A). In these models, markers of macrophage activation such as ED-1, MHC-II and Iba1 are increased from days to weeks in lumbar dorsal root ganglia (DRG) (Ristoiu, 2013). Additionally, microglia in the spinal cord exhibit similar activation kinetics as macrophages during the development of neuropathic pain following nerve injury (Ristoiu, 2013). However, it remains unclear what these cell types respective roles are in the onset and maintenance of neuropathic pain. Several inflammatory mediators such as chemokine (CC motif) ligand 2, interleukin (IL)-6, IL-1 β , matrix metalloproteinases-2, and -9, and CX₃CL1 are released following nerve injury

and facilitate the activation of macrophages and microglia (Zhao et al., 2017). Activation of these cells upregulates factors that increase central excitatory neurotransmission, decrease inhibitory tone of interneurons, and promote nociceptor plasticity (Bennett et al., 2016). During the pathogenesis of nerve injury, macrophages in periphery, and microglia in the spinal cord, polarize to facilitate inflammation, neuroprotection, and tissue-repair. These cells polarize into an M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotype. Typically, polarized macrophages are further classified into several subtypes which depend on the makeup of the inflammatory milieu (Martinez and Gordon, 2014). Despite substantive research regarding macrophage polarization in response to injury, it remains unclear how these cells, and their subtypes, are polarized during the development and resolution of injury-induced neuropathic pain. Clarifying this dynamic response to injury will help improve our understanding of sexual dimorphisms that have been implicated in neuropathic pain. Moreover, polarization of microglia in the central nervous system is controversial (Ransohoff, 2016). It is difficult to discern a distinct phenotype in these cells as they often concurrently express markers of both pro-inflammatory and anti-inflammatory polarization following injury. Noteworthy, microglia are similarly active in males and females following nerve injury, however, blocking microglial activity through inhibitors such as TAK 242 (TLR4 inhibitor), CSFR1, BDNF-TrkB and p38 MAPK shows reversal of pain behaviors only in males (Tsuda, 2019). This suggests the central immune system exhibits a “biased” signaling pathway in the development of injury-induced neuropathic pain that is different between sexes. Thus, a need exists to explore these biased-intracellular signaling cascades in the context of maladaptive plasticity.

Acute-to-chronic effect of neuropathic pain on macrophage and microglia: timing and signaling (biased): Microglia in the dorsal horn of the spinal cord facilitate chronic pain development in both sexes, but inhibiting certain second messenger systems has shown promise in males only (Tsuda, 2019). Surprisingly, no studies have found differences in altered genes between males and females in microglia after neuropathic injury, suggesting similar phenotypic profiles. It is important to note that although the authors did not map the estrus cycle over the course of the entire study, there are no differences in basal sensitivity throughout each phase of the cycle (Sorge et al., 2011). Moreover, there is evidence to suggest that levels of microgliosis are similar in the spinal cord of males in females after nerve injury and is not estrus cycle dependent (Taves et al., 2016; Tsuda, 2019). However, the

timing post-injury could reveal a therapeutic window that explains the etiology of sex differences. Based on this principle, a recent study revealed an increase in macrophage infiltration within the DRG in males 8 days after spinal nerve ligation, suggesting sexual dimorphic mechanisms in the recruitment of macrophages in the early phase of nerve injury (Lopes et al., 2017). While these immune cells are activated in both sexes after nerve injury, intracellular signaling cascades may facilitate maladaptive plasticity differently in males and females. Recent evidence highlights sex-biased signaling in various immune pathways. In males, elevated androgens enhance peroxisome proliferator activated receptor (PPAR) α expression that consequently inhibits nuclear factor- κ B activity and interferon- γ production. In contrast, estrogens in females induce PPAR γ to exhibit similar anti-inflammatory actions as PPAR α (Park and Choi, 2017) (Figure 1B). Activated macrophages in the DRG express proteins that help facilitate recruitment of various immune cells. This indicates alternative activation kinetics of the immune system where cell recruitment has a sexual dimorphic role in maladaptive plasticity. Thus, activation of nociceptors may be different in male and female following nerve injury. The mechanisms involved in the development and maintenance of neuropathic pain are different, however, the spatiotemporal role of macrophages and microglia in the DRG and spinal cord are poorly documented. A need exists to examine the early recruitment/activation of macrophages and microglia in both sexes, and how these cells' phenotypes are altered over the pathogenesis of neuropathic pain.

The importance of differentiating neuropathic *versus* post-surgery/injury (sham effects) and the importance of therapeutic window of opportunity in the early stage: Since most studies investigate the therapeutic potential of altering immune cell signaling and activation after injury-induced neuropathy has developed, we sit at an important crossroad where we are able to answer questions as to how microglia and macrophages contribute to the onset of neuropathic pain. Moreover, the maladaptive/plastic effects of post-operative pain are often overlooked in studies focusing on neuropathic pain. It is imperative to discern the etiology of neuropathic, as opposed to post-operative pain. This presents a potential point of unbiased therapeutic intervention where we may attenuate, or even prevent the manifestation of neuropathic pain behaviors in both sexes. Macrophages in the DRG and microglia in spinal cord are activated early in response to nerve injury and remain active throughout its pathogenesis. Chronic activation of macrophages and microglia facilitate recruitment of other immune cells which leads to long lasting neuroimmune interactions. This reciprocal signaling maintains chronicity in nociceptive plasticity. Moreover, our lab has shown the direct involvement of HMGB1-TLR4 signaling in female nociceptors during neuropathic pain development (Burton et al., 2019). Thus, modulating the early activation of macrophages and microglia may present a therapeutic approach to prevent neuropathic pain and avoid sexually biased signaling. However, we must first understand the respective contributions of these cells during the onset of injury to make conjecture as to how we should develop effective therapeutic approaches.

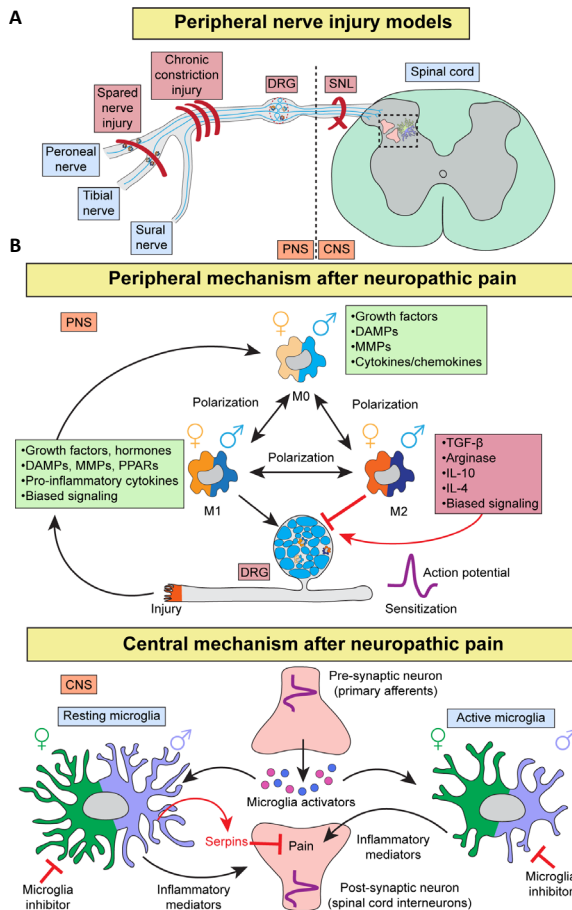


Figure 1 | A graphic depicting peripheral macrophage versus central microglia during a peripheral nerve injury and possible sex differences in biased signaling pathways.

(A) An illustration of different peripheral nerve injury experimental models of neuropathic pain. A prominent version of spared nerve injury (SN) is the ligation and transection of the peroneal and tibial nerve, leaving the sural nerve intact to be sensitized. Chronic constriction injury utilizes 3 loose ligatures around the sciatic nerve trunk. The spinal nerve ligation model ligates the spinal nerve. (B) A depiction of how early peripheral nerve injury depicts how early peripheral nerve injury recruits macrophages/monocytes to the site of injury and dorsal root ganglia (DRG), which are activated and release inflammatory mediators such as growth factors, cytokines, danger associated molecular patterns (DAMPs) and matrix metalloproteins (MMPs). These mediators bind to their respective receptors expressed on the DRGs neuron to mediate nociception. (C) A depiction of what happens at central terminals in the spinal cord where persistent activation of primary afferent neurons stimulate the release of inflammatory mediators that activate microglia in the dorsal horn of the spinal cord.

What are some differentiating factors between peripheral versus central immune cells and how they could reveal therapeutic targets? The mechanisms of microglia polarization are not well understood. Traditional views regarding immune cell polarization are not well adapted to microglia. These cells are dynamic and present a spectrum of polarization markers making it difficult to distinguish phenotypes. This is compounded by evidence that suggests microglia are similarly active during the onset of neuropathic injury in both sexes. Previous therapeutic approaches using tetracycline antibiotics to inhibit microglia were unspecific and alleviate pain only in males. More recent endeavors utilize specific markers to identify microglia, which allows for a more direct therapeutic approach. In nerve injury models, activation of Kir2.1 resulted in proliferation and activation of microglia in both males and females. Additionally, TMEM119 has been identified as a specific marker for microglia in mice and humans. This allows for clear dissection of microglia from infiltrating peripheral monocytes and perivascular macrophages (Bennett et al., 2016). Lastly, P2RY12 has also been identified

as a microglia specific marker and has been shown to be downregulated after nerve injury. Pharmacological manipulation of these proteins may help clarify the role of microglia specifically in central sensitization and maladaptive plasticity in response to nerve injury (Figure 1C).

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