Ru-Rong Ji · Jianguo Cheng ·
Jasmine Ji *Editors*

Neuroimmune Interactions in Pain

Mechanisms and Therapeutics

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Preface

Pain places an enormous burden on the healthcare system as well as the quality of life of individuals all around the world, with an economic cost over $600 billion in the United States alone. Despite this enormous financial burden on society, current treatments are inadequate and often produce severe side effects. Inflammation is the body's natural response to injury or inflection and is defined by five cardinal signs: redness, heat, swelling, loss of function, and pain. As a cardinal feature of pain, inflammation elicits pain through pro-inflammatory mediators. Meanwhile, recent evidence suggests that inflammation also leads to pain resolution by generating pro-resolving mediators, such as specialized pro-resolving mediators (SPMs), derived from molecules including omega-3 unsaturated fatty acids. When acute inflammation cannot be timely resolved, chronic inflammation will develop in many known disease conditions leading to chronic pain. While acute pain serves beneficial warning functions, chronic pain has no such protective purpose and severely degrades the quality of life of patients. A particular hallmark of the COVID-19 is the viral infection-triggered robust and widespread inflammatory responses, which could lead to the development of a wide range of new pain conditions as well as the exacerbation and prolongation of existing pains. A large body of literatures accumulated in the last three decades has demonstrated that immune system modulates pain via interactions with sensory neurons, as inflammatory mediators can either upregulate or downregulate the activity or sensitivity of pain-sensing neurons (nociceptors) in different physiological and pathological conditions. Paradoxically, some anti-inflammatory treatments, such as steroids, can inhibit acute pain but impair the resolution of pain, leading to chronic pain.

The editors have diverse research and clinical backgrounds: Dr. Ji is a basic science researcher who has been studying mechanisms of pain and neuroimmune interactions for 30 years. His research has elucidated the role of various inflammatory mediators and glial cells and immune cells in pain regulation using various animal models. Dr. Cheng is a clinician scientist with extensive expertise in managing tens of thousands of patients with diverse and complex pain conditions using multimodal and interdisciplinary approaches that include pharmacological, and non-pharmacological, interventional, and surgical treatments. Ms. Ji is a college

student majoring in neuroscience. She also has spent several years doing research projects related to pain, inflammation, and sex dimorphism in different institutes.

This book features 16 chapters that cover the key research and clinical aspects of pain and inflammation. We begin with a basic introduction to different classes of clinical pain, including nociceptive, nociplastic, and neuropathic pain in Chap. 1. These terms are important for clinical management of different pain disorders, including pharmacological, non-pharmacological, interventional, and surgical treatments. The advantages, as well as limitations of these treatments are highlighted. Finally, this chapter highlights the cause of the United States Opioid Crisis and chronic pain during the COVID-19 Pandemic, as well as patients identified as "long-haulers".

A historic perspective on inflammation and the positive and negative correlation of pain and inflammation in different disease conditions are provided and discussed in Chap. 2 and animal models that are commonly used in the study of pain are highlighted in Chap. 3. Notably, some findings and potential therapeutics that appear promising in animals have failed to translate to human pain conditions. Nevertheless, we review current methods for the measurements of mechanical pain, thermal pain, and spontaneous pain in mice and rats and highlight distinct effects of anti-inflammatory treatments in animal models of inflammatory and neuropathic pain. In Chap. 4, we discuss different types of inflammatory mediators, including but not limited to cytokines, chemokines, lipids (including SPMs), proteases, and miRNAs, and further discuss how these inflammatory mediators regulate the activity and sensitivity of nociceptors. In Chap. 5, we highlight the important role of non-neuronal cells, including immune cells and glial cells (e.g., microglia and astrocytes) in pain regulation. We also provide an update of the major research progress in the past two decades, emphasizing the change of neuron-centric view of pain control. Together, Chaps. 2, 3, 4, and 5 offer an in-depth mechanistic understanding of pain regulation by inflammation and neuroinflammation.

Sex dimorphism in pain is emerging as a hot topic in pain research. Although women suffer from more chronic pain than men, the majority of preclinical studies were conducted in male rats and mice. Compared to neuronal signaling in pain, sex dimorphism appears to be more striking in immune and glial regulation of pain. In Chap. 6, we discuss pain-related sex differences in neurons, immune cells, and glial cells and highlight sex dimorphism in neuro-immune interactions in rodent models of pain.

Itch or pruritus is also induced by inflammation and is commonly assessed by scratching behavior in rodents. Itch is frequently associated with skin injury and dermatitis. While pain can suppress itch, analgesics such as opioid can elicit itch. In Chap. 7, we discuss neuroimmune interactions in acute and chronic itch and highlight similarities and differences between pain and itch.

The Toll-like receptors (TLRs) are a family of proteins with deep evolutionary origins and play important roles in regulating both innate and adaptive immunity. Accumulating evidence suggests TLRs are critically involved in the activation of glial cells in chronic pain and itch conditions. In Chap. 8, we discuss how TLR-mediated proinflammatory signaling is coupled to pain or itch through

neuro-immune interactions. We also highlight emerging evidence that suggests non-canonical TLR signaling in sensory neurons.

In the remaining chapters (Chaps. 9, 10, 11, 12, 13, 14, 15, and 16), we focus on novel and emerging therapies, such as immunotherapies, cell and serum-based regenerative therapies, as well as non-pharmacological approaches (e.g., neuromodulation, diet, and exercise) that can effectively modulate inflammation and neuroinflammation for the management and resolution of acute and chronic pain.

Immunotherapy was developed as a method of treating various cancers through the use of the host's immune system. Immunotherapies with monoclonal antibodies against checkpoint inhibitors have saved the lives of many cancer patients. In recent years, immunotherapy is no longer limited to cancer treatment and is employed in the treatment of a wide variety of diseases. In Chap. 9, we cover several common forms of immunotherapies, as well as the emerging immunotherapies in the treatment of neurological diseases and pain.

Mesenchymal stem cells (MSCs), including those derived from the bone marrow, adipose tissue, and umbilical cord tissues, have shown long-lasting analgesic effects in preclinical models of pain including neuropathic pain. Their immunomodulatory and neuroprotective properties are distinct from the commonly assumed tissue regeneration potentials. In Chap. 10, we discuss the analgesic benefits conferred by MSCs through secreted mediators, including anti-inflammatory cytokines in animal models of pain. We also highlight extracellular vesicles and exosomes secreted from MSCs that mediate the analgesic effects previously attributed to MSCs themselves. Thus, MSCs and their secretome provide a promising treatment modality to resolve chronic pain conditions.

In Chap. 11, we discuss the preliminary evidence of MSC-based cell therapies for the management of clinical pain based on their remarkable immunomodulatory and analgesic effects in pre-clinical studies. There is a growing body of literature demonstrating promising results from randomized clinical trials for joint pain and prospective studies for neuropathic pain. The key to the success of future clinical trials is to increase the scientific rigor by using refined/standardized research protocols and cell quality control standards.

Osteoarthritis (OA) affects more than 50 million in the United States; however, surgical procedures are not options for the majority of OA patients. In Chap. 12, Dr. Buchheit discussed neuroimmune modulation-based OA treatment using platelet-rich plasma (PRP) and autologous conditioned serum (ACS). He has treated many OA patients with ACS at Duke Pain Clinic.

Lifestyle choices, such as exercise and diet, can play significant roles in mediating inflammation and consequently, pain. Healthy diets are enriched with omega-3 unsaturated fatty acids, which are precursors of SPMs that can potently inhibit pain in various animal models of inflammation. In Chap. 13, we discuss exercise and diet in the control of inflammation and pain. We highlight that exercise can profoundly change immune cell phenotypes and promote the resolution of inflammation and pain. We also speculate that a combination of exercise and health diet can facilitate the biosynthesis of SPMs and generate synergistic health benefits.

Understanding the complex mechanisms and causes of pain is crucial for precise diagnosis, adequate management, and improving outcomes. As we deepen our knowledge, new therapeutic targets are being identified, new treatment strategies are developed, and clinical management is becoming more mechanism-guided and evidence-based. In Chap. 14, we discuss current mechanism-based therapies including approaches to modulating the transduction, conduction, transmission, perception, and adaptation of pain through pharmacological, interventional, surgical, physical/psychological behavioral treatments. Increasing evidence suggests that some of these treatments not only alleviate pain symptoms but also modify disease progression through modulation of inflammation and neuroinflammation.

Neuromodulation through electrical stimulation is the most rapidly expanding area of pain management. The field is rapidly advancing in new technologies, clinical applications, and our understanding of its wide-ranging biological effects. In Chap. 15, we discuss neuromodulation to reduce the perception of pain through stimulation of peripheral nerves, the spinal column or brain, or the autonomic nervous system targets such as the vagus nerve. The most significant advancement is that, beyond its putative role of suppressing pain transmission, recent evidence indicates that neuromodulation can also influence neuroimmune mechanisms involved in the generation and maintenance of chronic pain states. We highlight novel waveforms and closed-loop systems of electrical stimulation that offer clinicians with highly configurable systems to optimize patient experience and maximize therapeutic benefits.

What do patients need to know about pain and its management? As patients begin to search such information, they may feel overwhelmed by the seemingly endless options available. In this era of social media and the Internet, there are countless “experts” with “advice” about almost everything. But, how will patients know what is fake and what is real? In Chap. 16, Dr. Rosenquist, an expert pain physician, will guide patients on the path to finding the right provider and give them direction on how they can be an active participant in their journeys to recovery.

In summary, neuro-immune interactions occur at all levels of the peripheral and central nervous systems. It has been widely recognized as one of the most important mechanisms underlying chronic pain development and maintenance. Our book will describe and discuss in detail the roles of the neuro-immune interactions in pain, inflammation, and infection, with specific focus on the non-neuronal cells such as glial cells and immune cells. Chronic inflammation and pain can be exacerbated in patients with long COVID, identified as “long-haulers”. Importantly, our book also includes multiple chapters discussing novel and emerging therapeutic strategies for effective control of inflammation for pain management.

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Contents

Chapter 1
Introduction to Acute, Chronic, and Episodic Pain

Laura Gil and Jianguo Cheng

Abstract Pain places an enormous burden on the healthcare system as well as the quality of life of individuals all around the world. With regard to the economic burden alone, it was found that the total annual cost of healthcare due to pain in the United States ranged from $560 to $635 billion. Pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Appropriate classification of pain is essential for research and clinical documentation, authorization of services, and facilitation of communications between healthcare providers as well as between clinicians and their patients. It is also important for determination of the appropriate therapeutic plan. Pain can be characterized as acute, chronic, or episodic based on its duration, and as nociceptive, neuropathic, and nociplastic based on its pathologic mechanism. We discuss current treatments such as pharmacological treatments, non-pharmacological treatments, interventional treatments, and surgical treatments, as well as limitations of these treatments. We highlight the cause of the United States Opioid Crisis and chronic pain during the COVID-19 Pandemic. COVID-19 can produce post-viral chronic pain syndromes in patients identified as "long-haulers." A particular hallmark of the COVID-19 infection is the robust, widespread inflammatory response triggered, which could play a role in the development of new pain conditions as well as the exacerbation of existing pain disorders.

Keywords Acute pain · Chronic pain · COVID-19-related pain · Definition of pain · Episodic pain · Neuropathic pain · Nociceptive pain · Nociplastic pain · Non-pharmacological treatments · Pharmacological treatments

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1.1 The Concept and Classification of Pain

Pain places an enormous burden on the healthcare system as well as the quality of life of individuals all around the world. With regard to the economic burden alone, Gaskin et al. found that the total annual cost of healthcare due to pain in the United States ranged from $560 to $635 billion, which exceeded the combined costs for cardiovascular, neoplastic, digestive, respiratory, and endocrinologic disorders (Gaskin and Richard 2022). This is largely due to the high incidence of chronic pain in the population at large. In fact, pain is one of the main reasons why patients seek medical care, with osteoarthritis and back pain being the top two painful conditions noted in a 2013 epidemiologic study by St. Sauver et al. (2013). The high prevalence of chronic pain carries further downstream ramifications, including increased risk of cardiovascular disease, gastrointestinal and hemostatic changes, diminished sleep quality, as well as higher rates of anxiety, depression, substance abuse, and disability. In fact, back pain, musculoskeletal disorders, and neck pain alone constitute three of the four leading causes of years lost to disability (Murray et al. 2013).

Recognition of the socioeconomic burden of pain has led to concerted efforts to effectively manage it. An integral part of effective management of pain is the ability to identify and properly classify the pain, which can then help guide the development of an appropriate therapeutic strategy. Thus, this chapter will serve as an introduction to pain and the appropriate classification of common painful states.

1.1.1 Defining Pain

In 1979, the International Association for the Study of Pain (IASP) proposed the definition of pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (IASP Subcommittee on Taxonomy 1979, p. 249). Since then, it has been widely adopted into practice and used by clinicians, researchers, and healthcare organizations. Despite further revisions to the IASP's publications on the taxonomy of pain in 1986, 1994, and 2011, this definition has remained unchanged. In recent years, many have advocated for an updated definition, which has been met with varying degrees of support and criticism. Therefore, in 2018, the IASP convened a 14-member panel of experts to evaluate the current definition and assess whether it should be preserved or altered. In 2020, Raja et al. published "The Revised IASP Definition of Pain: Concepts, Challenges, and Compromises" recommending the definition be changed to "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al. 2020, p. 1977).

This updated definition emphasizes the advances in our understanding of pain over the past four decades. Research into poorly understood chronic widespread pain disease states, such as fibromyalgia and headaches, has demonstrated the

possibility for pain to exist without tissue damage but instead due to altered function of pain-related sensory pathways in the peripheral and central nervous systems (PNS and CNS), causing increased sensitivity (Fitzcharles et al. 2021). In fact, microneurography studies in patients with fibromyalgia have shown that peripheral C-nociceptors in these patients exhibit hyperexcitability, spontaneous activity, and sensitization to mechanical stimuli (Serra et al. 2014). Cerebrospinal fluid (CSF) analyses in fibromyalgia patients have also demonstrated elevated levels of substance P and glutamate, two neurotransmitters known to have excitatory effects on pain receptors (nociceptors) and pathways (Becker and Schweinhardt 2012). This leads to an over-activation of *N*-methyl-*D*-aspartate (NMDA) receptors in specific spinal cord dorsal horn neurons that promote pain transmission (Littlejohn and Guymer 2020).

This definition as "an unpleasant sensory and emotional experience" acknowledges both the physical and emotional components of pain. Further investigations into the brain's pain-processing pathways have revealed two main ascending pathways – the medial and lateral pain pathways. The lateral pathways transmit the information that is most commonly associated with our understanding of pain. It involves the somatosensory cortex and processes the sensory information related to pain from the periphery, such as pain intensity, location, and character, through activation of C- and Aδ-fibers. The medial pathway involves the anterior cingulate and anterior insular cortex and is responsible for the unpleasant emotional component of pain through activation of C-fibers. A meta-analysis by Beissner el al. demonstrated that the medial pathway is involved in cognitive, emotional, somatosensory, and sympathetic autonomic processing (Beissner et al. 2013). Functional imaging studies have further supported these findings and confirmed that the structures of the medial pathways are responsible for the negative emotions associated with acute and chronic pain (Bushnell et al. 2013; De Ridder et al. 2021; Schreckenberger et al. 2005). These findings have in turn led to research on how our therapies, such as dorsal column neuromodulation, can help tamper the emotional suffering associated with painful states (De Ridder and Vanneste 2016).

1.1.2 Classification

Appropriate classification of pain is essential for research and clinical documentation, authorization of services, and to facilitate communication between healthcare providers, as well as between clinicians and their patients. As previously mentioned, it is also important for determination of the appropriate therapeutic plan. For example, nociceptive, axial low back pain may be treated with non-steroidal anti-inflammatory medications and physical therapy, while painful diabetic neuropathy would be better suited to treatment with an anticonvulsant or neuromodulation. Nevertheless, classification systems can vary significantly across different sources and can be arranged based on duration, etiology, affected anatomical system,

location, frequency, and intensity. This can produce confusion among clinicians and complicate documentation.

Given this disparity, the IASP published a classification system in 1986 in an attempt to standardize the classification of chronic pain. The second edition was published in 1994 with subsequent updates made to certain sections in 2011 and 2012. This classification system has since been implemented by the World Health Organization (WHO) through the *International Statistical Classification of Diseases and Related Health Problems (ICD)-11* that came into effect in January 2022 (World Health Organization (WHO 2022)) (Table 1.1). For the purposes of this chapter, pain will be classified per IASP guidelines as acute, chronic, or episodic based on its duration, and as nociceptive, neuropathic, or nociplastic based on its pathologic mechanism.

1.1.3 Acute Pain

Acute pain is defined as pain lasting less than 3 months, according to the new *ICD-11* coding recommendations. It tends to be sudden in onset and results from damage or injury to tissues, causing activation of nociceptive transducers. As such, the pain typically resolves after tissue damage is repaired and nociceptive input ceases. Acute pain serves an evolutionary purpose: it informs behavior in order to avoid harm and significant tissue damage. When the body perceives a painful nociceptive input (i.e., heat, cold, mechanical force, or chemical stimulation), biological mechanisms arise that retract from the environment, protect from further injury, and promote healing (Baliki and Apkarian 2015). In fact, disease processes in which individuals lack peripheral nociceptive afferents result in unrecognized infections and injuries and shortened life spans (Dubin and Patapoutian 2010).

Common examples of acute pain include postoperative pain and pain following a fracture. In these cases, direct tissue trauma leads to the release of potent inflammatory mediators. These mediators activate functionally distinct nociceptors in tissues, which then relay the information through electrical signals to higher brain processing centers. These mediators can also result in adaptive processes such as induction of hyperalgesia and allodynia of the surrounding region through decreased activation threshold of C-fibers and Aδ-fibers in an effort to avoid further harm to the area (Dubin and Patapoutian 2010).

1.1.4 Chronic Pain

Chronic pain, on the other hand, persists for at least 3 months beyond the expected disease course and healing time following the acute pathologic process. However, it does not always arise from an acute injury or pathological process. Often it has an insidious onset without an identifiable trigger, as in the case of arthritic pain and

Table 1.1 IASP *ICD-11* chronic pain classification

IASP ICD-11 chronic pain classification (possible table to include?)
- Chronic primary pain
° Chronic widespread pain
☐ Fibromyalgia
° Complex regional pain syndrome (CRPS)
☐ CRPS, Type 1
☐ CRPS, Type 2
° Chronic primary headache or orofacial pain
☐ Chronic migraine
☐ Chronic tension-type headache
☐ Trigeminal autonomic cephalgias (TACs)
☐ Chronic temporomandibular disorder pains
☐ Chronic burning mouth
☐ Chronic primary orofacial pain
° Chronic primary visceral pain
☐ Chronic primary chest pain syndrome
☐ Chronic primary epigastric pain syndrome
☐ Irritable bowel syndrome
☐ Chronic primary abdominal pain syndrome
☐ Chronic primary bladder pain syndrome
☐ Chronic primary pelvic pain syndrome
° Chronic primary musculoskeletal pain (other than orofacial)
☐ Chronic primary cervical pain
☐ Chronic primary thoracic pain
☐ Chronic primary low back pain
☐ Chronic primary limb pain
- Chronic cancer-related pain
° Chronic cancer pain
☐ Chronic visceral cancer pain
☐ Chronic bone cancer pain
☐ Chronic neuropathic cancer pain
° Chronic post-cancer treatment pain
☐ Chronic post-cancer medicine pain
• Chronic painful chemotherapy-induced polyneuropathy
• Other post-cancer medicine pain
• Post-cancer medicine pain, unspecified
☐ Chronic post-radiotherapy pain
- Chronic postsurgical or posttraumatic pain
° Chronic postsurgical pain
☐ Chronic pain after amputation

fibromyalgia. In contrast to acute pain, chronic pain is maladaptive and serves no biological purpose. Chronic pain is also unlikely to resolve with time and thus requires a different approach to its management.

Over the past few decades, we have transitioned away from the old adage that "pain never killed anyone" and learned to appreciate the immense biological and psychosocial effects that chronic pain has on the body. Pain can take an enormous toll on the body, disrupting nearly every organ system. Much like stress, chronic pain can lead to fatigue; anxiety; depression; irritability; dysphoria; weakness; lightheadedness; increased muscle tension; difficulty with memory, attention, and concentration; reduced appetite and libido; gastrointestinal changes like nausea and decreased gut motility; increased cardiac work due to elevations in heart rate and blood pressure; impaired wound healing; and non-restorative sleep (Chapman and Gavrin 1999; Fitzcharles et al. 2021; Weiner 2001). In fact, chronic pain has such a profound impact on the emotional well-being, function, and quality of life of patients that many have proposed pain as a disease in its own right rather than merely a symptom (Treede et al. 2019). Examples of chronic pain include arthritic pain, neuropathic pain, and fibromyalgia.

1.1.5 Episodic Pain

The precise definition of episodic pain is much debated. The term is often used synonymously with "recurrent pain" and "breakthrough pain." Dating back to 1983, it has been described as the acute flare-up of peripheral tissue pathology as a result of an underlying chronic pathological entity (Crue 1983). It implies recurrent, discrete, and acute episodes, such as those that occur in headaches, gastrointestinal motility disorders, degenerative disk and joint disease, collagen vascular disease, sickle cell disease, and other similar functional disorders. The temporal criteria for episodic pain vary from one disease state to another. For example, episodic migraines are characterized as those occurring on fewer than 15 days per month (Gobel 2022). Episodic cluster headaches and episodic paroxysmal hemicrania, on the other hand, are characterized as lasting from 7 days to 1 year and separated by pain-free remission periods of $\geq$3 months. Treatment of episodic pain can be challenging as painful episodes can be sudden and unpredictable. It often involves a strong emphasis on preventative measures as in the case of prophylactic therapies for migraines and other headaches.

In cancer pain literature, episodic pain describes a different entity: the type of pain known as "breakthrough pain." The European Association for Palliative Care has advocated for the use of the term episodic pain, as it provides a more universal terminology that can more easily be translated to other languages (Mercadante et al. 2002). In the context of cancer pain, episodic pain is described as short periods of higher pain intensity in the presence of otherwise stable, persistent, controlled background pain (Løhre et al. 2016). Episodic pain can be further divided into movement-related (bone pain, neuropathic pain, visceral pain, or somatic soft tissue pain) and

non-movement-related (neuropathic pain, visceral pain, or somatic soft tissue pain) episodic pain. Regardless of the underlying mechanism, there is consensus that episodic pain has no biological basis for benefits. As in the case of chronic pain (and in contrast to acute pain), the painful nociceptive signals in episodic pain do not serve to warn of tissue damage but instead reflect aberrant signals.

1.2 Taxonomy and Common Types of Pain

1.2.1 Nociceptive Pain

The IASP defines nociceptive pain as that which arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors (IASP Subcommittee on Taxonomy 1979). Nociceptive pain can be further divided into visceral pain, affecting the visceral organs, or somatic pain, affecting the skin, muscles, ligaments, tendons, joints, or bones. The characterization of nociceptive pain is highly variable but can be described as dull, aching, and throbbing. Visceral nociceptive pain is usually diffuse and poorly localized, often with associated referral patterns. In contrast, somatic nociceptive pain tends to be more discrete, though it may also be associated with referral patterns.

1.2.2 Nociplastic Pain

Following the IASP's adoption of the term nociceptive pain in 2005, pain came to be characterized as either nociceptive or neuropathic. However, in 2016, the term nociplastic pain was proposed as a third mechanistic descriptor. Kosek et al. offered this term to describe "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain" (Kosek et al. 2016, p. 1383). Since then, it has grown significantly in popularity within the pain medicine community and was subsequently adopted into the IASP's terminology (Raja et al. 2020).

In a recent publication series in *The Lancet*, Fitzcharles et al. and Cohen et al. expanded further on the pathophysiologic mechanism of nociplastic pain, describing it as a maladaptive, abnormal processing of nociceptive input or diminished inhibitory pathway activity that can arise de novo, be triggered by a painful stimulus, originate in the central or peripheral nervous systems, or be psychologically driven (Cohen et al. 2021; Fitzcharles et al. 2021). Integral to the diagnosis of nociplastic pain, however, is the absence of known tissue damage or discrete pathology.

Interestingly, based on these criteria, complex regional pain syndrome (CRPS) Type I would be classified as nociplastic pain, while CRPS Type II would fall under

the category of neuropathic pain, given the presence of known nerve injury. Other examples of nociplastic pain include fibromyalgia, irritable bowel syndrome, bladder pain syndrome, and some tension-type headaches. Nociplastic pain is best managed with anticonvulsants, analgesic antidepressants, behavioral interventions, exercise, or a combination thereof.

Examples of somatic nociceptive pain include osteoarthritis, bursitis, and muscle tears. Examples of visceral nociceptive pain include angina and pain associated with pancreatitis. Both types of pain can be managed with analgesic antidepressants, non-steroidal anti-inflammatory drugs, opioids, image-guided injections, neuromodulation, exercise, or a combination thereof depending on the underlying pathology and the body parts involved.

1.2.3 Neuropathic Pain

Neuropathic pain is defined, according to IASP, as that which is caused by a lesion or disease of the somatosensory nervous system (IASP Subcommittee on Taxonomy 1979). It can be further divided into central or peripheral neuropathic pain based on where in the somatosensory nervous system the lesion or disease lies. It is typically characterized as burning, shooting, or like pins and needles, often with associated paresthesia, allodynia, or hyperalgesia. In many cases, there may also be associated numbness or itching. Neuropathic pain typically arises spontaneously and without provocation, although it is often exacerbated by touch, heat, and cold. Based on these criteria, several screening tools, such as the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS), Neuropathic Pain Questionnaire (NPQ), Douleur Neuropathique en 4 questionnaire (DN4), painDETECT, and ID-Pain, have been developed to help identify patients with neuropathic pain and have demonstrated up to 80% sensitivity and specificity (Bennett et al. 2007).

Examples of neuropathic pain include painful diabetic neuropathy, postherpetic neuralgia, and complex regional pain syndrome Type II. Treatment of neuropathic pain can be a challenge but typically starts with identification of cause and ruling out reversible causes. Therapeutic options include anticonvulsants, analgesic antidepressants, opioids, image-guided injections, neuromodulation, behavioral interventions, or a combination thereof. The most robust data support pharmacologic therapies, of which antidepressants (particularly tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin) are considered first-line therapies (Gilron et al. 2015).

Importantly, pain exists on a continuum and patients' pain cannot always be classified into any one discrete category. Many disease states comprise elements of all three types of pain described above.

1.3 Current Treatments and Limitations

Current available mechanism-based pain therapies will be discussed in more detail in later chapters of this book. The following section, however, will serve as a brief introduction to available therapeutic options. Treatments can be divided into pharmacologic, non-pharmacologic, interventional, and surgical therapies. Pain management is highly complex, and no single therapeutic approach can be used to manage all types of pain. The U.S. Department of Health and Human Services (HHS) published the *Pain Management Best Practices Inter-agency Task Force Report* in 2019 to provide clinicians with recommendations on the management of acute and chronic pain encompassing these various therapies (U.S. Department of Health and Human Services 2019).

1.3.1 Pharmacologic Treatments

Pharmacologic therapies encompass several commonly used classes of medications known to have analgesic properties. These include anti-inflammatory non-steroidal drugs, corticosteroids, anticonvulsants, analgesic antidepressants, topical agents, antispasmodics, muscle relaxants, and opioids. Prior to prescribing one of these medications, it is important to discuss associated side effects with the patient. Often, the associated sedating effects of these medications can be helpful in patients whose sleep has been affected by chronic pain. The patient's age and medical comorbidities should always be taken into consideration when selecting among these drug classes.

1.3.2 Non-pharmacologic Treatments

Non-pharmacologic therapies include ice, heat, cognitive-behavioral therapy (CBT), biofeedback, physical therapy, occupational therapy, traction, therapeutic ultrasound, acupuncture, chiropractic manipulation, massage, yoga, tai chi, and transcutaneous electrical nerve stimulation (TENS). These treatment modalities have been found to be efficacious when used in combination with other therapies and can be of particular utility in nociplastic pain conditions. A 2017 Cochrane Review article by Geneen et al. studied the effects of various exercise programs on diverse pain conditions and showed favorable effects in pain reduction, improvement in physical function, and quality of life despite small sample sizes (Geneen et al. 2017). In additional studies, comprehensive, interdisciplinary pain rehabilitation programs have been shown to significantly improve function in patients with chronic pain while resulting in significant reductions in medical costs (Kurklinsky et al. 2016; Sletten et al. 2015).

1.3.3 Interventional Treatments

Interventional therapies include image-guided injections, radiofrequency denervation, chemical neurolysis, cryoneuroablation, neuromodulation, minimally invasive lumbar decompression, implantation of intrathecal drug delivery systems, regenerative and biologic therapies, and various percutaneous interventions (basivertebral nerve ablation, vertebral augmentation, and interspinous process spacer device placement). Patients are best suited for interventional procedures when their symptoms follow a neuroanatomical distribution, particularly if there are concordant imaging findings.

Neuromodulation is a treatment modality that has gained a lot of traction in recent years. It involves the application of electrical stimulation to peripheral nerves, spinal cord dorsal columns, dorsal root ganglia, motor cortex, or specific deep brain regions, which can be applied through a variety of waveforms, frequencies, and feedback control mechanisms, such as high-frequency, burst, and closed-loop stimulation. With regard to spinal cord stimulation, most of the randomized controlled trials studying these therapies have been used for neuropathic pain or CRPS, so their application to other pain states remains to be seen. Overall, there is good evidence showing superiority of neuromodulation over conventional medical management for the treatment of failed back surgery syndrome, CRPS, and painful diabetic neuropathy, and these studies have been largely industry-sponsored (Knotkova et al. 2021).

1.3.4 Surgical Treatments

Surgical therapies are typically reserved for patients who have failed conservative therapies, who have significant symptoms limiting function, or who have lesions causing specific tissue damage. Options for surgical therapies vary widely depending on the etiology of the pain but can include decompression or fusion in the case of spine pathologies, replacement in the setting of advanced osteoarthritis, or resection in the setting of malignancies.

Regardless of the therapy selected, however, there are general guidelines that should be followed in caring for patients suffering from chronic pain. In most cases, therapies are directed toward managing and attenuating symptoms rather than curing the disease. This often involves a thoughtful discussion between the clinician and the patient and setting realistic, attainable expectations. It is important to cultivate a strong clinician–patient relationship in order to promote patient's engagement and treatment compliance. It is also beneficial to promote an internal locus of control and remind patients of the things they can do for themselves as well through selection of healthy lifestyle habits.

In general, it is imperative to develop an individualized, patient-centered approach, typically through a multimodal, multidisciplinary approach (Cohen et al.

2021). This often begins with a thorough history and physical examination, and, when indicated, imaging studies in order to properly characterize the pain and thus select the appropriate therapeutic options. Careful consideration of the circumstances unique to each patient, such as age, comorbidities, and psychosocial issues such as financial limitations or barriers to care, is essential to this approach. Regardless of the treatment option selected, treatment should always focus on improving quality of life and restoring function.

1.4 Special Issues in Pain Management

1.4.1 The United States Opioid Crisis

Few things have impacted the field of pain management as severely as the United States opioid epidemic and several pivotal events have led to the rise of the nationwide health crisis. Following the release of Percocet and Vicodin to the market in the 1970–1980s, the pharmaceutical industry began pushing for opioids as safe, effective medications for the management of pain. The push from the industry was further supported by publications such as the 1980 *New England Journal of Medicine* letter "Addiction Rare in Patients Treated with Narcotics" and the 1986 *Pain* study "Chronic Use of Opioid Analgesics in Non-Malignant Pain: Report of 38 Cases" (Porter and Jick 1980; Portenoy and Foley 1986). In 1996, OxyContin was released and further marketed through campaigns claiming it was less addictive than its immediate-release counterparts. Supposedly the slow, sustained release formulation posed a lower risk for obtaining a "high" and thus had a very low risk of iatrogenic addiction (Lyden and Binswanger 2019). However, there were little data to support this claim.

The combination of these events caused substantial increases in opioid prescriptions, reaching a record 259 million in 2012 (Paulozzi et al. 2014). This was paralleled by an alarming rise in overdose deaths from prescription opioids, which nearly tripled from 1999 to 2015. In fact, in 2016, the Centers for Disease Control and Prevention (CDC) reported approximately 89 deaths per day and a total of 32,445 deaths in 2016 due to prescription opioid overdoses (Marshall et al. 2019). In recognition of the nationwide problem, in 2016 the CDC published the *CDC Guideline for Prescribing Opioids for Chronic Pain-United States, 2016* to provide guidance for primary care clinicians who were prescribing opioids for chronic pain outside of active cancer treatment, palliative care, and end-of-life care (Dowell et al. 2016). In 2017, the U.S. Department of Health and Human Services (HHS) declared a public health emergency and proposed a five-point strategy to combat the crisis. This focused on: (1) better data on the epidemic by strengthening public health surveillance, (2) better pain management, (3) improving access to treatment, prevention, and recovery services, (4) increasing the availability of overdose-reversing drugs, and (5) supporting cutting-edge research on pain and addiction (Price 2017).

Despite these efforts, opioid misuse continues to be an enormous problem in the United States. Opioid overdose deaths have continued to rise, reaching 93,331 in 2020, which is the highest number to date (Ahmad et al. 2022). On February 10, 2022, the CDC published an update to their 2016 guidelines for opioid prescriptions (Dowell et al. 2022). These recommendations no longer include specific dosage ceilings and abandon the previous three-day prescription limitations for acute pain. However, they continue to emphasize the importance of judicious opioid prescription and support the use of individualized clinical judgment in medical decision-making. The United States opioid epidemic remains a complex issue with devastating consequences that requires a concerted effort by pharmaceutical companies, government agencies, healthcare organizations, and clinicians of all medical specialties. As suggested by the HHS, it is imperative to prioritize additional research on pain and development of safe strategies to address chronic pain.

1.4.2 Chronic Pain and the COVID-19 Pandemic

The COVID-19 pandemic impacted the lives of people all around the world in many ways. It has caused significant and at times even life-threatening illness. Estimates from the United States at the peak of the epidemic placed the overall percentage of patients with laboratory-confirmed COVID-19 suffering from severe disease at 14% and those with fatal illness at 5% (Stokes et al. 2020). According to the current CDC data, there have been 78,855,000 total reported cases of COVID-19 and 947,882 deaths due to COVID-19 in the United States to date (CDC 2020). The data from around the world are even more staggering. The pandemic has also been a substantial psychosocial stressor for individuals through the associated isolation, economic hardships, and fear of illness.

In the healthcare community, the impact of the pandemic has been felt across all medical subspecialties, including in the field of pain management. At the height of the pandemic, with federal restrictions implemented to try to decrease transmission, many pain clinics were forced to suspend elective procedures, which include essentially all interventional pain therapies. The suspension of these procedures limited access to care for chronic pain patients who needed these interventions to return to function, subsequently driving up rates of medication prescriptions, including opioids, for pain management. With the closure of many facilities came limited access to gyms, pools, and physical therapy centers, which further decreased activity and thus function in these patients. The situation of chronic pain patients was further exacerbated by decreased social interactions and an inability to leave the house, leading to escalating anxiety and depression levels that drove increases in pain. Overall, these secondary effects of the pandemic resulted in higher rates of chronic pain, which led to a greater burden on pain management physicians once clinics, many of which had become short-staffed, were able to reopen. In response, medical societies have published recommendations for pain interventionalists on how to safely and responsibly reinitiate pain-related care with an emphasis on

understanding the value of interventional pain therapies to avoid additional harm to chronic pain patients (Deer et al. 2020).

The COVID-19 pandemic has undoubtedly caused an increase in chronic pain rates worldwide. Several mechanisms for the rise in numbers have been proposed, including exacerbation of pre-existing pain due to infection-induced inflammation by the virus, emergence of new post-viral chronic pain syndromes, and development of new pain as a result of increased risk factors related to the secondary effects of the pandemic (increased anxiety and depression, inactivity, poor sleep, and decreased socialization) in non-infected patients (Clauw et al. 2020). Of particular interest in recent research is the possibility that COVID-19 can produce post-viral chronic pain syndromes in patients identified as "long-haulers," characterized by having prolonged (often >12 weeks) symptoms following the initial infection. A large 2020 study by Bowles et al. on 1409 patients admitted to home health care following COVID-19 infection reported that the most common symptoms included daily or constant pain (42%), confusion (47%), anxiety (50%), and dyspnea with any exertion (84%) (Bowles et al. 2021). Risk factors for the development of chronic pain and fatigue identified in this study include: pre-existing comorbidities, history of chronic pain or previous pain experience, history of mental health problems, disadvantaged socioeconomic status, social isolation, and ICU-related specific factors (prolonged stay, ventilation, proning, sepsis, immobility, neuromuscular block). Among the reported painful conditions seen in post-viral COVID-19 syndromes are persistent generalized pain, joint pain/arthralgias, chest pain, and low back pain (Korompoki et al. 2021). These new chronic pain conditions have been observed in patients who experienced severe disease as well as those with even mild to moderate illness and can often be poorly localized making the treatment more challenging (Korompoki et al. 2021).

Post-infection pain syndromes lasting over 12 months have been reported in the past following microbial infections with *Coxiella burnetii* (Q fever), Epstein-Barr virus (mononucleosis), and *Borrelia burgdorferi* (Lyme disease). Thus, it is reasonable to hypothesize that the SARS-CoV-2 virus has similar capabilities. A particular hallmark of the COVID-19 infection is the robust, widespread inflammatory response triggered, which could play a role in the development of new pain conditions as well as the exacerbation of existing pain pathologies. This widespread inflammatory response has also been postulated to be the result of the organ-specific damage that may preferentially occur in individuals with fragile stress response systems (Clauw et al. 2020). Further research is needed to better understand the inflammation-driven pathophysiology behind this post-viral pain syndrome, in order to guide mitigation strategies to curtail further consequences of this pandemic.

1.5 Concluding Remarks

In summary, pain places an enormous burden on the society and healthcare system. According to IASP, pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage. Appropriate classification of pain is essential for research, treatments, and communications between healthcare providers and patients. Pain is characterized as acute, chronic, or episodic based on its duration, or as nociceptic, neuropathic, and nociplastic based on its pathologic mechanism. We highlight the cause of the United States Opioid Crisis and chronic pain during the COVID-19 Pandemic. COVID-19 can produce post-viral chronic pain syndromes in patients identified as "long-haulers." Notably, inflammation could be a major driver of this chronic disorder.

References

Ahmad F, Rossen L, Sutton P. Provisional drug overdose death counts. CDC National Center for Health Statistics; 2022.

Baliki MN, Apkarian AV. Nociception, pain, negative moods and behavior selection. Neuron. 2015;87(3):474–91.

Becker S, Schweinhardt P. Dysfunctional neurotransmitter systems in fibromyalgia, their role in central stress circuitry and pharmacological actions on these systems. Pain Res Treat. 2012;2012:741746.

Beissner F, Meissner K, Bär KJ, Napadow V. The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. J Neurosci. 2013;33(25):10503–11.

Bennett MI, Attal N, Backonja MM, Baron R, Bouhassira D, Freynhagen R, Scholz J, Tölle TR, Wittchen HU, Jensen TS. Using screening tools to identify neuropathic pain. Pain. 2007;127(3):199–203.

Bowles KH, McDonald M, Barrón Y, Kennedy E, O'Connor M, Mikkelsen M. Surviving COVID-19 after hospital discharge: symptom, functional, and adverse outcomes of home health recipients. Ann Intern Med. 2021;174(3):316–25.

Bushnell MC, Ceko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci. 2013;14(7):502–11.

CDC. United States COVID-19 Cases, Deaths, and Laboratory Testing (NAATs) by State, Territory, and Jurisdiction. Centers for Disease Control and Prevention. 2020, March 28. https://covid.cdc.gov/covid-data-tracker. Accessed 2 Mar 2022.

Chapman CR, Gavrin J. Suffering: the contributions of persistent pain. Lancet. 1999;353(9171):2233–7.

Clauw DJ, Häuser W, Cohen SP, Fitzcharles MA. Considering the potential for an increase in chronic pain after the COVID-19 pandemic. Pain. 2020;161(8):1694–7.

Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet. 2021;397(10289):2082–97.

Crue BL. The neurophysiology and taxonomy of pain. In: Crue BL, editor. Management of patients with chronic pain. Springer; 1983. p. 21–31.

De Ridder D, Vanneste S. Burst and tonic spinal cord stimulation: different and common brain mechanisms. Neuromodulation. 2016;19(1):47–59.

De Ridder D, Adhia D, Vanneste S. The anatomy of pain and suffering in the brain and its clinical implications. Neurosci Biobehav Rev. 2021;130:125–46.

Deer TR, Sayed D, Pope JE, Chakravarthy KV, Petersen E, Moeschler SM, Abd-Elsayed A, Amirdelfan K, Mekhail N. ASPN COVID workgroup. Emergence from the COVID-19 pandemic and the care of chronic pain: guidance for the Interventionalist. Anesth Analg. 2020;131(2):387–94.

Dowell D, Haegerich TM, Chou R. CDC guideline for prescribing opioids for chronic pain – United States, 2016. MMWR Recomm Rep. 2016;65(1):1–49.

Dowell D, Ragan KR, Jones CM, Baldwin GT, Chou R. CDC clinical practice guideline for prescribing opioids-United States, 2022, February 10. https://www.federalregister.gov/documents/2022/02/10/2022-02802/proposed-2022-cdc-clinical-practice-guideline-for-prescribing-opioids. Accessed 25 Feb 2022.

Dubin AE, Patapoutian A. Nociceptors: the sensors of the pain pathway. J Clin Invest. 2010;120(11):3760–72.

Fitzcharles MA, Cohen SP, Clauw DJ, Littlejohn G, Usui C, Häuser W. Nociplastic pain: towards an understanding of prevalent pain conditions. Lancet. 2021;397(10289):2098–110.

Gaskin DJ, Richard P. The economic costs of pain in the United States. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK92521/. Accessed 7 Feb 2022.

Geneen LJ, Moore RA, Clarke C, Martin D, Colvin LA, Smith BH. Physical activity and exercise for chronic pain in adults: an overview of cochrane reviews. Cochrane Database Syst Rev. 2017;4(4):CD011279.

Gilron I, Baron R, Jensen T. Neuropathic pain: principles of diagnosis and treatment. Mayo Clin Proc. 2015;90(4):532–45.

Gobel H. International headache society classification. ICHD-3. https://ichd-3.org/classification-outline/. Accessed 25 Feb 2022.

IASP Subcommittee on Taxonomy. Pain terms: a list with definitions and notes on usage. Recommended by the IASP Subcommittee on taxonomy. Pain. 1979;6(3):249.

Knotkova H, Hamani C, Sivanesan E, Le Beuffe MFE, Moon JY, Cohen SP, Huntoon MA. Neuromodulation for chronic pain. Lancet. 2021;397(10289):2111–24.

Korompoki E, Gavriatopoulou M, Hicklen RS, Ntanasis-Stathopoulos I, Kastritis E, Fotiou D, Stamatelopoulos K, Terpos E, Kotanidou A, Hagberg CA, Dimopoulos MA, Kontoyiannis DP. Epidemiology and organ specific sequelae of post-acute COVID19: a narrative review. J Infect. 2021;83(1):1–16.

Kosek E, Cohen M, Baron R, Gebhart GF, Mico JA, Rice ASC, Rief W, Sluka AK. Do we need a third mechanistic descriptor for chronic pain states? Pain. 2016;157(7):1382–6.

Kurklinsky S, Perez RB, Lacayo ER, Sletten CD. The efficacy of interdisciplinary rehabilitation for improving function in people with chronic pain. Pain Res Treat. 2016;2016:7217684.

Littlejohn G, Guymer E. Key milestones contributing to the understanding of the mechanisms underlying fibromyalgia. Biomedicine. 2020;8(7):223.

Løhre ET, Klepstad P, Bennett MI, Brunelli C, Caraceni A, Fainsinger RL, Knudsen AK, Mercadante S, Sjøgren P, Kaasa S, European Association for Palliative Care Research Network. From "breakthrough" to "episodic" cancer pain? A European association for palliative care research network expert Delphi survey toward a common terminology and classification of transient cancer pain exacerbations. J Pain Symptom Manag. 2016;51(6):1013–9.

Lyden J, Binswanger IA. The United States opioid epidemic. Semin Perinatol. 2019;43(3):123–31.

Marshall B, Bland MK, Hulla R, Gatchel RJ. Considerations in addressing the opioid epidemic and chronic pain within the USA. Pain Manag. 2019;9(2):131–8.

Mercadante S, Radbruch L, Caraceni A, Cherny N, Kaasa S, Nauck F, Ripamonti C, De Conno F, Steering Committee of the European Association for Palliative Care (EAPC) Research Network. Episodic (breakthrough) pain: consensus conference of an expert working group of the European Association for Palliative Care. Cancer. 2002;94(3):832–9.

Murray CJL, Atkinson C, Bhalla K, et al. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. JAMA. 2013;310(6):59–608.

Paulozzi LJ, Mack KA, Hockenberry JM. Vital signs: variation among states in prescribing of opioid pain relievers and benzodiazepines – United States, 2012. MMWR Morb Mortal Wkly Rep. 2014;63(26):563–8.

Portenoy RK, Foley KM. Chronic use of opioid analgesics in non-malignant pain: report of 38 cases. Pain. 1986;25(2):171–86.

Porter J, Jick H. Addiction rare in patients treated with narcotics. N Engl J Med. 1980;302(2):123.

Price T. Secretary Price announces HHS strategy for fighting opioid crisis. HHS.gov. 2017, December 4. https://www.hhs.gov/about/leadership/secretary/speeches/2017-speeches/secretary-price-announces-hhs-strategy-for-fighting-opioid-crisis/index.html. Accessed 25 Feb 2022.

Raja SN, Carr DB, Cohen M, Finnerup NB, Flor H, Gibson S, Keefe FJ, Mogil JS, Ringkamp M, Sluka KA, Song XJ, Stevens B, Sullivan MD, Tutelman PR, Ushida T, Vader K. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain. 2020;161(9):1976–1982. https://doi.org/10.1097/j.pain.0000000000001939. PMID: 32694387; PMCID: PMC7680716.

Schreckenberger M, Siessmeier T, Viertmann A, Landvogt C, Buchholz HG, Rolke R, Treede RD, Bartenstein P, Birklein F. The unpleasantness of tonic pain is encoded by the insular cortex. Neurology. 2005;64(7):1175–83.

Serra J, Collado A, Solà R, Antonelli F, Torres X, Salgueiro M, Quiles C, Bostock H. Hyperexcitable C nociceptors in fibromyalgia. Ann Neurol. 2014;75(2):196–208.

Sletten CD, Kurklinsky S, Chinburapa V, Ghazi S. Economic analysis of a comprehensive pain rehabilitation program: a collaboration between Florida blue and Mayo Clinic Florida. Pain Med. 2015;16(5):898–904.

St Sauver JL, Warner DO, Yawn BP, et al. Why patients visit their doctors: assessing the most prevalent conditions in a defined American population. Mayo Clin Proc. 2013;88(1):56–67.

Stokes EK, Zambrano LD, Anderson KN, et al. Coronavirus disease 2019 case surveillance – United States, January 22–May 30, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(24):759–65. https://doi.org/10.15585/mmwr.mm6924e2.

Treede RD, Rief W, Barke A, et al. Chronic pain as a symptom or a disease: the IASP classification of chronic pain for the International Classification of Diseases (ICD-11). Pain. 2019;160(1):19–27.

U.S. Department of Health and Human Services. Pain management best practices inter-agency task force report: updates, gaps, inconsistencies, and recommendations. 2019, May 9. https://www.hhs.gov/ash/advisory-committees/pain/reports/index.html. Accessed 9 Feb 2022.

Weiner RS. Pain management: a practical guide for clinicians. 6th ed. CRC Press; 2001.

World Health Organization (WHO). ICD-11 for mortality and morbidity statistics. International Statistical Classification of Diseases and Related Health Problems (ICD-11). https://icd.who.int/browse11/l-m/en. Accessed 7 Feb 2022.

Chapter 2
Inflammation and Pain

Jasmine Ji, Matthew Yuan, and Ru-Rong Ji

Abstract Inflammation is the body's natural response to injury or the infiltration of a foreign substance. The process is defined by five cardinal signs: redness, heat, swelling, loss of function, and pain. Because of its relationship with pain and injury, various treatments for inflammation have been historically documented. During inflammation, pro-inflammatory mediators can be produced by infiltrating and resident immune cells. These pro-inflammatory mediators can induce pain. On the other hand, recent evidence suggests that inflammation also resolves pain by generating anti-inflammatory and pro-resolving mediators. The inflammatory process, when properly mediated via cellular mechanisms, eliminates pathogens and damaged or dead cells from the body. Thus, inflammation was proposed to resolve pain by producing specialized pro-resolving mediators (SPMs), derived from omega-3 unsaturated fatty acids. However, dysregulated inflammation, such as chronic inflammation, can lead to various pathological conditions associated with chronic pain. While acute pain, which is temporary and serves a protective purpose, is beneficial, chronic pain has no such protective purpose and severely degrades the quality of life of patients.

Keywords Acute inflammation · Acute pain · Anti-inflammatory mediators · Chronic inflammation · Chronic pain · COVID-19 · Infection · Inflammatory mediators · Specialized pro-resolving mediators (SPMs) · Resolution of inflammation

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2.1 What Is Inflammation?

Inflammation is the body's response to infection and damage to tissues, with the end goal of eliminating any foreign pathogens and repairing any tissue damage. Inflammatory stimuli can be broadly viewed as anything that causes infection (e.g., viruses, bacteria, microbes, other pathogens) or tissue damage (e.g., physical trauma, toxic chemicals, radiation) (Medzhitov 2008). In response to an inflammatory stimulus, the body will initiate an inflammatory response; this response typically involves the recognition of the stimulus via cell surface pattern receptors, the release of inflammatory mediators that subsequently activate inflammatory pathways, and the release of inflammatory markers that lead to the recruitment of inflammatory cells (Ji et al. 2016). Once foreign pathogens have been eliminated and the tissues have been repaired, the inflammatory response will have served its purpose and be subsequently resolved. In some cases, however, the inflammatory response may not be properly mediated, morphing into a chronic process; unlike its helpful, acute counterpart, chronic inflammation is harmful to the body, and, indeed, a cause and symptom of various inflammatory diseases (Donnelly et al. 2020; Medzhitov 2008). Chronic inflammation is also often closely associated with chronic pain (although notably there are also several painless or partially painless chronic inflammatory conditions) (Ji et al. 2014, 2018; Lewis et al. 2020; Martinez Quintero et al. 2021). Inflammation can be broadly divided into neurogenic inflammation and neuroinflammation, based on the type of pathological pain it underlies (Chiu et al. 2012; Ji et al. 2014, 2018; Matsuda et al. 2019). The following chapter will consider the cardinal signs of inflammation, its history, the biological processes involved in inflammation, the close correlation between pain and inflammation, and the implications of chronic inflammation.

2.2 A History of Inflammation

The existence of inflammation has been known for millennia (Fig. 2.1), described early on in the medical texts of the ancient Greeks and Egyptians. This is no wonder, as the condition is easily observable, chaperoning injuries and infections, often lethal in ancient times. The Greek physician Hippocrates described signs of inflammation in the fifth century, using terms such as *oidēma* (swelling), which we today call edema. He also seemed to appreciate the role of inflammation in the healing process following injury. The first four cardinal signs—redness, swelling, heat, and pain—were described by the fifth-century Roman writer Aulus Cornelius Celsus, in the oft-quoted phrase, "calore et tumor cum calore et dolore" (redness and swelling with heat and pain), from his medical treatise, *De Medicina*, written during the first century (Plytycz and Seljelid 2003).

The next major advancement in understanding inflammation came much later, with British neurophysiologist Augustus Waller (1846) and German pathologist

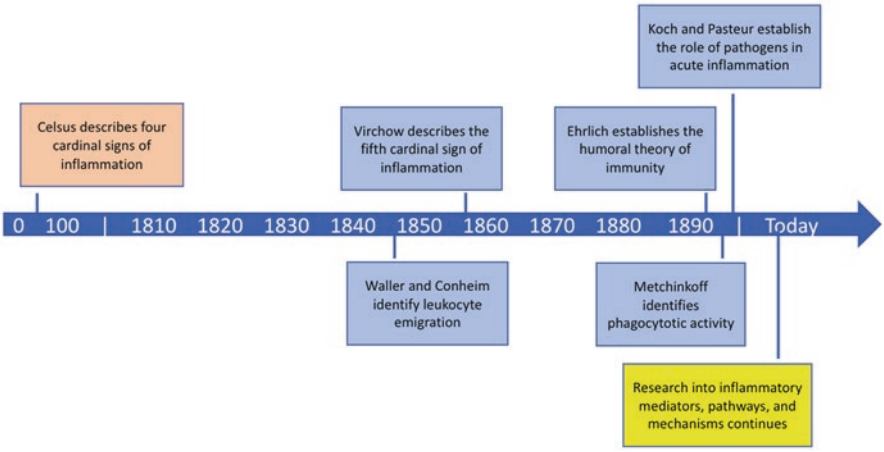

Fig. 2.1 Major events that led to our modern understanding of inflammation

Julius Cohnheim (1867). Both scientists independently described the emigration of leukocytes from blood vessels, as well as other vascular structures, during an acute inflammatory response. Using a microscope, they were able to observe the vasodilation, plasma leakage, and the movement of leukocytes from the blood vessels into neighboring tissue. In other words, they were able to identify some of the most critical events that occur during the inflammatory process (Rochae e Silva 1978).

In 1858, Rudolph Virchow, the German physicist often known as the "father of modern pathology," described the fifth cardinal sign of inflammation in his book *Cellularpathologie*: disturbance of function. This final sign differs from the previous four in that it is the only sign that appears in all inflammatory processes, not just in acute processes like the previous four. Virchow's work also established the concept that pathologies arise from cells and not the four humors, as was previously believed (Heidland et al. 2006).

In 1892, there came the discovery of phagocytes and phagocytosis by Russian zoologist Élie Metchnikoff, who was awarded a Nobel Prize for his discovery. The theory of cellular immunity, which states that cellular immunity is based on the activity of phagocytes, followed. This theory emphasized the importance of macrophages and neutrophils in defending and maintaining the homeostasis of tissues (Plytycz and Seljelid 2003).

In 1890, German physiologist Emil von Behring and Japanese bacteriologist Shibasaburo Kitasato co-authored a paper describing the production of "antitoxins" against diphtheria and tetanus toxins, one of the earliest examples of serum therapy (Plytycz and Seljelid 2003). Their paper inspired German physician Paul Ehrlich's humoral theory of immunity, also known as antibody-mediated immunity, which describes the ability of B-cells, a type of immune cell, to produce antibodies, large proteins that respond to and counteract antigens, proteins found on many pathogens (Rochae e Silva 1978). Belgian immunologist Jules Bordet followed-up in 1896

with his work examining the role of serum components in immunity. German physician Robert Koch and French microbiologist Louis Pasteur contributed greatly to the modern understanding of inflammation by specifying microbial agents as a major inducer of the acute inflammatory response (Rochae e Silva 1978).

Today, the study of inflammation accelerates and expands as researchers probe the different classes of inflammatory mediators, discover the cells and pathways that regulate their production, and reveal their mechanisms of action. We now understand that inflammation has many different forms, and can be induced, regulated, and resolved in multiple different ways. As modern medicine becomes increasingly effective at resolving acute inflammation and increasing lifespan, chronic inflammation has emerged as the next great topic of interest. Inflammatory conditions, driven by chronic inflammation, plague many people, especially in old age. These conditions include atherosclerosis, asthma, type 2 diabetes, neurodegenerative diseases (e.g., Alzheimer's disease), and cancer. Studying inflammation would provide insights into these debilitating and widespread conditions (Scrivo et al. 2011).

A PubMed search shows that doctors were interested in inflammation as early as 1791 (though PubMed itself was only founded in January of 1996; Fig. 2.2a) when Mr. Thomas Mainwaring, a doctor, wrote a letter to his patient who had been attacked and subsequently suffered from severe inflammation in his throat (Mainwaring 1791). Up until 1970, inflammation research remained relatively rare until the development of endothelial cell research in the 1970s, which quickly exploded, contributing greatly to inflammatory research due to the enormous role that endothelial cells play in inflammation. Aided by endothelial cell research, the number of papers on inflammation has grown exponentially in the past 50 years. Development of gene-targeted knockout technology has allowed scientists to study the roles of various immune-cell-derived mediators, such as cytokines and chemokines (small cytokines), and endothelial-cell-derived adhesion molecules, as well as their receptors in inflammation. These studies have helped scientists create useful models of inflammation. Further methodologies developed in immunology, including antibody blocking, in mice have also contributed significantly to the progress of inflammation research (Kvietys and Granger 1997). Since 2000, the number of papers on inflammation has increased steadily every year, showing the lingering relevance and importance of inflammation in our lives (Fig. 2.2a). Correlation with inflammation has been demonstrated in almost all types of human diseases.

2.3 Inflammation and Pain

2.3.1 The Correlation Between Inflammation and Pain: Clinical Relevance and Social Impact

Inflammation and pain are two closely interrelated concepts that often go hand in hand in research (Calvo et al. 2012; Ji et al. 2016; Fig. 2.2b). It is of critical importance to investigate their correlation and interactions not only in acute pain

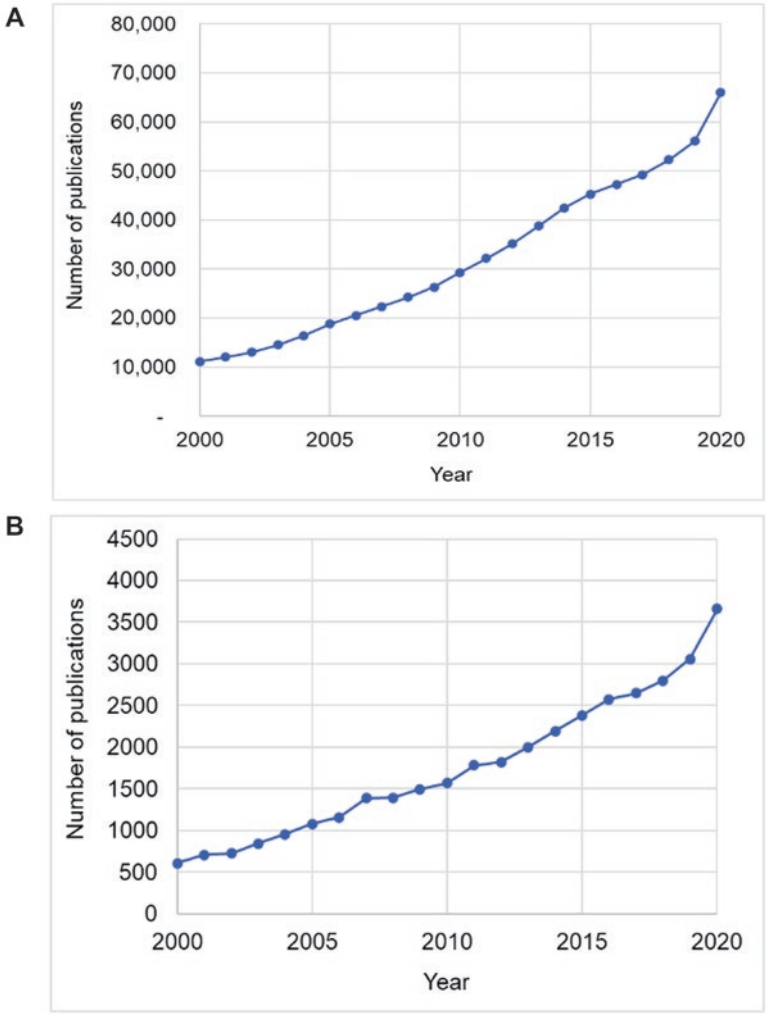

Fig. 2.2 A PubMed search shows (**a**) an increasing number of publications using the keyword "inflammation" over the last two decades and (**b**) an increasing number of publications using the keywords "inflammation" and "pain" over the last two decades

conditions but also in chronic pain conditions. Chronic pain conditions plague tens of millions of Americans, with annual costs on pain management and labor loss exceeding 600 billion US dollars per year (Gereau et al. 2014). Inflammation-associated pain is typically treated with local anesthetics, opioids, and non-steroidal anti-inflammatory drugs (NSAIDs). Each of these has obvious problems, however (Brigham et al. 2021). Opioids function by blocking pain signals in the spinal cord and brain (Corder et al. 2018). Thus, people treated with opioids are temporarily completely incapable of feeling pain. Local anesthetics work similarly; by binding

to the sodium channels, they prevent sodium ions from entering, in turn preventing nerve signals from being conducted to the brain. Being completely incapable of feeling pain is dangerous. Acute pain exists as a protective mechanism—to prevent you from reaching into a hot oven without mitts, or to encourage you to take immediate action after dropping your textbooks on your foot, for example. Thus, blocking pain signals is hardly an ideal solution. Addiction is also a problem, which can lead to breathing suppression and subsequently death. Opioid abuse, in particular, is an enormous problem that has led to America's Opioid Crisis. The third treatment option, NSAIDs, does not block pain signals. Instead, NSAIDs block enzymes like cyclooxygenases (Cox-1 and Cox-2), which produce prostaglandins (e.g., PGE2), lipids that promote swelling and pain in inflammation. While NSAIDs are effective in treating acute inflammatory pain and headache, they have their own issues; they can damage your stomach lining and even result in internal bleeding (Brigham et al. 2021).

Lack of effective treatments for chronic pain is associated with the ongoing crisis of opioid use disorder (OUD) (Volkow and Collins 2017). In 2020, more than 92,000 Americans died from drug overdoses, a nearly 30% increase over 2019, according to a report from the Centers for Disease Control and Prevention. Thus, there is an urgent need to develop non-opioid medicine that can control excessive inflammation and neuroinflammation for the prevention and resolution of chronic pain (Ji et al. 2018).

Though pain and inflammation often co-exist, some inflammatory conditions, especially chronic inflammatory conditions, may not involve pain (Fig. 2.3). Periodontal disease refers to a severe gum infection that can result in inflammation of the gums, as evidenced by their red, tender, and swollen appearance. Notably periodontal disease is frequently painless, either during early stages or throughout the entire process. Atherosclerosis occurs due to the build-up of fats, cholesterol, and other substances in and on the artery walls, which obstruct blood flow and can rupture, causing acute occlusion of the artery by clotting. Despite chronic inflammation of the artery walls, this condition has no obvious painful symptoms, until a build-up becomes severe enough to block blood flow. Asthma is a rather common condition, in which inflamed airways cause additional mucus to be produced, which interferes with breathing. Asthma can cause wheezing, which forces the chest to constrict and produces chest pain. Even so, the actual location of inflammation, the lungs, does not experience pain. Lastly, early-stage melanoma, alongside some other early-stage cancers, also is not painful. Interestingly, melanoma cells produce specific mediators called immune checkpoint inhibitors that can suppress pain (Chen et al. 2017a). This lack of pain is especially misleading, as patients cannot be aware of the cancer in an earlier stage and take necessary precautions. These painless inflammatory conditions present two facts: the first is that inflammation does not necessarily induce pain, despite drastic tumor growth that causes significant skin lesion; the second is that inflammation without pain can be quite dangerous, as pain serves as a warning sign. It is noteworthy that many types of cancers are diagnosed by doctors after patients have reported pain.

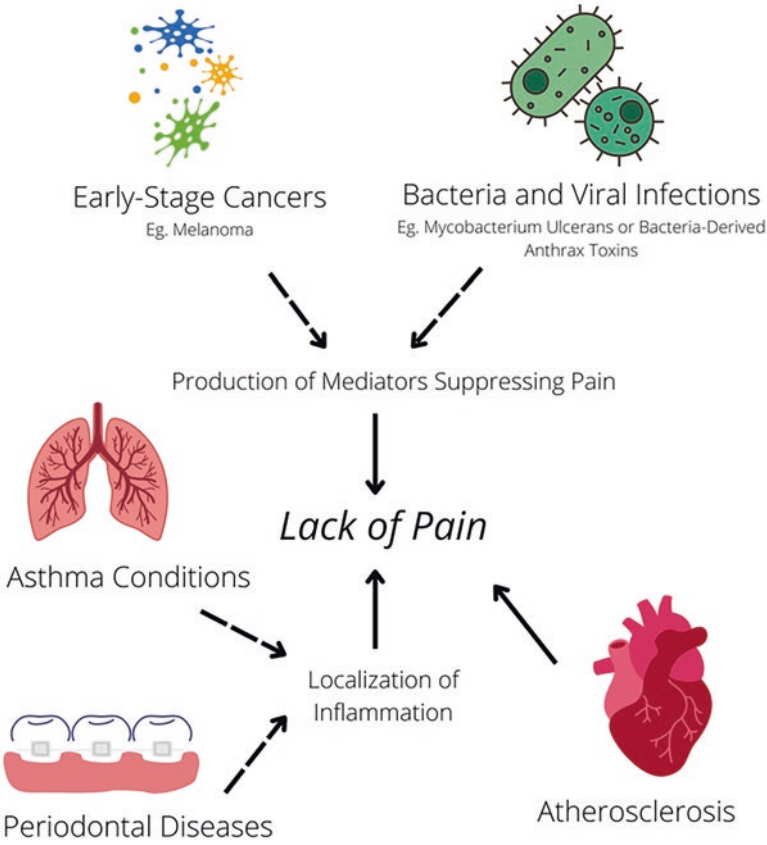

Fig. 2.3 There are also painless inflammatory conditions due to the production of anti-nociceptive mediators at the inflammatory sites

Bacterial and viral infections are typically painful (Chiu 2018; Chiu et al. 2013), but are not always painful (Ji et al. 2016). For example, *Mycobacterium ulcerans*, the etiological agent of Buruli ulcer, produces extensive and severe skin lesions but not pain. Instead, *M. ulcerans* produces remarkable analgesia by producing analgesic mediators (Marion et al. 2014). Several types of viruses, such as herpes simplex virus (HSV) and varicella zoster virus (VZV), are capable of infecting sensory neurons and evoke severe pain. However, during reactivation of HSV, patients frequently experience paresthesia (numbness, tingling), signs of analgesia, prior to the development of acute pain following ulceration (Donnelly et al. 2020). Recently, it was found that bacteria-derived anthrax toxins produce potent pain inhibition by binding ANTXR2, the high-affinity receptor for anthrax toxins, which are expressed by nociceptors (Yang et al. 2021). Thus, infections can be both painful and non-painful (Donnelly et al. 2020) (Fig. 2.3).

2.4 The Inflammatory Process

Despite its inconveniences, inflammation is an enormously important process during which the body cleans up the pathogens and dead cells at the site of injury and begins the healing process. Acute inflammation is activated by resident immune cells already present in the involved tissue, mainly resident macrophages, dendritic cells, and mast cells. These cells have surface receptors called pattern recognition receptors (PRRs), which bind two kinds of molecules: pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are substances associated with various pathogens, but can still be distinguished from host molecules. DAMPs are substances associated with host-related injury and cell damage (Akira et al. 2006). In addition, PRRs are also activated by microbe-associated molecular patterns (MAMPs) (Boller and Felix 2009). When an infection, burn, or other injury occurs, these cells are activated (one of the PRRs recognizes a PAMP, DAMP, or MAMP) and release inflammatory mediators that result in the aforementioned cardinal signs of inflammation (Donnelly et al. 2020). Vasodilation and the resulting increase in blood flow cause redness and increased heat. Increased permeability of the blood vessels leads to the leakage of plasma proteins and fluid into the tissue in a condition known as edema, which then causes swelling. Different types of mediators that are released during this process, such as bradykinin, prostaglandins, cytokines, and chemokines, can cause pain or hyperalgesia (Gold and Gebhart 2010; Ji et al. 2014). Mediator molecules, such as chemokines (small cytokines), also change blood vessels to allow the migration of leukocytes, such as neutrophils and macrophages, out of the blood vessels into tissue in a process called extravasation. Plasma extravasation can also be induced by neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), released after stimulation of pain-sensing nerve fibers during neurogenic inflammation (Xanthos and Sandkuhler 2014). The neutrophils move along a chemotactic gradient (moving from an area of low chemical concentration to an area of high chemical concentration) made by local cells in order to reach the site of injury. The fifth cardinal sign of inflammation, loss of function, could be related to both injury and illness. For example, an inflamed joint cannot be moved properly due to acute injury or chronic diseases such as arthritis. Inflammation can make it difficult for patients to breathe due to a respiratory infection, which has been experienced by many COVID patients. Accumulating evidence suggests that PRRs such as toll-like receptors (TLRs) are also present in primary sensory neurons and play important roles in regulating neuronal activity and excitability and sensory functions such as pain and itch (Diogenes et al. 2011; Donnelly et al. 2020; Liu et al. 2010). Furthermore, neuronal TLR signaling in nociceptive neurons was shown to regulate local immunity (Liu et al. 2014). Thus, neuron–immune interactions may be involved in fundamental inflammatory processes.

Additionally, there are several biochemical cascades that do not involve cells. Instead, they consist of preformed plasma proteins that work together to start and spread the inflammatory response. These systems include the complement system,

which is activated by bacteria and the coagulation and fibrinolysis (breaking down fibrin) cascades, which are activated by necrosis (i.e., a burn or blow). Acute inflammation can be seen as the first line of defense against injury. Acute response to inflammation needs constant stimulation to persist. Inflammatory mediators last only temporarily; afterward, they are rapidly degraded in the tissue. It was generally believed that acute inflammation comes to a halt once the stimulus is removed. Recent progress has demonstrated that resolution of inflammation is not a passive process but an active process, which can generate specialized pro-resolution mediators (SPMs) that promote the resolution of inflammation, as well as pain (Ji et al. 2011; Serhan 2007). Below, we will be discussing some of the mechanisms of inflammatory mediators more in depth.

2.5 Cellular Mechanisms

Cellular mechanisms involve a number of different leukocytes, which carry out leukocyte extravasation and phagocytosis during inflammation to rid the body of any foreign substances (Fig. 2.4). Extravasation is the process leukocytes use to reach the site of injury. During this process, leukocytes, which are usually located at the center of blood vessels, will move toward the walls of the vessels. Activated macrophages in tissue will release cytokines including interleukin (IL)-1β and tumor necrosis factor (TNF)-α (or TNF, as this is the only member), which causes the production of chemokines that bind to proteoglycans (a major component in extracellular matrices). This forms a gradient in both the inflamed tissue and along the endothelial wall. Inflammatory cytokines then rapidly induce the expression of P-selectin (a cell adhesion molecule) on the surfaces of endothelial cells. P-selectin binds (albeit weakly) to carbohydrate ligands on leukocyte surfaces, moving across the endothelial surface during which bonds are made and broken. Injured cells will release cytokines that induce E-selectin (which functions similarly to P-selectin) expression on endothelial cells. Cytokines also induce integrin ligand, such as CADM-1 (cell adhesion molecule 1) and VCAM-1 (vascular cell adhesion molecule1) expression on endothelial cells, which control the adhesion of the selections and contribute to slowing leukocytes. The weakly bound leukocytes can then detach if they remain non-activated by chemokines produced in injured tissue following signal transduction through G-protein-coupled receptors (GPCRs). These receptors activate integrins on leukocytes, increasing the bound integrin receptor affinity for ICAM-1 (intercellular adhesion molecule 1) and VCAM-1 on the endothelial cell surface, thus firmly binding the leukocytes to the endothelium. Once the leukocytes have been bound to the endothelium, they will transmigrate, or move across the endothelium in a process called diapedesis. In this process, also termed chemotaxis, chemokine gradients will cause the leukocytes to move in between adjacent endothelial cells. The endothelial cells in turn will retreat, allowing the leukocytes to move into surrounding tissue (Fig. 2.4). After the leukocytes have reached the tissue interstitium, they will bind to the extracellular matrix. A variety

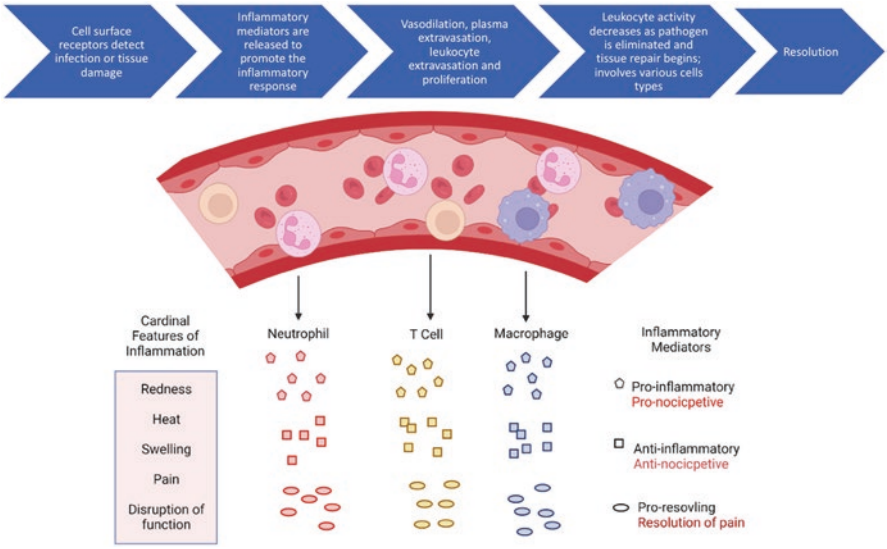

Fig. 2.4 Basic flowchart of the inflammatory process, induced by infection or tissue damage. Inflammation is associated with infiltration of immune cells, such as neutrophils, macrophages, and T-cells. Acute inflammation results in resolution and wound healing. There are five cardinal features of inflammation. After inflammation, immune cells produce pro-inflammatory mediators that are pro-nociceptive. Inflammation also produces anti-inflammatory and pro-resolving mediators that are anti-nociceptive and promote pain resolution

of chemoattractant (e.g., C3a or C5) will then guide the leukocytes along chemotactic gradients toward the inflammatory source.

Mounting evidence suggests that inflammation is also regulated by primary sensory neurons such as pain-sensing nociceptive neurons (nociceptors) (Talbot et al. 2016). Nociceptors include unmyelinated C-fiber neurons and myelinated Aδ-fiber neurons, with their cell bodies localized in dorsal root ganglion (DRG) and trigeminal ganglion (TG) and axons/terminals innervating the skin, muscle, and joint (Julius and Basbaum 2001; Woolf and Ma 2007). Upon activation, nociceptors release neuropeptides, cytokines, chemokines, and amino acids from their terminals that potently shape the function of innate and adaptive immune cells in the local environment (Baral et al. 2019; Liu et al. 2014). For some pathogens, neuron–immune interactions enhance host protection from infection, but for other pathogens, neuron–immune signaling pathways can be exploited to facilitate pathogen survival, depending on specific disease conditions, disease progression stages, and mediators released from nociceptors (Baral et al. 2019). For example, C-fiber nociceptors have been shown to activate dendritic cells and subsequent activation of T-cells, leading to the pathogenesis of psoriasis, a skin disease associated with autoimmune dysfunction and chronic itch, via the release of the neuropeptide CGRP (Riol-Blanco et al. 2014; Zhang and He 2020). On the other hand, recent study also

shows that activation of TRPV1 (transient receptor potential vanilloid 1) positive nociceptive neurons are sufficient for host defense against infections (Cohen et al. 2019; Lei et al. 2022).

The discovery of glial cells and neuroinflammation revolutionized how scientists understood chronic inflammation. Glial cells, immune cells found in the central nervous system (CNS), are key players in the process known as neuroinflammation, or inflammation of tissue in the peripheral nervous system (PNS) and CNS. Activation of glial cells, which include Schwann cells in peripheral nerves (e.g., sciatic nerve), satellite glial cells in the DRG and TG, and microglia, astrocytes, and oligodendrocytes in the spinal cord and brain, leads to the release of glial mediators that can modulate pain sensitivity (Gosselin et al. 2010; Hanani and Spray 2020; Ji et al. 2016). In other words, glia-produced neuromodulators can increase pain sensitivity in neuroinflammation, which in turn can sustain sensitization in nociceptive neurons. By maintaining signaling interactions between neurons and glial cells, neuroinflammation can thus modulate chronic pain (Ji et al. 2014). Please see more details in Chap. 5.

2.6 From Inflammation to Infection and Sterile Inflammation

2.6.1 From Inflammation to Infection

The inflammatory process described above is the standard reaction to inflammation by infection. Infection, it should be noted, is defined by the entrance of a foreign substance into the body, often through a wound, and can be broadly divided into four categories, including bacterial, viral, fungal, and prion. Inflammation to infection is a common form of inflammation, though it is actually not the only form of inflammation (Medzhitov 2010, 2021).

COVID-19, the respiratory condition caused by the coronavirus, is a notable example of an inflammatory condition. The virus causes severe inflammation in the lungs that can result in the excessive mucus production and, consequently, an inability to breathe. This excessive inflammation has been noted to cause damage to the alveoli of the lungs, which are responsible for the exchange of oxygen and carbon dioxide. Specifically, COVID-19 enters alveolar type II cells, through the receptor protein angiotensin-converting enzyme 2 (ACE2), which is found in the plasma membrane of cells, including human sensory neurons (Shiers et al. 2020). As the disease progresses, a mark of severity is demonstrated when the linings of the pleura, which are the tissue layers that surround the lungs, become irregular, caused by interstitial thickening and worsening inflammatory conditions.

2.6.2 *Sterile Inflammation*

During tissue damage, cells that die can cause an inflammatory response quite similar to infection-induced inflammation. Note that sterile injury and injury with infection are quite different, as injury with infection involves microbes. Most injuries are associated with infection, as they often involve breaking the skin, the first barrier of the body, which allows microbes to enter the body. More rarely, sterile inflammation will occur, during which cells are damaged or die in a sterile setting, most often due to ischemia (lack of blood supply to a particular body part), ischemia-reperfusion (tissue damage that occurs as blood returns to oxygen-deprived tissue due to ischemia), and trauma that can be found in the nerve, spinal cord, and brain. In these cases, inflammation occurs without infection, but still causes events such as neutrophil and monocyte infiltration, in addition to dendritic cell activation. In more severe cases, sterile inflammation can advance into circulatory shock or even multiple organ failure. Stunningly, how tissue injury without infection is detected is poorly understood, though TLRs and other innate receptors may play a role in discovering sterile injuries. One possibility, however, is that endogenous molecules that are released with cell death following necrosis may be a marker for sterile cell injury, including β-defensin, heat shock proteins, hyaluronan, uric acid crystals, as well as many other biomolecules, such as high-mobility group box protein 1 (HMGB1) and microRNAs (e.g., let-7b). Members of the TLR family are thought to be responsible for recognizing these molecules, such as activation of TLR4 by HMGB1 and activation of TLR7 by miRNA-let-7b (Liu et al. 2012), but it should be noted that relevant studies always run the risk of containing microbial contamination. Nonetheless, the capacity to stimulate inflammation of these endogenous molecules/ligands in response to microbial contamination is difficult to deny. Studies of TLR-deficient mice with various models of tissue injury are likely the best evidence for the fact that TLRs participate in detection of tissue injury. Various TLRs are not only expressed by immune cells, but also by glial cells, as well as neurons, such as DRG and TG neurons. TLRs play crucial roles in the pathogenesis of pain in animal models of inflammatory pain and nerve injury (Christianson et al. 2011; Donnelly et al. 2020; Liu et al. 2012). Please see more details in Chap. 8.

2.7 Tissue Injury, Microbes, and Pathogens: How Do They Affect the Host?

2.7.1 *Degrees of Inflammation: Tissue Injury and Pathogens*

We previously discussed the different types of inflammation and some mechanisms that cause them. However, it is also worthy to ask whether or not the severity of inflammation depends on the type of injury/infection. Do tissue injury, microbes, and pathogens cause different degrees of inflammation in the host?

Inflammation, if not induced or regulated properly, can cause enormous damage to the host. Though this inflammation is intended to protect the host, it also causes significant "collateral damage." A complex network of regulatory signals usually determines the degree of inflammation according to the original and the continuing cues. Inflammation signals have been described as "stop and go" signals, which play a role in determining when to escalate, dampen, or resolve an inflammatory response. Infection and injury, as discussed before, both cause inflammatory responses, but with different effects. The ligands responsible for signaling to them, however, converge on similar innate pathways. This seems to point to a lack of discrimination between qualitatively different stresses, which is rather confusing and requires further research. The existing complex feedback mechanisms suggest that there are additional mechanisms to address this, though, once again, very little is known. Pathogenic microbes, for example microbes with virulence mechanisms, seem to present a third qualitatively distinct challenge that requires an escalated response. It seems tempting to think that inflammation escalates or dampens based on the characteristics of the trigger, though specific gene induction downstream of innate receptors suggests that such a scenario may be plausible.

Does the host view tissue injury and microbial injury as different stresses? The extent to which the endogenous ligands produced during tissue damage stimulate the inflammatory signal induced by microbes remains unclear. It is possible that the responses to tissue damage and infection are similar, maybe due to the high likelihood of concomitant infection during tissue damage. Severe trauma as a method of sterile inflammation was mentioned above; sometimes, such trauma can lead to hemorrhagic shock, just as overwhelming infection can lead to sepsis. Nevertheless, these severe examples do not accurately represent if the overall purpose of each response is similar. It seems strange to think that the response to tissue damage would be the same to infection. The response to tissue damage has a purpose of healing, while the response to infection is first to rid the body of the infection and microbes. Tissue repair requires tissue remodeling, breakdown of extracellular matrix, and proliferation of cells to reestablish homeostasis. Many of these are also seen in the resolving phase of inflammation in response to infection, yet much of the collateral damage caused by activated neutrophils and macrophages during microbial infection might be unnecessary to heal a sterile wound. So far, research into the TLRs shows that an individual TLR is capable of differential signaling in response to different ligands, although the mechanisms responsible for these distinct responses are still unclear (Donnelly et al. 2020).

2.7.2 Gut Microbiota in Inflammation and Pain

Recent studies have pointed to a critical involvement of microbiota in health and disease. Microbiota include a range of microorganisms that may be commensal, symbiotic, or pathogenic found in and on all multicellular organisms, including bacteria, parasites, fungi, and viruses. Increasing evidence suggests microbiota play

a crucial role in regulating immunologic, hormonal, and metabolic homeostasis of their host, and gut–brain interaction is emerging as a hot topic in neuroscience and medicine (Erny et al. 2015). The recent progress in gut microbiota has expanded our knowledge on PAMPs. PAMPs are conserved microbial structures that are present in all microorganisms (including hot bacteria) and, thus, are not restricted to pathogens. Given our growing appreciation of the commensal microbiota (Erny et al. 2015), which activate the same host PRRs using the same ligands (e.g., lipopolysaccharide [LPS] and flagellin), it was suggested that these molecules should be renamed microbe-associated molecular patterns (MAMPs) (Donnelly et al. 2020). However, there should be functional distinction between MAMPs and PAMPs, as only the term PAMP emphasizes pathogenic microorganism, as opposed to MAMPs that focus on the commensal microbiota.

Emerging evidence has revealed an important role of microbiota in pain regulation. Microbiota have been implicated not only in visceral pain, but also in other types of pain including inflammatory pain, headache, and neuropathic pain, as well as opioid-induced anti-nociceptive tolerance (Guo et al. 2019). A study from Harvard Medical School has demonstrated that gut microbiota is critical for the induction of chemotherapy-induced pain, through LPS-mediated activation of TLR4 in the DRG (Shen et al. 2017). Furthermore, in the spinal cord and brain, gut-microbiota-derived mediators may regulate chronic pain and neuroinflammation through immune cells and microglia (Chen et al. 2018; Guo et al. 2019).

2.7.3 Antimalarial Drugs: Targeting Pathogens or Hosts?

According to the World Health Organization 2019 Report, malaria claims more than 400,000 lives every year. Artemisinin (ART) and its semisynthetic derivatives are used to treat malaria due to *Plasmodium falciparum* infection. Artemisia plants consist of 300 species and are distributed in temperate, warm temperate, and subtropical regions. Initial scientific efforts worldwide ended in failure after screening over 200,000 compounds against malaria. In the 1960s, Youyou Tu and her team screened over 2000 traditional Chinese recipes and made 380 herbal extracts, leading to the discovery of ART and its derivatives in 1972. This breakthrough in the twentieth-century tropical medicine has saved millions of lives in the world and led to the 2015 Nobel Prize in Physiology or Medicine. ART and its derivatives artemisinins (ARTs) might have a therapeutic value for several other diseases beyond malaria, including cancers, inflammatory diseases, and autoimmune disorders, as well as pain relief (Cao et al. 2020; Park 2019).

Malaria remains a major public health threat, especially in Southeast Asia, where artemisinin-based combination therapies (ACTs) are losing their efficacy (Wang et al. 2019). Artesunate (ARU) is a derivative of ART and the first-line treatment for children or adults with severe malaria. It was believed that the antimalarial

mechanism of action of ART is based on a direct action on parasites, involving activation of the endoperoxide bridge by ferrous heme. This reactive species may alkylate parasite proteins and lipids to cause lethality (Blasco et al. 2017). Recent studies have shown that ARU has an anti-inflammatory role in animal models, such as experimental cerebral malaria and experimental colitis, as well as human rheumatoid arthritis (RA) (An et al. 2017; Bang et al. 2021; Miranda et al. 2013; Xu et al. 2007; Yang et al. 2012). These studies suggest the possibility that the anti-malaria drugs may achieve their therapeutic effects by simultaneously targeting the pathogens (parasites) and treating the host (immune cells of the affected patients).

However, these studies failed to demonstrate the molecular targets (e.g., receptors) of ART and ARU on immune cells. Interestingly, it was proposed that in vivo ARU treatment causes rapid reduction in parasitemia by promoting phagocyte-mediated clearance (phagocytosis) of parasitized red blood cells (Khoury et al. 2017). Recent work from Duke University has identified a novel receptor for ARU and its anti-malaria actions (Bang et al. 2021). Computer simulations revealed ARU binding to GPR37, an orphan GPCR that was previously implicated in controlling macrophage phagocytosis (Bang et al. 2018). ARU promotes phagocytosis in macrophages in vitro and robustly improves survival and decreases hallmarks of sepsis, such as hypothermia, cytokine storm, and septic death.

The risk of death from sepsis is as high as 30% affecting about 49 million people worldwide in 2017, with 11 million deaths (Rudd et al. 2020). Thus, macrophage activation by GPR37 agonists such as ARU may also be of therapeutic benefit in sepsis. Notably, administration of ARU-primed macrophages was also sufficient to mitigate sepsis (Bang et al. 2021).

Infections of certain bacteria, such as *Staphylococcus aureus* and *Listeria* bacteria, result in severe pain in mice (Bang et al. 2021; Chiu et al. 2013). Intriguingly, *Listeria* bacteria-induced pain can be rapidly suppressed by ARU within 1 h of treatment. The severity and duration of the infection-induced pain are enhanced in mice lacking GPR37 (Bang et al. 2021). Furthermore, treatment of the infected animals with the ARU-stimulated macrophages could promote pain resolution (Bang et al. 2021). These findings strongly suggest that ARU-mediated activation of GPR37 in macrophages promotes pain resolution after infection, in further support of the emerging evidence showing the important role of macrophages in the resolution of inflammation and pain (Chen et al. 2020; Ji et al. 2011; Niehaus et al. 2021).

Last but not least, emerging studies suggest that ARU and/or its derivatives could inhibit SARS-CoV2 replication in vitro (Gendrot et al. 2020), leading to clinical evaluation of ARU in patients with mild COVID-19 (Kapepula et al. 2020). It will be of great interest and critical importance to investigate whether ARU may produce protective effects on COVID-19 through modulation of immune cells such as macrophages.

2.8 Inflammation Throughout the Body

Inflammation has long been recognized as a major cause of disease. An estimated 15% of human cancers are associated with chronic infection and inflammation. Acute and chronic tissue injury arising from inflammation has been found in many organ systems, including the heart, pancreas, liver, kidney, lung, and brain (Chen et al. 2017b).

Cardiovascular disease, and its associated pathology, atherosclerosis, is a major cause of death and disability throughout the world, and 23.6 million people are projected to die annually from cardiovascular disorders by 2030. Naturally, inflammatory mediators play very important roles in atherosclerosis, beginning with leukocyte recruitment and ending with the rupturing of the atherosclerotic plaque. Inflammation also contributes to cardiac stress. Increased levels of inflammatory cytokine and chemokine production and release are regularly found in affected cardiac tissues.

Pancreatitis, an inflammatory disease of the pancreas, has various causes, including the obstruction of the pancreatic duct, a mutation in the trypsinogen gene, or alcoholism. Acute pancreatitis (AP) occurs in 4–45 out of 100,000 patients per year and increases every year by approximately 1.3–4.0% in most developed countries. AP is one of the most common gastrointestinal-related causes for hospitalization in the USA. Chronic pancreatitis (CP) is less common than AP; however, CP patients suffer from chronic abdominal pain and exocrine and/or endocrine insufficiency, resulting in a reduced quality of life. Acinar cell destruction and activation of inflammatory cells, including macrophages, neutrophils, and granulocytes, which secrete inflammatory cytokines, are all seen in pancreatitis. Notably, pancreatitis is very painful, and pancreatic cancer is one of the most severe conditions of pain. Recent research has suggested that seeking surgery treatment early, among patients where surgical intervention is recommended, provides improved quality of life through more resistant pain relief (Skube and Beilman 2018).

Inflammation usually protects the liver from infection and injury, but excessive inflammation can cause extensive loss of hepatocytes, ischemia-reperfusion injury, metabolic alterations, and can eventually cause permanent hepatic damage. Inflammation can destroy hepatic parenchymal cells, increasing the risk of chronic liver diseases, such as non-alcoholic fatty liver disease (NAFLD) or viral hepatitis, which are a major cause of morbidity and mortality in the USA. Hepatocellular carcinomas, which make up 70–95% of all liver tumors, are known to cause abdominal pain in some patients and may cause intense abdominal pain in later stages in some patients (Christian-Miller and Frenette 2018).

Lung inflammatory diseases involve complex interactions between cells of the lungs and immune cells. Lung inflammation results primarily from tissue exposure to bacterial and viral pathogens, and/or environmental pollutants. Too much acute inflammation and resulting lung injury can cause pulmonary fibrosis and make gas exchange difficult. Unresolved lung injury and chronic inflammation are often seen

in acute respiratory distress syndrome, cystic fibrosis, chronic obstructive pulmonary disease (COPD), and asthma. About 90% of COPD cases are associated with cigarette-smoking-induced inflammation in small airways and lung parenchyma. Smoking cigarettes is a major risk factor for COPD, which involves both systemic and pulmonary inflammation. COVID-19 is a respiratory condition that often features excessive inflammation and leukocyte dysfunction caused by the coronavirus. Severe COVID-19 can result in acute respiratory distress syndrome. Patients with severe COVID-19 are known to have alveolar damage. In addition, post-mortem samples often reveal lung lesions (Shi et al. 2020). Body aches (including chest pain), headaches, and muscle pains may be an early symptom of COVID-19. COVID-19-associated persistent chest pain is listed as an emergency symptom by Centers for Disease Control and Prevention (CDC) and requires urgent medical care.

Inflammatory responses occur in the brain in many CNS diseases, including autoimmune diseases, neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD), and epilepsy. Inflammation in the brain can increase the excitability of neurons, injure cells, and increase blood-brain-barrier permeability to various molecules. CNS diseases associated with neuroinflammation are caused by the activation of the brain's resident immune cells and microglia, which produce pro-inflammatory mediators (Ji et al. 2014, 2018). These neuroinflammatory processes also involve both the innate and adaptive immune systems and may resemble immune responses to systemic infection (Chen et al. 2017a). Notably, neuroinflammation is a driving force of chronic pain (Ji et al. 2014).

2.9 Pain in Autoimmune and Inflammation-Related Diseases

We will discuss painful systemic autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), neuromyelitis optica spectrum disorder (NMOSD), and multiple sclerosis (MS) (Lee et al. 2021), as well as inflammation-associated vascular diseases such as Erythromelalgia (Waxman and Dib-Hajj 2005).

2.9.1 Complex Regional Pain Syndrome (CRPS)

Autoinflammatory and autoimmune contributions to CRPS have been documented (Clark et al. 2018). CRPS typically develops after injury or surgery to a limb, and severe pain and disability are common. Human studies have revealed changes in cytokines and other inflammatory mediators in the skin of affected limbs. CRPS research has been facilitated by the development of animal bone fracture models that can mimic chronic pain lasting for several months (Wei et al. 2016). The

autoinflammatory components of CRPS are regulated by neuropeptide-containing peripheral nerve fibers and the sympathetic nervous system (Li et al. 2009). Pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α are identified in the peripheral tissues (e.g., skin) during the acute phases of the syndrome. Interestingly, IL-1β in the spinal cord also contributes to the maintenance of pain in the late-phase (Wei et al. 2016). Emerging evidence from human, animal, and translational studies has demonstrated the production of autoantibodies and the potential targets of those antibodies (Clark et al. 2018). Autoimmunity in skin and muscle of the affected limb has been revealed from CRPS patients and laboratory animals. More recently, autoimmune contributions have been implicated by the presence of pain-promoting IgG and IgM antibodies in CRPS patients and animal models (Clark et al. 2018). Notably, CRPS can be characterized as CRPS-I and CRPS-II, with the latter involving neuropathic pain.

Erythromelalgia, or Mitchell's disease (named after Silas Weir Mitchell), is a rare vascular peripheral pain disorder in which blood vessels, usually in the lower extremities or hands, are episodically blocked (frequently on and off daily), then become hyperemic and inflamed (Waxman and Dib-Hajj 2005). Patients with erythromelalgia suffer from severe burning pain and display skin redness. Pain attacks are periodic and triggered by heat, pressure, mild activity, exertion, insomnia, or stress. Erythromelalgia may result from a primary or secondary disorder. Secondary erythromelalgia can be caused by small fiber neuropathy of any cause, polycythemia vera, essential thrombocytosis, hypercholesterolemia, mushroom or mercury poisoning, and some autoimmune disorders. A major progress in human genetics of pain has revealed that primary erythromelalgia is caused by mutation of the voltage-gated sodium channel α-subunit gene *SCN9A*, encoding Nav1.7 subunit (Bennett and Woods 2014; Waxman 2013).

Finally, fibromyalgia is a chronic and widespread musculoskeletal pain condition and has been shown to demonstrate fiber neuropathy with chronic neuroinflammation (Ji et al. 2018; Sommer et al. 2018). The degree to which the acute and chronic pain is mediated by the neuroinflammatory response is still an area for investigation. Also, associated with fibromyalgia is the decreased activity of enzymes that metabolize catecholamines, such as epinephrine and norepinephrine. Subsequently, pain conditions are exacerbated and maintained for a longer period of time, which may be associated with observed increased levels of pro-inflammatory cytokines in patients with chronic pain conditions. It was found that reduction in skin innervation is associated with a severe fibromyalgia phenotype (Evdokimov et al. 2019). Unbiased immune profiling has revealed a natural killer (NK) cell–peripheral nerve axis in fibromyalgia patients. NK cells may contribute to the loss of skin nerve innervation in these patients (Verma et al. 2022). A recent study from Sweden has demonstrated that transfer of serum/autoantibodies from fibromyalgia patients to naïve mice is sufficient to induce fibromyalgia-like pain and neuroinflammation (Goebel et al. 2021).

2.9.2 Multiple Sclerosis

Multiple sclerosis is characterized by the debilitating loss of myelin. Though the pathogenesis of multiple sclerosis remains poorly understood, studies suggest that T-cell-mediated inflammation directed against myelin may contribute to the condition. Pain from severe multiple sclerosis can be immensely disabling, often requiring patients to have a caretaker (Garg and Smith 2015). The cause of the activation of T-cells against the host is unclear but may involve infectious agents from the environment. Corticosteroids in conjunction with adrenocorticotropic hormone have immunomodulatory effects that can be acute therapeutic options. Additionally, interferon injections can limit the entry of T-cells into the CNS, stabilizing the blood-brain barrier, but may cause side effects including flu-like symptoms, fluctuations in liver enzyme levels, and injection site reactions, among others (Berkovich et al. 2017; Tan et al. 2021).

2.9.3 Neuromyelitis Optica Spectrum Disorder (NMOSD)

Neuromyelitis optica spectrum disorder (NMOSD) is a disease that affects the central nervous system. In NMOSD, inflammation characteristically appears in stretches of the spinal cord and/or in the optic nerve. Patients with NMOSD often become blind and paralyzed with the progression of the disease. Although the precise cause of NMOSD is unclear, patients with NMOSD are noted to have antibodies against aquaporin-4, a channel for water in astrocytes. NMOSD is a debilitating painful disease for approximately 80% of patients, resulting in significant reduction in quality of life (Bradl et al. 2014). Antibodies against AQP4 lead to interleukin-6 (IL-6) production, resulting in decreased blood-brain-barrier functions. Additionally, astrocytes expressing AQP4 become debilitated and support for nearby oligodendrocytes and neurons become limited, leading to granulocyte infiltration and demyelination as a result of damaged oligodendrocytes. Acute treatment primarily involves high-dose steroids while long-term treatment uses immunosuppressants including anti-IL-6 treatment (Bradl et al. 2014; Ji et al. 2019).

2.9.4 Rheumatoid Arthritis (RA)

Rheumatoid arthritis is a common form of inflammatory arthritis. Although the mechanisms of pain are not fully understood, research indicates that TNF-α and interleukin-1 cytokines are present in affected synovial fluid and tissues at higher levels. Accordingly, anti-TNF-α interventions have emerged as a treatment option to target aggravated inflammatory pathways. Severe inflammation of the diarthrodial joint is seen in rheumatoid arthritis, resulting in significantly decreased mobility

and ongoing pain. Rheumatoid arthritis is a major health issue in the USA, where it afflicts 1.3 million adults. In addition to producing major costs in terms of productivity and treatment costs, the condition significantly reduces quality of life, making it difficult for patients to perform many day-to-day tasks (Lee and Weinblatt 2001). Immunotherapies such as monoclonal antibodies against cytokines and cytokine receptors have been approved to treat RA symptoms (Kalpachidou et al. 2022). Majority of RA patients complain about pain such as joint stiffness. The effects of the immunotherapies on RA pain remain to be validated.

2.9.5 Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus is a chronic autoimmune disorder. Research has demonstrated that microparticles (MPs), small membrane-bound vesicles, can contribute to the pathogenesis of SLE. In MPs, DNA and RNA may initiate and sustain autoantibody production. Additionally, the particles can source extracellular nuclear molecules to form pathogenic immune complexes, leading to elevated inflammatory responses through multiple receptor systems. Inflammation in lupus can also be visible in red, swollen joints. SLE is also a risk factor for atherosclerosis, a disease marked by fatty plaques in arteries, in adolescents, increasing the risk of myocardial infarction. Consequently, dyslipidemia patterns in pediatric SLE should be monitored routinely. In SLE, the body produces antibodies against its own antigen in cell nuclei and cytoplasm, causing damage in numerous organs. SLE can cause significant pain, usually widespread muscle stiffness or aches. Although treatable, the pain is recurrent and requires continuous treatment (Pisetsky et al. 2021).

2.10 Concluding Remarks

As a cardinal feature of inflammation, pain is naturally associated with inflammation. During inflammation, pro-inflammatory mediators are produced that can evoke pain. Thus, anti-inflammatory treatments can effectively alleviate acute pain. Acute inflammation is also beneficial for wound healing, and inflammation-produced pro-resolving mediators such as SPMs are potent inhibitors of pain. However, chronic inflammation, including neuroinflammation, can lead to various pathological conditions associated with chronic pain. It is generally believed that chronic pain persists after the observable signs and symptoms of inflammation have resolved. However, recent advances in understanding of neuroinflammation are changing this perspective. We have begun to appreciate that neuroinflammation is associated with and perhaps mediates the transition from acute to chronic pain, as well as chronification of human pain conditions. Thanks to the proximity to pain neurocircuit in the PNS and CNS, neuroinflammation-associated mediators or neuromodulators (e.g., cytokines and chemokines) are highly effective in modulating pain sensitivity.

Importantly, neuroinflammation contributes to the pathophysiology of chronic overlapping pain conditions, including but not limited to fibromyalgia, headache, temporomandibular disorder, back pain, irritable bowel syndrome, primary headaches, pelvic pain, and vestibulodynia (Ji et al. 2018). Thus, control of neuroinflammation may help to alleviate chronic overlapping pain conditions.

References

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124(4):783–801.

An J, Minie M, Sasaki T, Woodward JJ, Elkon KB. Antimalarial drugs as immune modulators: new mechanisms for old drugs. Annu Rev Med. 2017;68:317–30.

Bang S, Xie YK, Zhang ZJ, Wang Z, Xu ZZ, Ji RR. Gpr37 regulates macrophage phagocytosis and resolution of inflammatory pain. J Clin Invest. 2018;128(8):3568–82.

Bang S, Donnelly CR, Luo X, Toro-Moreno M, Tao X, Wang Z, Chandra S, Bortsov AV, Derbyshire ER, Ji RR. Activation of gpr37 in macrophages confers protection against infection-induced sepsis and pain-like behaviour in mice. Nat Commun. 2021;12(1):1704.

Baral P, Udit S, Chiu IM. Pain and immunity: implications for host defence. Nat Rev Immunol. 2019;19(7):433–47.

Bennett DL, Woods CG. Painful and painless channelopathies. Lancet Neurol. 2014;13(6):587–99.

Berkovich R, Bakshi R, Amezcua L, Axtell RC, Cen SY, Tauhid S, Neema M, Steinman L. Adrenocorticotropic hormone *versus* methylprednisolone added to interferon β in patients with multiple sclerosis experiencing breakthrough disease: a randomized, rater-blinded trial. Ther Adv Neurol Disord. 2017;10(1):3–17.

Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking plasmodium falciparum parasite biology to the clinic. Nat Med. 2017;23(8):917–28.

Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol. 2009;60:379–406.

Bradl M, Kanamori Y, Nakashima I, Misu T, Fujihara K, Lassmann H, Sandkühler J. Pain in neuromyelitis optica – prevalence, pathogenesis and therapy. Nat Rev Neurol. 2014;10(9):529–36.

Brigham NC, Ji RR, Becker ML. Degradable polymeric vehicles for postoperative pain management. Nat Commun. 2021;12(1):1367.

Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. Lancet Neurol. 2012;11(7):629–42.

Cao R, Hu H, Li Y, Wang X, Xu M, Liu J, Zhang H, Yan Y, Zhao L, Li W, et al. Anti-sars-cov-2 potential of artemisinins in vitro. ACS Infect Dis. 2020;6(9):2524–31.

Chen G, Kim YH, Li H, Luo H, Liu DL, Zhang ZJ, Lay M, Chang W, Zhang YQ, Ji RR. Pd-l1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via pd-1. Nat Neurosci. 2017a;20(7):917–26.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017b;9(6):7204–18.

Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100(6):1292–311.

Chen O, Donnelly CR, Ji RR. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020;62:17–25.

Chiu IM. Infection, pain, and itch. Neurosci Bull. 2018;34(1):109–19.

Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. Nat Neurosci. 2012;15(8):1063–7.

Chiu IM, Heesters BA, Ghasemlou N, von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR, et al. Bacteria activate sensory neurons that modulate pain and inflammation. Nature. 2013;501(7465):52–7.

Christian-Miller N, Frenette C. Hepatocellular cancer pain: impact and management challenges. J Hepatocell Carcinoma. 2018;5:75–80.

Christianson CA, Dumlao DS, Stokes JA, Dennis EA, Svensson CI, Corr M, Yaksh TL. Spinal tlr4 mediates the transition to a persistent mechanical hypersensitivity after the resolution of inflammation in serum-transferred arthritis. Pain. 2011;152(12):2881–91.

Clark DJ, Tawfik VL, Tajerian M, Kingery WS. Autoinflammatory and autoimmune contributions to complex regional pain syndrome. Mol Pain. 2018;14:1744806918799127.

Cohen JA, Edwards TN, Liu AW, Hirai T, Jones MR, Wu J, Li Y, Zhang S, Ho J, Davis BM, et al. Cutaneous TRPV1(+) neurons trigger protective innate type 17 anticipatory immunity. Cell. 2019;178(4):919–32 e914.

Corder G, Castro DC, Bruchas MR, Scherrer G. Endogenous and exogenous opioids in Pain. Annu Rev Neurosci. 2018;41:453–73.

Diogenes A, Ferraz CC, Akopian AN, Henry MA, Hargreaves KM. LPS sensitizes TRPV1 via activation of TLR4 in trigeminal sensory neurons. J Dent Res. 2011;90(6):759–64.

Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends Neurosci. 2020;43(10):822–38.

Erny D, Hrabe de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18(7):965–77.

Evdokimov D, Frank J, Klitsch A, Unterecker S, Warrings B, Serra J, Papagianni A, Saffer N, Meyer Zu Altenschildesche C, Kampik D, Malik RA, Sommer C, Üçeyler N. Reduction of skin innervation is associated with a severe fibromyalgia phenotype. Ann Neurol. 2019 Oct;86(4):504–16.

Garg N, Smith TW. An update on immunopathogenesis, diagnosis, and treatment of multiple sclerosis. Brain Behav. 2015;5(9):e00362.

Gendrot M, Duflot I, Boxberger M, Delandre O, Jardot P, Le Bideau M, Andreani J, Fonta I, Mosnier J, Rolland C, et al. Antimalarial artemisinin-based combination therapies (act) and covid-19 in Africa: In vitro inhibition of sars-cov-2 replication by mefloquine-artesunate. Int J Infect Dis. 2020;99:437–40.

Gereau RW, Sluka KA, Maixner W, Savage SR, Price TJ, Murinson BB, Sullivan MD, Fillingim RB. A pain research agenda for the 21st century. J Pain. 2014;15(12):1203–14.

Goebel A, Krock E, Gentry C, Israel MR, Jurczak A, Urbina CM, Sandor K, Vastani N, Maurer M, Cuhadar U, Sensi S, Nomura Y, Menezes J, Baharpoor A, Brieskorn L, Sandström A, Tour J, Kadetoff D, Haglund L, Kosek E, Bevan S, Svensson CI, Andersson DA. Passive transfer of fibromyalgia symptoms from patients to mice. J Clin Invest. 2021;131(13):e144201.

Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med. 2010;16(11):1248–57.

Gosselin RD, Suter MR, Ji RR, Decosterd I. Glial cells and chronic pain. Neuroscientist. 2010;16(5):519–31.

Guo R, Chen LH, Xing C, Liu T. Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential. Br J Anaesth. 2019;123(5):637–54.

Hanani M, Spray DC. Emerging importance of satellite glia in nervous system function and dysfunction. Nat Rev Neurosci. 2020;21(9):485–98.

Heidland A, Klassen A, Rutkowski P, Bahner U. The contribution of Rudolf Virchow to the concept of inflammation: what is still of importance? J Nephrol. 2006;10(Suppl):S102–9.

Ji RR, Xu ZZ, Strichartz G, Serhan CN. Emerging roles of resolvins in the resolution of inflammation and pain. Trends Neurosci. 2011;34(11):599–609.

Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533–48.

Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science. 2016;354(6312):572–7.
Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology. 2018;129(2):343–66.
Ji RR, Donnelly CR, Nedergaard M (2019) Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20(11):667–685. https://doi.org/10.1038/s41583-019-0218-1
Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413(6852):203–10.
Kalpachidou T, Riehl L, Schöpf CL, Ucar B, Kress M. Proinflammatory cytokines and their receptors as druggable targets to alleviate pathological pain. Pain. 2022;163(Suppl 1):S79–98.
Kapepula PM, Kabengele JK, Kingombe M, Van Bambeke F, Tulkens PM, Sadiki Kishabongo A, Decloedt E, Zumla A, Tiberi S, Suleman F, Tshilolo L, Muyembe-TamFum JJ, Zumla A, Nachega JB. *Artemisia* Spp. derivatives for COVID-19 treatment: anecdotal use, political hype, treatment potential, challenges, and road map to randomized clinical trials. Am J Trop Med Hyg. 2020;103(3):960–4.
Khoury DS, Cromer D, Elliott T, Soon MSF, Thomas BS, James KR, Best SE, Aogo RA, Engel JA, Gartlan KH, et al. Characterising the effect of antimalarial drugs on the maturation and clearance of murine blood-stage plasmodium parasites in vivo. Int J Parasitol. 2017;47(14):913–22.
Kvietys PR, Granger DN. Endothelial cell monolayers as a tool for studying microvascular pathophysiology. Am J Phys. 1997;273(6):G1189–99.
Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet. 2001;358(9285):903–11.
Lee DSW, Rojas OL, Gommerman JL. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. Nat Rev Drug Discov. 2021;20(3):179–99.
Lei V, Handfield C, Kwock JT, Kirchner SJ, Lee MJ, Coates M, Wang K, Han Q, Wang Z, Powers JG, Wolfe S, Corcoran DL, Fanelli B, Dadlani M, Ji RR, Zhang JY, MacLeod AS. Skin injury activates a rapid TRPV1-dependent antiviral protein response. J Invest Dermatol. 2022;142(8):2249–59.e9.
Lewis WD, Lilly S, Jones KL. Lymphoma: diagnosis and treatment. Am Fam Physician. 2020;101(1):34–41.
Li WW, Guo TZ, Liang D, Shi X, Wei T, Kingery WS, Clark JD. The nalp1 inflammasome controls cytokine production and nociception in a rat fracture model of complex regional pain syndrome. Pain. 2009;147(1–3):277–86.
Liu T, Xu ZZ, Park CK, Berta T, Ji RR. Toll-like receptor 7 mediates pruritus. Nat Neurosci. 2010;13(12):1460–2.
Liu T, Gao YJ, Ji RR. Emerging role of toll-like receptors in the control of pain and itch. Neurosci Bull. 2012;28(2):131–44.
Liu XJ, Zhang Y, Liu T, Xu ZZ, Park CK, Berta T, Jiang D, Ji RR. Nociceptive neurons regulate innate and adaptive immunity and neuropathic pain through MyD88 adapter. Cell Res. 2014;24(11):1374–7.
Mainwaring T. An account of an uncommon inflammation of the epiglottis: Communicated in a Letter to Everard Home, Esq. F.R.S., and by Him to Dr. Simmons. Med Facts Obs. 1791;1:40–42. PMID: 29106131; PMCID: PMC5072801.
Marion E, Song OR, Christophe T, Babonneau J, Fenistein D, Eyer J, Letournel F, Henrion D, Clere N, Paille V, et al. Mycobacterial toxin induces analgesia in buruli ulcer by targeting the angiotensin pathways. Cell. 2014;157(7):1565–76.
Martinez Quintero B, Yazbeck C, Sweeney LB. Thyroiditis: evaluation and treatment. Am Fam Physician. 2021;104(6):609–17.
Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. J Anesth. 2019;33(1):131–9.
Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428–35.
Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771–6.
Medzhitov R. The spectrum of inflammatory responses. Science. 2021;374(6571):1070–5.

Miranda AS, Brant F, Rocha NP, Cisalpino D, Rodrigues DH, Souza DG, Machado FS, Rachid MA, Teixeira AL Jr, Campos AC. Further evidence for an anti-inflammatory role of artesunate in experimental cerebral malaria. Malar J. 2013;12:388.

Niehaus JK, Taylor-Blake B, Loo L, Simon JM, Zylka MJ. Spinal macrophages resolve nociceptive hypersensitivity after peripheral injury. Neuron. 2021;109(8):1274–82 e1276.

Park JY. Artemisinin and the Nobel Prize in physiology or medicine 2015. Korean J Pain. 2019;32(3):145–6.

Pisetsky DS, Eudy AM, Clowse MEB, Rogers JL. The categorization of pain in systemic lupus erythematosus. Rheum Dis Clin N Am. 2021;47(2):215–28.

Plytycz B, Seljelid B. From inflammation to sickness: historical perspective. Arch Immunol Ther Exp. 2003;51(2):105–9.

Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, Wood JN, von Andrian UH. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. Nature. 2014;510(7503):157–61.

Rochae e Silva M. A brief history of inflammation. In: Vane JR, Ferreira SH, editors. Inflammation. Handbook of experimental pharmacology (continuation of Handbuch der experimentellen Pharmakologie), vol. 50/1. Berlin/Heidelberg: Springer; 1978. https://doi.org/10.1007/978-3-642-66888-3_2.

Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kissoon N, Finfer S, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the global burden of disease study. Lancet. 2020;395(10219):200–11.

Scrivo R, Vasile M, Bartosiewicz I, Valesini G. Inflammation as "common soil" of the multifactorial diseases. Autoimmun Rev. 2011;10(7):369–74.

Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol. 2007;25:101–37.

Shen S, Lim G, You Z, Ding W, Huang P, Ran C, Doheny J, Caravan P, Tate S, Hu K, et al. Gut microbiota is critical for the induction of chemotherapy-induced pain. Nat Neurosci. 2017;20(9):1213–6.

Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, Fan Y, Zheng C. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020 Apr;20(4):425–34.

Shiers S, Ray PR, Wangzhou A, Sankaranarayanan I, Tatsui CE, Rhines LD, Li Y, Uhelski ML, Dougherty PM, Price TJ. ACE2 and SCARF expression in human dorsal root ganglion nociceptors: implications for SARS-CoV-2 virus neurological effects. Pain. 2020;161(11):2494–501.

Skube ME, Beilman GJ. Surgical treatment of pain in chronic pancreatitis. Curr Opin Gastroenterol. 2018 Sep;34(5):317–21.

Sommer C, Leinders M, Üçeyler N. Inflammation in the pathophysiology of neuropathic pain. Pain. 2018;159(3):595–602.

Talbot S, Foster SL, Woolf CJ. Neuroimmunity: physiology and pathology. Annu Rev Immunol. 2016;34:421–47.

Tan PH, Ji J, Yeh CC, Ji RR. Interferons in pain and infections: emerging roles in neuro-immune and neuro-glial interactions. Front Immunol. 2021 Nov 5;12:783725. https://doi.org/10.3389/fimmu.2021.783725. eCollection 2021

Verma V, Drury GL, Parisien M, Özdağ Acarli AN, Al-Aubodah TA, Nijnik A, Wen X, Tugarinov N, Verner M, Klares R 3rd, Linton A, Krock E, Morado Urbina CE, Winsvold B, Fritsche LG, Fors EA, Piccirillo C, Khoutorsky A, Svensson CI, Fitzcharles MA, Ingelmo PM, Bernard NF, Dupuy FP, Üçeyler N, Sommer C, King IL, Meloto CB, Diatchenko L, HUNT-All In Pain. Unbiased immune profiling reveals a natural killer cell-peripheral nerve axis in fibromyalgia. Pain. 2022 July 1;163(7):e821–36. https://doi.org/10.1097/j.pain.0000000000002498.

Volkow ND, Collins FS. The role of science in addressing the opioid crisis. N Engl J Med. 2017;377(4):391–4.

Wang J, Xu C, Liao FL, Jiang T, Krishna S, Tu Y. A temporizing solution to "artemisinin resistance". N Engl J Med. 2019;380(22):2087–9.

Waxman SG. Painful Na-channelopathies: An expanding universe. Trends Mol Med. 2013;19(7):406–9.

Waxman SG, Dib-Hajj SD. Erythromelalgia: a hereditary pain syndrome enters the molecular era. Ann Neurol. 2005;57(6):785–8.

Wei T, Guo TZ, Li WW, Kingery WS, Clark JD. Acute versus chronic phase mechanisms in a rat model of CRPS. J Neuroinflammation. 2016;13:14.

Woolf CJ, Ma Q. Nociceptors – noxious stimulus detectors. Neuron. 2007;55(3):353–64.

Xanthos DN, Sandkuhler J. Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. Nat Rev Neurosci. 2014;15(1):43–53.

Xu H, He Y, Yang X, Liang L, Zhan Z, Ye Y, Yang X, Lian F, Sun L. Anti-malarial agent artesunate inhibits TNF-alpha-induced production of proinflammatory cytokines via inhibition of NF-kappaB and pi3 kinase/Akt signal pathway in human rheumatoid arthritis fibroblast-like synoviocytes. Rheumatology (Oxford). 2007;46(6):920–6.

Yang Z, Ding J, Yang C, Gao Y, Li X, Chen X, Peng Y, Fang J, Xiao S. Immunomodulatory and anti-inflammatory properties of artesunate in experimental colitis. Curr Med Chem. 2012;19(26):4541–51.

Yang NJ, Isensee J, Neel DV, Quadros AU, Zhang HB, Lauzadis J, Liu SM, Shiers S, Belu A, Palan S, et al. Anthrax toxins regulate pain signaling and can deliver molecular cargoes into ANTXR2(+) DRG sensory neurons. Nat Neurosci. 2021;25(2):168–79.

Zhang X, He Y. The role of nociceptive neurons in the pathogenesis of psoriasis. Front Immunol. 2020;11:1984.

Chapter 3
Animal Models of Pain and Anti-inflammatory Treatments

Aidan McGinnis, Michael Wang, and Ru-Rong Ji

Abstract Animal models are critical to the field of pain research, both for the study of mechanisms and the testing of novel therapeutics. Unfortunately, many findings that appear promising in animals fail to translate to human disease, underscoring the criticality of thoughtful model establishment and meaningful behavioral testing. In this chapter, we review current methods for the measurements of mechanical pain, thermal pain, and spontaneous pain in mice and rats. We then discuss different models with a focus on rat and mouse models of inflammatory and neuropathic pain. While most of these models involve significant inflammation, not all respond similarly to anti-inflammatory treatments. We examine the varying efficacy of common anti-inflammatory treatments in this range of models and highlight promising pro-resolution and/or immunoregulatory treatments where relevant.

Keywords Animal models · Anti-inflammatory treatment · Behavioral testing · Inflammatory pain · Mice · Neuropathic pain · Nerve injury · Neuropathy · Non-steroidal anti-inflammatory drugs (NSAIDs) · Rats · Rodents · Specialized pro-resolving mediators (SPMs)

3.1 Introduction

Researchers are both scientifically and morally obliged to make the most of every animal that passes through their laboratory (Zimmermann 1983). Choosing a model carefully and evaluating effects on pain accurately are critical steps in maximizing the odds of achieving clinical relevance.

Mice and rats remain the animals most commonly used to model pain (Sadler et al. 2021) (Fig. 3.1). Rats are anecdotally calmer in demeanor than mice and their larger scale means that model establishment and behavioral measurement can be

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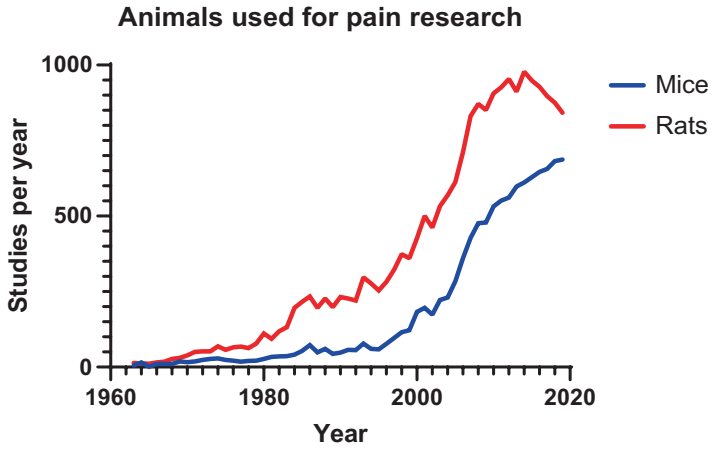

Fig. 3.1 Comparison of rodent species used in pain research in the past 40 years. Note an exponential increase in mouse research since 2000

more convenient. Rats also require less time to habituate to novel environments. Unsurprisingly then, most classical pain models were initially established in rats (Bennett and Xie 1988; Decosterd and Woolf 2000; Kim and Chung 1992; Seltzer et al. 1990; Stein et al. 1988). Of late, mice have become an increasingly popular choice (Fig. 3.1). Nearly all rat models can be established in mice with enough care. Mice, and their housing, can be less expensive, allowing investigators to work with larger sample sizes. Most critically, transgenic mice are both more available than transgenic rats and more convenient due to their short breeding turnarounds (productive breeders can produce pups every 3 weeks). Transgenic mice have now been used in pain research for more than 20 years and have greatly expanded our knowledge of the molecular mechanisms of pain (Caterina et al. 2000; Malmberg et al. 1997; Sadler et al. 2021).

There are critical shortcomings of murine models that must be considered when designing experiments or drawing conclusions from the literature. There are important differences between rodents and humans, many of which are not fully understood, making translation unpredictable. For example, human genetics has strongly implicated the sodium channel subunit Nav1.7 (encoded by *SCN9A*) in human pain syndromes (Bennett and Woods 2014; Waxman and Dib-Hajj 2005), but there is a striking species difference in Nav1.7 expression: human sensory neurons exhibit much higher Nav1.7 expression than mouse sensory neurons (Chang et al. 2018). Many more genes are differentially expressed in mouse vs. human dorsal root ganglion (DRG), where primary sensory neurons are localized (Shiers et al. 2020). Leaving aside murine–hominoid differences, even different varieties of inbred mice have been demonstrated to respond divergently to painful stimuli (Mogil et al. 1999). Behavior that is not consistent between two mouse strains cannot be assumed to be relevant to human patients. Murine pain models chosen for ease of establishment or convenient testing can fall short of approximating conditions found in

humans, limiting the relevance of any work done using them. Despite the fact that chronic pain is more commonly diagnosed in women, more than half of the murine-model-based studies published in *PAIN* between 2016 and 2020 used only male mice or rats (Sadler et al. 2021). The use of inbred rodents (inbred mice in particular are commonly used), the use of young rodents (likely to save on housing costs), and the standardization of life experiences prior to model establishment also fail to properly model the diverse and, on average, older population of chronic pain patients. Finally, accurately measuring pain behavior has been a key challenge for the field. Even the most relied upon tests, while clearly measuring something, are only guessing at the animal's lived experience. Many tests elicit responses unlike the pain actually experienced by human patients (Blackburn-Munro 2004).

Pain is multifaceted, but, simplistically, arises for one of several reasons. Evoked pain occurs in response to a stimulus, which can be mechanical (mechanical pain), hot (thermal pain), or cold (cold pain). Evoked pain can manifest as physical withdrawal and/or vocalization. Evoked pain can further be divided into allodynia and hyperalgesia. Allodynia is pain caused by a normally benign/innocuous stimulus; hyperalgesia, aka hypersensitivity, is the experience of increased pain in response to a normally painful stimulus. Allodynia and hyperalgesia can be highly distressing and are reported by some chronic pain patients. Spontaneous pain arises without a clear transient stimulus. In rodents, it is commonly observed as licking, lifting, flinching, and/or guarding of an injured/painful area, behaviors that are collectively referred to as "nocifensive." Changes in facial expression, altered gait, and/or reduction in physical activity can also indicate spontaneous pain. Millions of Americans combat spontaneous, often ongoing pain. Pain research has been criticized for relying too heavily on the more convenient measures of evoked pain.

3.2 Assessment of Mechanical Pain

The pain most measured in animal research is mechanical pain (Sadler et al. 2021), which in healthy animals is defined as nociceptive pain occurring in response to a mechanical stimulus.

3.2.1 The Von Frey Test (VFT)

The most used test for mechanical pain assessment is the Von Frey filament test (Fig. 3.2a). A Von Frey filament is a thin fiber with a known stiffness (measured in grams). To perform the Von Frey test, series of filaments are applied to the area of interest, commonly (but not necessarily) the animal's hindpaw, and the responses to each are recorded. Von Frey filaments have also been used to measure facial pain (application to the periorbital region) and visceral pain (application to the thoracic region). Results are often reported as a threshold value calculated using the

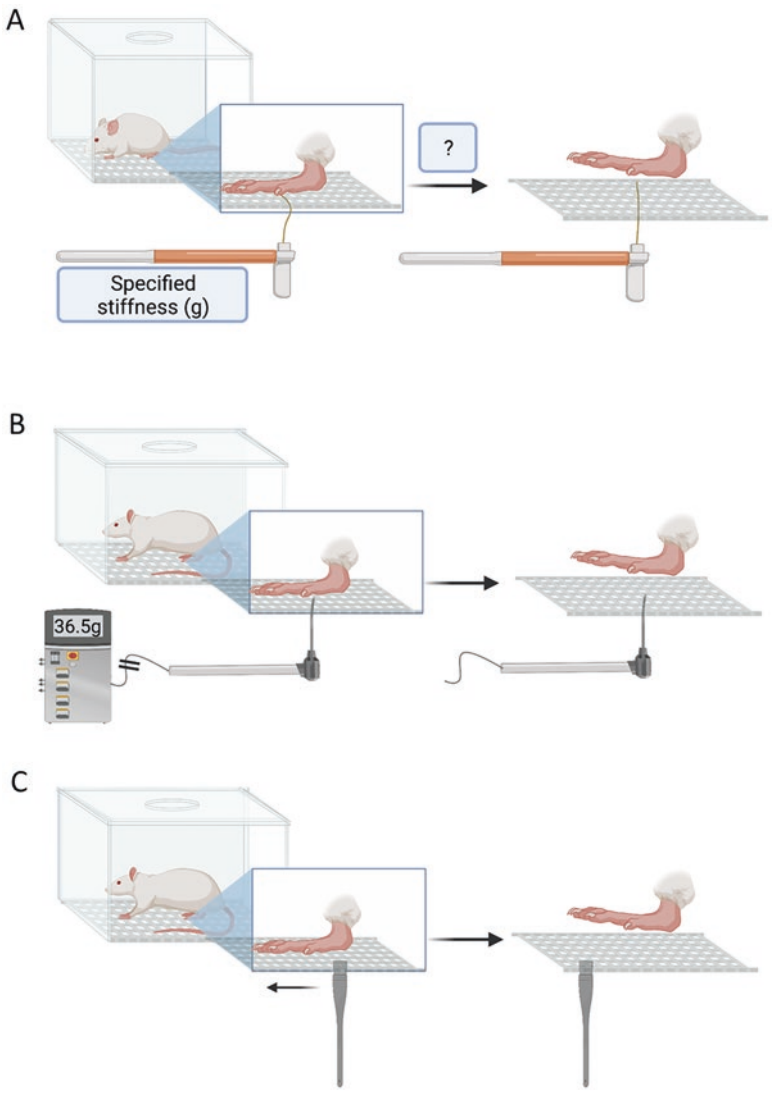

Fig. 3.2 Testing mechanical pain in rodents: (**a**) Von Frey hair test; (**b**) electronic Von Frey filament test; and (**c**) brush test for dynamic mechanical allodynia

"up–down method" (Chaplan et al. 1994; Dixon 1980). The experimenter applies increasingly thicker filaments until the animal responds, at which point the experimenter drops to the last filament that did not elicit a response and continues the process. An equation is used to estimate the amount of force that would cause a withdrawal 50% of the time. Other methods for determining a withdrawal threshold have also been validated; the exact method is likely of less importance than the skill of the experimenter. Von Frey filaments are also used to calculate the frequency of withdrawals in response to a fixed stimulus. An electronic device is available for rat

Von Frey testing (Fig. 3.2b), which applies continuously increasing force and reports the grams elicited when contact with the animal is lost (Tao et al. 2021). This method requires a steady hand but removes the need for threshold estimation and has become a mainstay in rat studies of mechanical allodynia.

Altogether, the Von Frey test has several advantages. It is quick, inexpensive, and easily understood. Since arrays of fiber widths are available, hypersensitivity and allodynia can be tested. The filaments cause little or no paw damage, allowing for time point testing as frequently as hourly. The Von Frey test has important limitations. Allodynia is a feature of chronic pain, but it is rarely caused by a pinprick—the dynamic brush of clothing and simply pain from walking are much more typical examples. The field's blanket application of the up–down quantification method has also been criticized (Bradman et al. 2015; Christensen et al. 2020). The most common series of filaments (0.16 g, 0.4 g, 0.6 g, 1.0 g, 2.0 g) used for mice only approximately fit the logarithmic relationship for which the equations used were developed; solutions have been proposed (Christensen et al. 2020) but are yet to be widely adopted. Finally, the Von Frey test is highly subjective; experimenter blindness is critical to meaningful data.

3.2.2 Dynamic Mechanical Allodynia

To test for mechanical allodynia in response to dynamic stimuli more closely resembling real-world physical contact, a brush can be used (Fig. 3.2c). This sensation is distinct from the fine-point pressure caused by a Von Frey filament. The plantar hindpaw (other locations are very seldom reported) is stimulated using a paintbrush by stroking in the heel-to-toe direction (Cheng et al. 2017). Results are presented as the frequency of or latency to a withdrawal response. Another method quaternizes responses: 0 = no response, 1 = paw withdrawal, 2 = shaking of the paw, and 3 = licking of the paw.

3.2.3 Paw-Pressure Test

The paw-pressure test (PPT), also known as the Randall-Selitto test (Randall and Selitto 1957), functions similarly to the electronic Von Frey test. Briefly, the experimenter restrains the animal to the apparatus then slowly applies a blunt point, also attached to the machinery, to the target region. The apparatus measures the increasing pressure exerted in grams. A common endpoint reported is the pressure threshold to attempted withdrawal/struggling. Pressure needed to evoke vocalization is also a common measurement and can be performed on the second paw. A mean of several test values is often taken to reduce variability (Kayser 2013). The paw-pressure test is useful in that it produces a single and easily interpretable value. Less judgment is required on the part of the experimenter than in the VFT. Great care should be made to standardize restraint procedures and minimize animal handling.

3.3 Assessment of Thermal Pain

Thermal pain is simply evoked pain occurring in response to a warm or noxiously hot stimulus (Fig. 3.3). Animals sensitive to mechanical stimuli are commonly but not necessarily sensitive to thermal stimuli, and vice versa. There are three exceedingly common tests for thermal pain, each with its own advantages and disadvantages.

3.3.1 Paw Withdrawal to Radiant Heat (Hargreaves) Test

The Hargreaves test, developed by Dr. Kenneth Hargreaves in 1988, is a convenient way to measure thermal hyper- or hypoalgesia (Hargreaves et al. 1988) (Fig. 3.3a). The animal is first habituated to a transparent box placed over a glass surface. A device is then used to concentrate a radiant or infrared source of thermal heat onto a hindpaw. The animal is monitored for withdrawal from the heat stimuli and latency to withdrawal is recorded. In order to prevent heat-induced tissue injury, the apparatus is shut off after a short period, i.e., 20 s (the cut-off value), if there is no response. Proper habituation is critical to successful data collection.

3.3.2 Hot Plate Test

The hot plate test, pioneered for behavioral research as far back as 1953 (Eddy and Leimbach 1953), also uses noxious heat to test for hyperalgesia (or hypoalgesia) (Fig. 3.3b). The animal is placed onto a hot surface enclosed by transparent walls at a temperature typically between 50 and 55 °C. Latency to nocifensive behavior (including escape attempts) is recorded. This test is highly convenient; most animals respond within 30 s (the cut-off value; if they do not, the test is ended to prevent tissue damage). A single, easily understandable number is produced. Unfortunately, the hot plate test is highly subjective—perhaps the most subjective of the classical pain tests. This is likely why the Hargreaves test has surpassed the hot plate test as the most common assay for thermal pain (Sadler et al. 2021). However, the hot plate test may be more suitable for testing the supraspinal cord mechanisms of pain.

3.3.3 Tail Flick Test

First reported in 1941 when it was used to compare opioid-induced analgesia to the effects of cobra venom (D'Amour and Smith 1941), the tail flick test is often used in conjunction with one of the above tests (Fig. 3.3c). To perform the tail flick test,

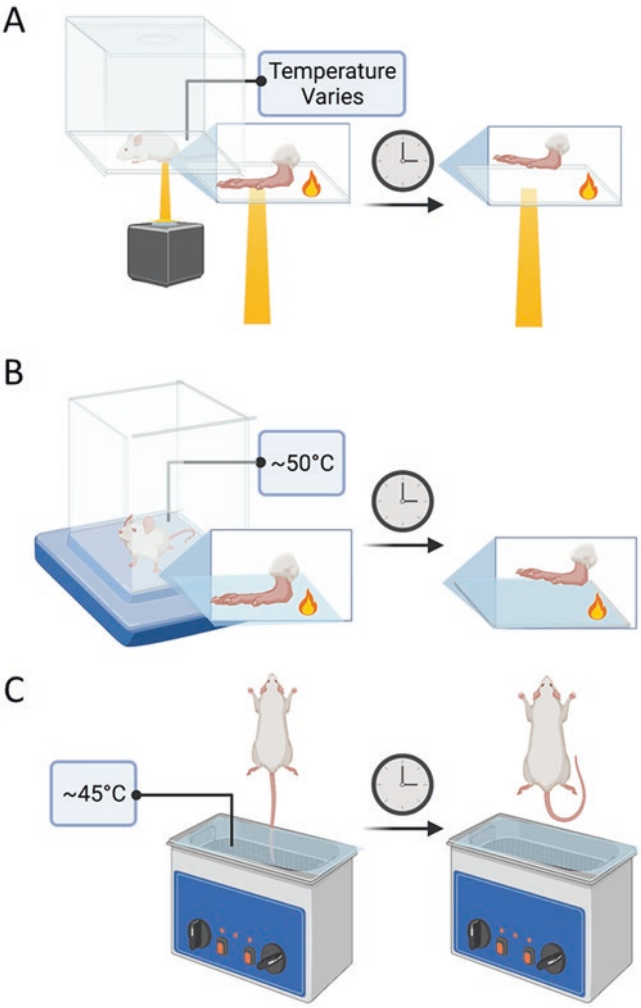

Fig. 3.3 Testing thermal pain in rodents: (**a**) Hargreaves test; (**b**) hot plate test; and (**c**) tail flick test

the animal is grasped securely, and the tail is either inserted into water between 45 and 50 °C or exposed to a beam of condensed heat. Latency to rapid flicking or withdrawal of the tail is recorded. Unlike typical nocifensive behavior, the tail flick reflex occurs at the spinal level, meaning that treatments such as opioids affect it differently than other tests of thermal hyper/hypoalgesia. Given this, tail flick testing is the gold standard for testing opioid analgesia (Wang et al. 2020). Like the hot plate test, it can be performed very rapidly. Special care should be taken to minimize stress and standardize the restraint technique used.

3.4 Cold Pain

Like heat pain, cold pain is evoked pain occurring in response to a cold or noxiously cold stimulus (Fig. 3.4). Cold pain too has its own receptors and can occur independently of other modalities. Although cold pain is important to chronic pain patients, the pain field studies it less than heat pain and significantly less than mechanical pain (Sadler et al. 2021).

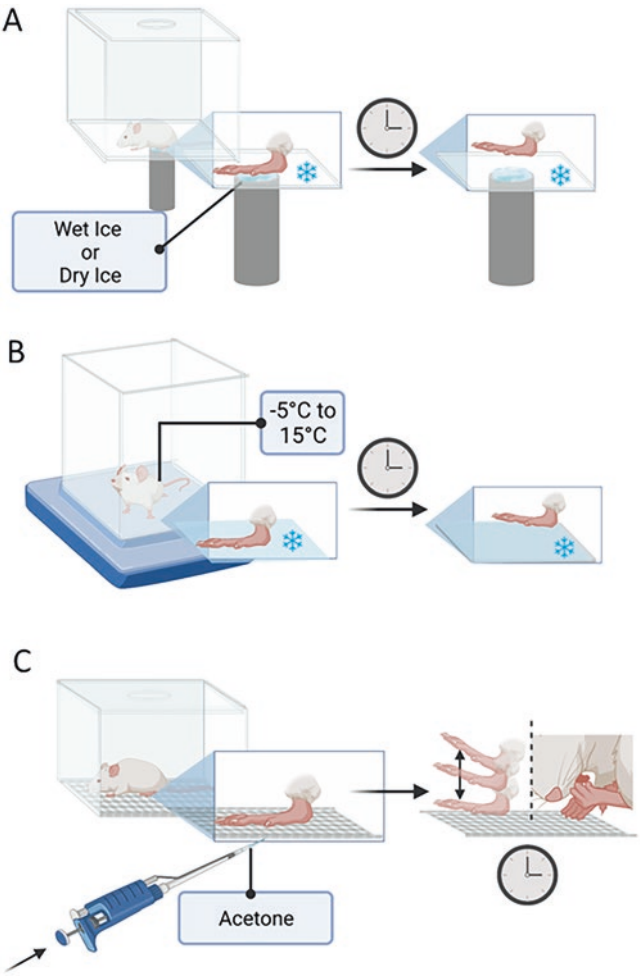

Fig. 3.4 Testing cold pain: (**a**) cold plantar assay; (**b**) cold plate test; and (**c**) acetone test

3.4.1 Cold Plate Test

The cold plate test resembles the hot plate test, but the plate used is set between −5 and 15 °C. Several protocols exist, each measuring something slightly different. Latency to nocifensive behavior can be recorded (Fig. 3.4a). The flinch rate over a fixed period can be measured so long as the plate temperature is not noxious. Finally, a progressively cooling plate can be used to determine the temperature threshold at which the animal responds nocifensively. Thus, the cold plate apparatus can be used to measure both cold allodynia and cold hyper/hypoalgesia. Because the animal is less frantic and thereby easier to observe than in the hot plate test, it is possible to report unilateral responses. Still, all variations of the cold plate test are highly subjective.

3.4.2 Cold Plantar Assay

A recently developed test for both cold hyper/hypoalgesia and cold allodynia, referred to as the cold plantar assay (Fig. 3.4b) or simply the cold test, makes use of ground up ice or dry ice (Brenner et al. 2012). Glass flooring interchangeable with the setup for the Hargreaves test is used—animals are situated and habituated similarly. A vessel such as a cut-off syringe is filled with ice and held against the glass under the animal's hindpaw; latency to response is recorded. If available to the experimenter, glass barriers of varying thicknesses can allow for a variety of stimuli, as thicker barriers allow less thermal conductivity. Glass can also be heated or cooled using coils. Just as the Hargreaves test has become the predominant test for heat pain, the cold plantar assay may soon be the gold standard for cold pain assessment.

3.4.3 Acetone Test

The acetone test, developed in 1994, uses a phenomenon called evaporative cooling to test cold allodynia (Carlton et al. 1994; Yoon et al. 1994) (Fig. 3.4c). Acetone rapidly absorbs heat from warm tissues and then evaporates, causing paw temperature to transiently drop by approximately 10–12 °C (Colburn et al. 2007). Experimenters place animals on a mesh rack and habituate them as per the Von Frey test. Acetone is then applied to the hindpaw, commonly with a pipette (care must be taken to avoid direct contact or misapplication to another region). Direct dabbing with a cloth or a very focused spray are alternative methods. Volumes used vary; many groups use 20–30 μL while others have used a milliliter. Naïve animals respond very briefly if at all; animals experiencing cold allodynia may display a few to a dozen seconds of additional nocifensive behavior. Data are often reported in

seconds of nocifensive behavior but can also be condensed into a “cold score” from 0 to 3 based on response severity (Colburn et al. 2007). Warm water can be used as a negative control. While the acetone test is a measure of cold allodynia, ethyl chloride causes a much more substantial decrease in paw temperature and, when substituted, can be considered a measure of cold hyperalgesia (Leith et al. 2010).

3.5 Assessment of Spontaneous or Ongoing Pain

It is increasingly recognized that relying on evoked pain measured by withdrawal reflexes has limitations (Mogil 2009; Rosenberg et al. 2013). Non-reflexive ongoing pain is an important feature of chronic pain (Cobos et al. 2012; Huang et al. 2013; King et al. 2009; Langford et al. 2010) and has been argued to be a more clinically relevant measurement for many chronic pain conditions experienced by patients. For these reasons, the measurement of so-called spontaneous pain is at minimum a very useful addition to most animal studies. The primary difficulties with its measurement are time requirements and ambiguity. Visible evidence of spontaneous pain arises randomly, necessitating long periods of observation. Video recordings are typically made and graded by a blinded technician. Scoring is tedious, and there is a risk of substantial inter- and intrarater variability. Machine learning methods promise a rapid and rigorous solution to these problems but are not yet widely available.

3.5.1 Conditioned Place Preference (CPP)

Conditioned place preference (CPP) is a classical paradigm commonly used to study addiction. Animals are subjected to a multi-day habituation period in a biased compartment paradigm (two chambers, one dark and one bright) (Fig. 3.5a). After habituation, video is recorded and reviewed to confirm that animals spent roughly equivalent time in the white- vs. the black-walled chamber, indicating no pre-existing preference. On the conditioning day, animals receive a vehicle control paired with a randomly chosen chamber in the morning, and then the appropriate drug (e.g., clonidine, a non-addictive analgesic) treatment paired with the other chamber 4 h later. Chamber pairings will be counterbalanced. On the test day, 20 h following the afternoon pairing, animals will be placed in the CPP box with access to both chambers. Animal behaviors are video-recorded for 15 min and analyzed, often by ANY-maze software (Stoelting Co.), for chamber preference.

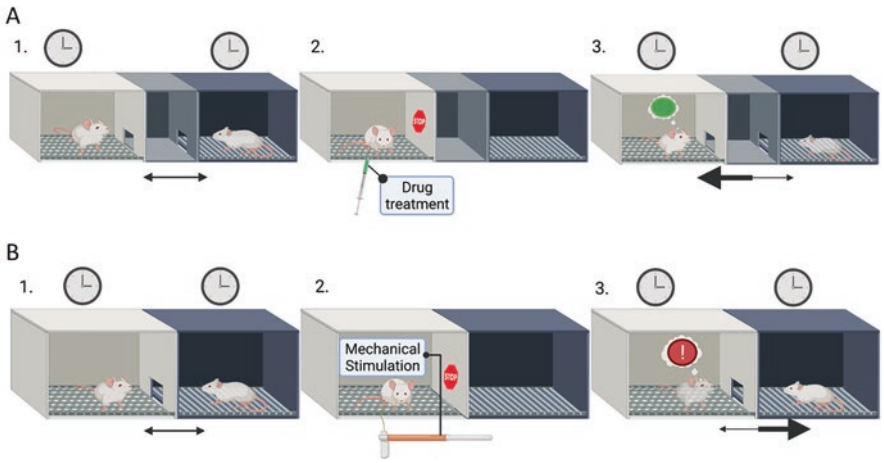

Fig. 3.5 Spontaneous pain assessment by conditioned place preference (CPP, **a**) and conditioned place avoidance (CPA, **b**)

3.5.2 Conditioned Place Aversion (CPA)

Like CPP, the CPA paradigm uses a biased compartment paradigm, trains the animal to associate a specific event with a chamber, then measures the amount of time spent in the chamber after association. In the pain field, a negative valence provided via a painful stimulus (e.g., Von Frey hair, acetone) is typically assessed using CPA (Fig. 3.5b). For example, a mouse can be sequestered in a certain chamber (after habituation and baseline preference testing) and then be repeatedly stimulated with a light Von Frey filament (i.e., 0.07 g) for 10 min. After this, animals experiencing mechanical hypersensitivity will avoid the box they were sequestered in significantly more than naïve animals. Results are presented in terms of an aversion score, calculated as the net difference in time (seconds) spent in the stimulation-associated compartment during pre-testing vs. post-testing (Cheng et al. 2017).

3.5.3 Facial Expression (Grimace Scales)

First reported in 2010, the mouse grimace scale is a simple and validated method of determining ongoing pain in rodents while minimizing experimenter contact (Langford et al. 2010). The mouse should be recorded before and after pain establishment with its face clearly visible. Frames from these videos are grabbed and graded by a trained experimenter based on the trimerized severity (not present = 0, moderate = 1, severe = 2) of five distinct features: orbital tightening, nose bulge, cheek bulge, ear position, and whisker change. The sum score can be used as a proxy for the animal's pain experience. Following the development of the mouse

grimace scale, a rat grimace scale was produced by the same group (Sotocina et al. 2011), accompanied by software allowing semi-automation. Considerations for the rat grimace scale are almost identical, but only four of the five expressions are included (nose bulge and cheek flattening are combined into a single factor). Both scales provide an accessible, although imperfect, window into the animal's lived experience independent of outside stimuli. The investigators who created both scales predicted that their methods would not be well equipped to measure pain caused by acute stimuli or long-term pain occurring in paroxysmal bouts—notably, this second category includes most neuropathic pain models. They are, however, very well suited to models of inflammatory pain or repeated application of noxious stimuli.

3.5.4 Vocalization

Mice emit audible distress calls when in pain (Williams et al. 2008). To record and study such vocalizations, a microphone is placed above the behavior testing apparatus. A webcam with audio can also enable correlation of vocalization timing with evoked pain behavior (e.g., Von Frey filament or acetone application). The recorded audio is then analyzed to obtain quantified data, such as the number of pips or duration of the distress calls, which can be used to compare between groups (Rodriquez et al. 2017). A distress call likely indicates the presence of pain, but mice will not always vocalize in response to even the most painful acute stimuli.

3.5.5 Voluntary Exercise

Another recent non-reflexive pain assay, reported in 2012, is the voluntary exercise assay (Cobos et al. 2012). Mice are habituated to an individual habitat fitted with a self-propelled running wheel before model establishment. After a pain or sham intervention, testing is performed over a 1-h time course. A computer records the time spent exercising using the wheel; mice experiencing prohibitive pain choose to exercise less. This model has proven to be a sensitive test for pain experience or related comorbidity in models of inflammatory pain. Anti-inflammatory analgesic treatment can reverse the pain-induced exercise deficit even when tests of mechanical pain remain below baseline.

3.5.6 Testing Pain Comorbidities

Pain reaches beyond physical discomfort; chronic pain is correlated with increased incidence of mood disorders such as anxiety and depression (Dersh et al. 2002; Fishbain et al. 1997; McWilliams et al. 2003), as well as cognitive impairment (Moriarty et al. 2011). These conditions greatly impact patients and are appropriately receiving more attention from the field. In animal studies, relief from these symptoms can be both supplemental evidence that an intervention is efficacious toward treating pain and meaningful endpoints in their own right.

3.6 Animal Models of Inflammatory Pain

Inflammatory pain is defined as pain caused by immune responses to tissue injury or infection (Woolf 2010). Importantly, these injuries typically involve peripheral tissues and do not affect the nervous system directly. The most common inflammatory pain models are established by injecting an immunogenic or noxious substance into a rodent's hindpaw (Fig. 3.6a). Although other peripheral targets are also used, the hindpaw has been favored because it allows for unilateral testing at a distinct, accessible site. Following injection, the affected tissue can be expected to swell in size and have a slightly higher temperature (Ren and Dubner 1999).

3.6.1 Formalin Model

Pioneered in 1977 (Dubuisson and Dennis 1977), the formalin model is a classic inflammatory pain model still widely used today. It is established by injecting 1–5% formalin (dilute formaldehyde) into the rodent's hindpaw. The resulting pain occurs in a biphasic manner over the course of the next hour (Hunskaar and Hole 1987). The first, beginning shortly after injection and lasting 5–10 min, is a direct result of first-order sensory neuron activation. A short period of reduced pain follows, typically lasting 5 min, before the longer second phase begins. The second phase lasts 20–50 min (reports vary) and is driven not only by peripheral activation but also by central sensitization caused by the inflammatory response. Some analgesics such as the mu opioid receptor agonist DAMGO (Dickenson and Sullivan 1987) and the local anesthetic lidocaine (Coderre et al. 1990) can prevent both phases of pain when administered prior to formalin injection. Many other analgesics, such as inhibitor of extracellular signal-regulated kinase (ERK), are effective against the second phase, which could be mediated by spinal cord mechanisms via central sensitization (Ji et al. 1999), but not against the first phase.

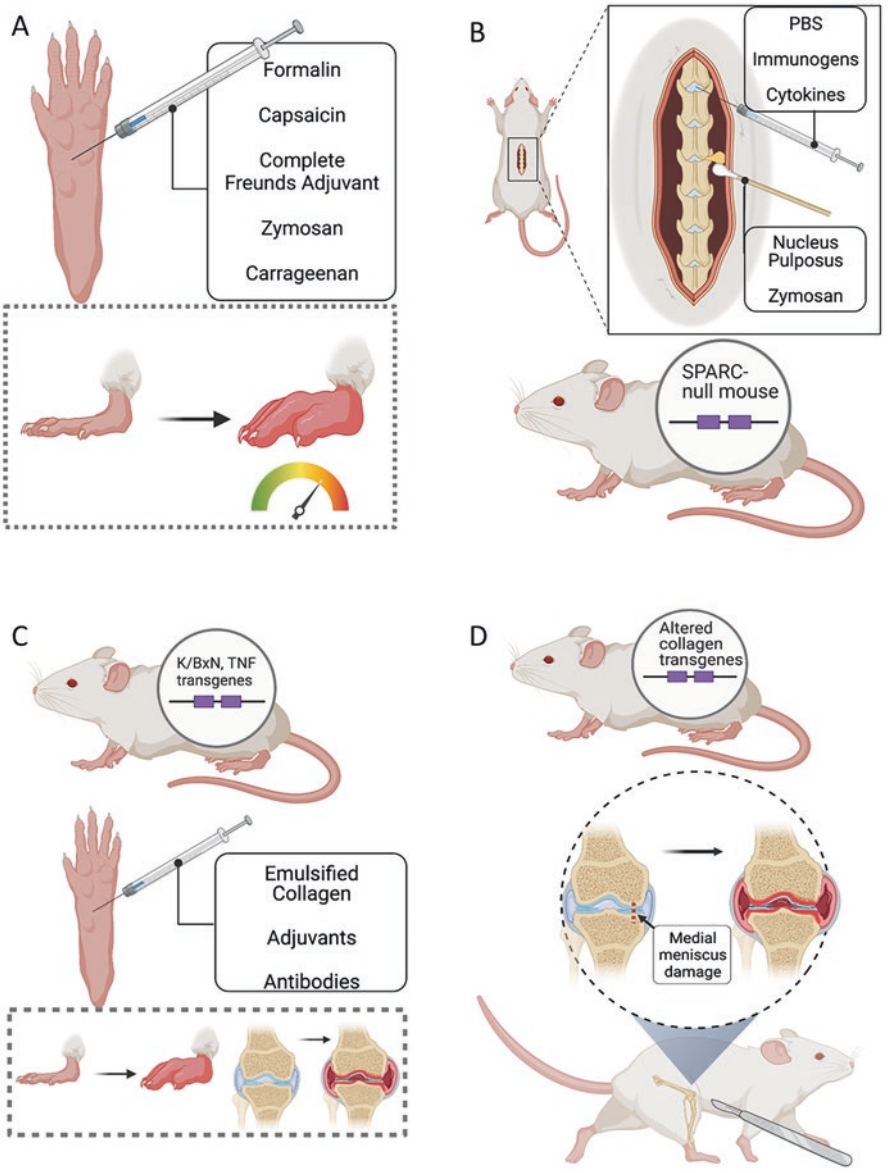

Fig. 3.6 Animal models of inflammatory pain: (**a**) acute and chronic inflammatory pain models; (**b**) inflammatory low back pain models; (**c**) rheumatoid arthritis (RA) pain models; and (**d**) osteoarthritis (OA) pain models

3.6.2 Capsaicin Model

Capsaicin has been used to study pain as far back as the 1970s (Szolcsanyi 1977). Interestingly, it was humans who were first treated with the compound in an experimental setting, typically via cream or intradermal injection (Carpenter and Lynn 1981; Simone et al. 1987). To date capsaicin remains one of the only models that can ethically be performed on human volunteers because pain rapidly and completely resolves. It took until 1996 for this model to be characterized in rodents (Gilchrist et al. 1996). Intraplantar capsaicin produces spontaneous pain over a short time course (~5 min) in a manner very similar to the first phase of formalin-induced pain, but no second phase occurs. While spontaneous pain dissipates rapidly, thermal hypersensitivity is present following low or moderate doses and lasts on the order of 10–60 min depending on dose (Gilchrist et al. 1996), and mechanical hypersensitivity has been reported to linger for several hours (Ren and Dubner 1999). Both primary mechanical hyperalgesia (within the injection site) and secondary mechanical hyperalgesia (distal to the injection site) were reported in the capsaicin model, but the secondary mechanical hyperalgesia is mediated by central sensitization and can be blocked by intrathecal injection of an ERK inhibitor (Kawasaki et al. 2004).

3.6.3 Complete Freund's Adjuvant (CFA) Model

Complete Freund's adjuvant (CFA) is inactivated bacteria, most commonly *Mycobacterium tuberculosis*, in a solution of mineral oil. It was first utilized in pain research in 1988 (Stein et al. 1988). Administration provokes an immune response, triggering lasting and painful inflammation. As with the formalin model, mechanical and thermal pain both manifest. In contrast, these symptoms last for between 1 and 3 weeks, with the most severe pain occurring days to weeks after induction. Pain remains localized to the inflamed paw, which becomes measurably enlarged, remaining inflamed even for a time after pain subsides. CFA-induced inflammation should be chosen over the formalin model by investigators seeking a medium- to long-term model, especially if they hope to compare resolution times between groups. A similar model, which combines CFA with immunogenic collagen, has been used as a model of rheumatoid arthritis (RA) (Brand et al. 2007) (for more on arthritis models, see below). In contrast to unilateral heat hyperalgesia, mechanical allodynia appears bilaterally in some CFA models (Gao et al. 2010).

3.6.4 Zymosan and Carrageenan Models

Zymosan is a glycan isolated from fungi, most typically yeast, which provokes a strong inflammatory response. Carrageenan is found in algae and is similarly immunogenic. Intraplantar administration of zymosan as a model of inflammatory pain was first reported in 1997 (Meller and Gebhart 1997); the carrageenan model was developed slightly earlier (Hylden et al. 1991). Both models cause pain on a timescale greater than that of formalin but less than that of CFA. Pain begins after 30 min (zymosan) or 60 min (carrageenan) and lingers for approximately 24 h (Ren and Dubner 1999). Hypersensitivity and hyperalgesia to mechanical and thermal stimuli are induced and reach their peak intensity approximately 4 h post injection. Spontaneous pain also occurs within the first 4 h. Unless the investigator is interested in mechanistic specifics, these models are effectively interchangeable.

3.6.5 Osteoarthritis (OA) Models

Small animal OA models remain important for mechanistic science and screening; findings are then validated in larger animals with more similar joints to humans such as dogs, sheep, goats, or horses (Kuyinu et al. 2016). Several strains of laboratory mice, including but not limited to STR/ort and C57BL/6, may spontaneously develop OA. Investigators need only screen their mice for symptoms at frequent intervals (McCoy 2015). Transgenic mice are also used in order to hasten and increase the penetrance of OA onset—mice with altered type II or IX collagen are common choices (Helminen et al. 1993; Hu et al. 2006), but other transgenic lines exist with varying effects on OA development (Cope et al. 2019). Murine OA can also be induced surgically (Fig. 3.6b). Although most surgical models are performed in larger mammals, rodents are commonly used for models involving the medial meniscus. Transection of structural collagen and/or cartilage leads to bone-on-bone contact, triggering synovitis and, later, hypersensitivity (Gowler et al. 2020). In mice, medial partial meniscectomy (Clements et al. 2003) leads to biphasic pain, first up to 3 weeks post operation and then recurring after ~9 weeks (Knights et al. 2012). The medial meniscal tear model involves transection of both the medial collateral ligament and the medial meniscus and rapidly leads to mechanical pain at the joint and in the affected limb (Bove et al. 2006).

3.6.6 Rheumatoid Arthritis (RA) Models

Like OA models, rodent models of RA can be separated into spontaneous and induced. Spontaneous RA does not occur commonly in wild type mice, but several transgenic strains exist, which develop RA with notable consistency: two common lines are K/BxN and tumor necrosis factor (TNF)-transgenic mice (Fischer et al.

2017). The former model causes expression of a transgenic T-cell receptor that reacts with a very widely expressed enzyme, triggering autoimmunity as early as 3–4 weeks and resulting in notable RA symptomology 4–8 weeks later (Kouskoff et al. 1996; Monach et al. 2007). TNF-transgenic mouse strains simply overexpress a human TNF transgene that has been altered to lack certain regulatory binding domains (Keffer et al. 1991; Li and Schwarz 2003). Inducible models of RA onset more rapidly than spontaneous models (Fischer et al. 2017). Intradermal injection of emulsified collagen, other adjuvants such as Freund's adjuvant, antibodies, or other immunizing compounds can all lead to immune-driven RA symptomology (Bolon et al. 2010; Brand et al. 2007; Fischer et al. 2017) (Fig. 3.6c). In most induced models, early symptoms appear within days and peak after weeks, although the severity varies between models and between animals (Fischer et al. 2017).

3.6.7 Inflammatory Models of Low Back Pain (LBP)

Discogenic pain is back pain caused by the disruption of at least one intervertebral disk (IVD). Experimental IVD disruption is still being studied in murine models (Fig. 3.6d), but there is little consensus regarding which are most useful (Lyu et al. 2021; Shi et al. 2018). The secreted protein acid rich in cysteine (SPARC)-null mouse is a transgenic mouse that spontaneously develops discogenic LBP with age (Millecamps et al. 2011, 2012). In most successfully established discogenic models, bilateral mechanical and thermal pain is measurable. Markers of disk damage, cytokine levels, and changes in locomotion are also valuable assessments for the severity of disk damage (Shi et al. 2018).

Radicular pain is a form of back pain often described as radiating down the spine, low back, hips, and/or upper legs. It can but does not always occur alongside discogenic back pain; other causes include stenosis or cancer metastasis (Knezevic et al. 2021). Some models use an inflammation-driven approach to model radicular pain. The IVD is filled with nucleus pulposus (NP), a shock-absorbing jelly made of water and collagen. NP elicits a strong immune response if it escapes the disk. NP application to the epidural space of rats can produce inflammation-driven mechanical hypersensitivity after 1–2 weeks (Kawakami et al. 1996). Along similar lines, depositing zymosan (in incomplete Freund's Adjuvant) in the epidural space surrounding the L5 DRG of rats has been shown to cause bilateral mechanical hyperalgesia and allodynia (Xie et al. 2006).

3.6.8 Inflammatory Pain Models: Responses to Anti-inflammatory Treatments

Intuitively, anti-inflammatory treatments typically perform well against inflammatory pain models (Table 3.1). The biphasic formalin model provides an interesting test case. The first phase is not affected by non-steroidal anti-inflammatory drugs

Table 3.1 Efficacy of important treatment types in the classical pain models

Pain model	NSAIDs	Minocycline	Steroids	SPMs	p38 inhibitor	References
Acute inflammatory pain						
Formalin (phase 1)	–	–	–	++	–	Cho et al. (2006), Fan et al. (2018), and Xu et al. (2010)
Formalin (phase 2)	++	++	+++	+++	++	Cho et al. (2006), Fan et al. (2018), and Xu et al. (2010)
Capsaicin	++	+/–	??	??	??	Joshi et al. (2006)
Carrageenan	++	+	++	+++	++	Bang et al. (2018) and Bastos et al. (2007)
Zymosan	++	+	++	+++	++	Bang et al. (2018) and Bastos et al. (2007)
Chronic inflammatory pain						
CFA	++	++	++	+++	+++	Ji et al. (2002) and Serhan and Levy (2018)
OA	++	??	++	+++	++	Brown et al. (2008) and Zaninelli et al. (2021)
RA	+/–	??	??	+++	??	Serhan and Levy (2018)
LBP (inflammatory)	+	??	++	??	??	Cornefjord et al. (2002) and Ibrahim et al. (2018)
Neuropathic pain						
CCI—Early phase	++	+++	++	+++	+++	Clatworthy et al. (1995), Parisien et al. (2022), and Xu et al. (2013b)
CCI—Late phase	+/–	+	+/–	+	+/–	Medeiros et al. (2020), Parisien et al. (2022), and Xu et al. (2013a)
PSNL—Early phase	++	+++	++	+++	+++	Ma et al. (2002)
PSNL—Late phase	+/–	+	+/–	+++	+/–	Ma et al. (2002)
SNL—Early phase	++	+++	++	+++	+++	Li et al. (2007)
SNL—Late phase	–	+	+/–	+++	+/–	Xu et al. (2013b)
SNI—Early phase	++	+++	++	++	++	Lee et al. (2010) and Sorge et al. (2015)
SNI—Late phase	–	+	–	??	–	Sorge et al. (2015)
Neuropathic radiculopathy	+	??	++	??	??	Gu et al. (2007)
SCI (generalized)	+	++	??	?/+	??	Schmidt et al. (2021)

(continued)

Table 3.1 (continued)

Pain model	NSAIDs	Minocycline	Steroids	SPMs	p38 inhibitor	References
TBI (generalized)	+/?	+/?	??	++/?	+/?	Lew et al. (2006)
CIPN	+	+	??	++	+	Starobova et al. (2019)
DPN	+	++	??	++	++	Zychowska et al. (2013)
Postoperative pain						
Paw-incision	+	+/−	??	+++	??	Huang et al. (2013), Ito et al. (2009), and Whiteside et al. (2004)
Tibial fracture	+	++	??	++	+	Cottrell et al. (2009)
Thoracotomy	??	??	??	++	??	Wang and Strichartz (2017)
Visceral pain						
Acute colonic	+	??	??	??	??	Baskın et al. (2016)
Chronic colonic	−	++	??	???	??	Kannampalli et al. (2014)
Bone cancer pain						
Early phase (generalized)	+	++	??	??	++/?	Song et al. (2016)
Late phase (generalized)	−	+	??	??	??	Pacharinsak and Beitz (2008)

+++, very highly effective; ++, highly effective; +, effective; +/−, mixed results; −, ineffective; ??, unknown; +/?, extrapolation based on studies that reported non-pain endpoints

(NSAIDs) or steroids, but both treatments can prevent second-phase pain (Hunskaar and Hole 1987). NSAIDs often drop in efficacy, however, against chronic inflammatory conditions such as low back pain. Steroids such as dexamethasone have also proven effective in these models. Strikingly, however, recent data suggest that despite inhibiting the inflammatory response by way of NSAIDs or dexamethasone improving short-term pain outcomes, these treatments lead to remission of symptomology and worsened long-term outcomes in mice by blocking neutrophil invasion (Parisien et al. 2022). Further investigation into this phenomenon is of critical importance as NSAIDs remain in high use.

Immune modulators are quite efficacious against inflammatory pain, but with some caveats. Inhibition of spinal p38 mitogen-activated protein (MAP) kinase prevents the second phase of formalin-induced pain just as an NSAID might—but only in male mice (Taves et al. 2016). Minocycline, an inhibitor of microgliosis, also has a sex-dependent effect on inflammatory pain; male CFA mice treated with minocycline saw almost triple the reduction in nocifensive behavior that females did (Sorge et al. 2015).

Special pro-resolution mediators (SPMs) have shown significant effects against inflammatory pain. Resolvins such as RvD1 and RvE1 outperform morphine in the

formalin test at 1/100th the dose; these effects have been repeated in other models (Serhan and Levy 2018; Xu et al. 2010). The other two families of SPMs, protectins and maresins, have also become attractive targets for treating acute inflammation (Bang et al. 2018; Serhan et al. 2015).

3.7 Animal Models of Neuropathic Pain

Neuropathic pain is caused by a disease or lesion to the somatosensory system (Jensen et al. 2011; Woolf and Mannion 1999), the sources of which can be quite diverse. Symptoms of neuropathic pain are both positive and negative; the gain of pain signaling is often accompanied by a loss of function or other sensations in the affected tissues (Colloca et al. 2017). Neuropathic pain models cause inflammation, but, unlike the inflammatory models discussed above, this inflammation is secondary to direct nerve damage (Fig. 3.6). Nerve injury causes a loss of signaling from the damaged primary sensory neurons coupled with hypersensitivity of the remaining first-order neurons. Typically, second-order spinal neurons become hyperactive soon after, a phenomenon termed "central sensitization" (Ji et al. 2003; Woolf 1983).

The nerves innervating the hindlimb are often chosen for nerve injury models due to the convenience of unilateral paw testing for pain. This is not without cost; the field's intense focus on this region does not reflect the distribution of nerve injuries in patients. Neuropathies, spinal cord injuries, and traumatic brain injuries (TBIs) can also cause neuropathic pain (Fig. 3.7a).

3.7.1 Chronic Constriction Injury (CCI) Model (Aka the Bennett Model)

The chronic constriction injury (CCI) model of neuropathic pain was first established in rats (Bennett and Xie 1988) (Fig. 3.7a). A small region of the sciatic nerve proximal to its trifurcation is tied loosely, or "constricted," by three or four ligatures with about 1 mm of spacing in between. The CCI model induces thermal and mechanical hyperalgesia as well as cold and mechanical allodynia. These changes in sensitivity to stimuli start approximately 3–7 days post operation and typically last a minimum of 7–10 weeks (Bennett and Xie 1988). A noticeable limp, ventroflexed toes, and overgrown claws on the injured side are also observed. In addition, CCI may cause weight loss, muscle atrophy, hindpaw guarding, and autotomy. Recently, the CCI model has gained in popularity (Sadler et al. 2021). The incomplete loss of innervation to the hindpaw makes this model particularly useful because some degree of sensation is preserved in all plantar regions. The pain induced in this model may not be as severe as in models that completely damage at least one nerve.

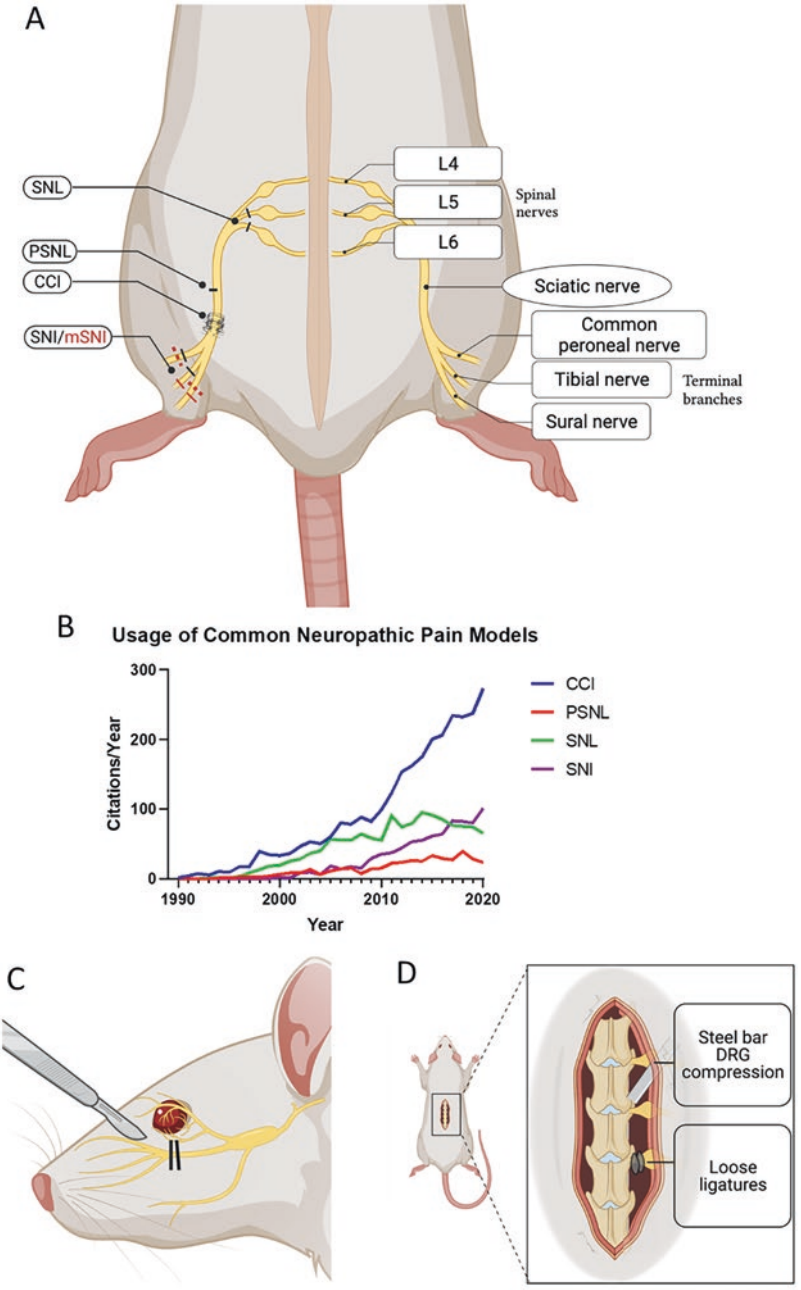

Fig. 3.7 Animal models of neuropathic pain: (**a**) the anatomy of common nerve injury models—nerve injury models with chronic constriction injury (CCI), partial sciatic nerve ligation (PSNL), spinal nerve ligation (SNL), spared nerve injury (SNI), and modified SNI (mSNI); (**b**) frequency of common neuropathic pain models with nerve injury each year by PubMed search; (**c**) infraorbital nerve CCI (IoN-CCI); and (**d**) two methods of inducing radicular neuropathic back pain

3.7.2 Partial Sciatic Nerve Ligation Model (or Seltzer Model)

The partial sciatic nerve ligation (PSNL) model was developed in 1990 (Seltzer et al. 1990) and first established in mice later that decade (Malmberg and Basbaum 1998). Like the CCI model, the sciatic nerve is exposed and damaged without complete transection (Fig. 3.7a). Instead of tightly constricting a region, however, PSNL is established at a single point by ligating the nerve to 1/3 to 1/2 of its original diameter. PSNL causes marked mechanical allodynia and hyperalgesia as well as thermal hyperalgesia and cold sensitivity (Seltzer et al. 1990). Symptoms onset as soon as an hour post operation and can last for months (Seltzer et al. 1990); mechanical pain reportedly outlasts thermal pain (Malmberg and Basbaum 1998). A shifting of weight onto the uninjured side and spontaneous licking of the injured paw and limb is noticeable as well. Unlike some other models, contralateral pain may occur, although it is not always reported to. The PSNL model has been a mainstay of neuropathic pain research since its establishment. The damage caused by the ligature does not affect any fiber type preferentially, perhaps more consistent with nerve injury caused by blunt trauma. The pain resulting from PSNL is typically more intense and more lasting than CCI.

3.7.3 Spinal Nerve Ligation (SNL) Model (or Chung Model)

The spinal nerve ligation (SNL) model, first developed by Kim and Chung in 1992, involves ligation of the L4 and L5 spinal nerves that connect the sciatic nerve to individual DRG (Kim and Chung 1992) (Fig. 3.7a). The sciatic nerve branches into the L4, L5, and L6 spinal nerves as it approaches the spinal cord, but damage to the L4 spinal nerve has been found to cause motor deficits and reduced mechanical sensitivity, rendering the model unusable (Kim and Chung 1992). The initial rat model ligated the L5 and L6 nerves; a modified SNL model was developed later in which only the L5 nerve is ligated with similar results (Kawasaki et al. 2008; Tanga et al. 2005). Mechanical allodynia begins 12–20 h postoperatively and persists for months without improvement (Kim and Chung 1992). Thermal hyperalgesia begins roughly 3 days postoperatively and lasts for over 5 weeks. Animals avoid placing weight on the injured side and display hindpaw guarding; there is no obvious autotomy, a self-mutilation behavior typically seen after complete transection of the sciatic nerve (Ji et al. 1994). Using the L5-SNL model, a sequential activation of ERK signaling pathway in neurons (within minutes to hours), microglia (within days), and astrocytes (within weeks) of the spinal cord was shown to regulate neuropathic pain in the early and late phases (Zhuang et al. 2005). A variation has been established in which L5 is partially ligated ala PSNL, resulting in analogous symptoms but spontaneous, slow recovery over weeks 4–8 (Guan et al. 2010). While SNL is more standardized than PSNL or CCI, a total ligation of a single spinal nerve is exceedingly unlikely in real-world situations.

3.7.4 Spared Nerve Injury Model

The spared nerve injury (SNI) model, first developed in 2000 (Decosterd and Woolf 2000), is similar in approach to SNL but targets the distal branches of the sciatic nerve. Classically, the common peroneal and tibial nerves are transected, leaving the sural nerve intact (Fig. 3.7a). Variations have since been established in which other combinations of one or two of the sciatic nerves' branches are transected (Bourquin et al. 2006; Shields et al. 2003). Taken together, these models have gained significant popularity in the pain field and may soon be the most common nerve injury paradigm. SNI causes mechanical allodynia and hyperalgesia developing within hours to a few days and peaking approximately 2 weeks after operation. This model is long lasting; rats can experience pain for over a year and may never recover (Decosterd and Woolf 2000). Altered gait, spontaneous paw withdrawal, and cold allodynia are observed. Heat allodynia was not found in the initial model (Decosterd and Woolf 2000); reports differ depending on which nerve branch is spared (Bourquin et al. 2006; Pertin et al. 2012; Shields et al. 2003). No variation produces autotomy or a mirror image effect (Bourquin et al. 2006; Decosterd and Woolf 2000). Care must be taken to avoid contact with the nerve branch intended to be spared; if all three branches are injured, leg paralysis can occur, rendering the model useless. Animals also lose much of their receptive field; experience and care is necessary to assess only the area retaining sensation during behavioral testing.

A PubMed search (1990–2020) for the CCI, PSNL, SNL, and SNI nerve injury models reveals significant increases in the applications of these rodent models of neuropathic pain in the last three decades. The past 10 years especially have seen remarkable increase in the citations of the CCI and SNI models (Fig. 3.7b).

3.7.5 Infraorbital Nerve Branch Chronic Constriction (IoN-CCI) Model

This orofacial neuropathic pain model is similar to CCI of the sciatic nerve but targets a branch of the trigeminal facial nerve (Vos et al. 1994) (Fig. 3.7c). It is typically performed in rats, but recently a mouse model has been pioneered with good success despite the smaller scale (Castro et al. 2017). A Harvard group has reported a more convenient method that instead ligates the distal IoN ("dIoN-CCI") through a facial incision near the cheek pad (Ding et al. 2017). IoN-CCI causes mechanical allodynia and heat hyperalgesia in the region innervated by the injured IoN (Vos et al. 1994). The onset of mechanical hypersensitivity begins after a 6–12-day period of hyperresponsivity post operation (Deseure and Hans 2015; Vos et al. 1994). This latent period contrasts with many classical rat nerve injury models (Bennett and Xie 1988; Decosterd and Woolf 2000; Kim and Chung 1992; Seltzer et al. 1990). Heat hyperalgesia appears to onset sooner, at around 4 days postoperatively (Imamura et al. 1997). In rats, symptoms can last as long as 120 days (Deseure

and Hans 2015). While ipsilateral responses are more frequent, contralateral hypersensitivity to both heat and mechanical stimuli has also been observed. Spontaneous pain behavior occurs and can be quantified in the form of increased grooming of the referred facial region, beginning as soon as recovery from anesthesia. Significant weight loss is observed, perhaps mainly due to pain when eating (Imamura et al. 1997; Vos et al. 1994). Time spent feeding has itself been reported and correlated to treatment response. The mouse grimace scale has been validated in both mouse and rat IoN-CCI models (Akintola et al. 2017).

3.7.6 Models of Neuropathic/Radicular Low Back Pain

Not all models of low back pain are driven by inflammation; others approximate stenosis and other compression-related radiculopathies by surgically damaging the DRG and/or nerve root. In one method, termed chronic compression of the DRG (CCD), a stainless steel rod is inserted such that it continually presses against the L5 DRG (Fig. 3.7d). This leads to lasting unilateral mechanical and thermal hypersensitivity (Hu and Xing 1998). Application of loose ligatures to the DRG nerve root causes shorter-term radiculopathy characterized by initial motor dysfunction, mechanical hypoalgesia, and thermal hypersensitivity (Kawakami et al. 1994).

In addition to peripheral nerve injury, damage to the spinal cord and certain brain regions will also cause neuropathic pain, which can be termed “central neuropathic pain” (Fig. 3.8).

3.7.7 Spinal Cord Injury Models

The need for effective spinal cord injury (SCI) treatments expands beyond pain therapy. Still, pain occurs in roughly two-thirds of SCI patients and can be both severe and persistent (Siddall and Loeser 2001; Van Gorp et al. 2015). In the SCI models in which it has been characterized, pain typically develops caudally to the injured spinal cord segment (Hutchinson et al. 2004) (Fig. 3.8A). Alongside typical assays used to quantify neuropathic pain, SCI rodents can also be assessed for indicators of injury severity such as the blood-brain barrier (BBB) (Basso et al. 1995) and the ladder rung test (Metz and Whishaw 2009). Of note, nearly all pain research on SCI is performed in rats. The scale of the mouse spinal cord may not be prohibitive but is certainly inhibitive.

The first characterization of pain in an animal model of pain found that experimental ischemia of the spinal cord caused significant allodynia (Hao et al. 1991). This model is established by intravenously injecting rats with a fluorescein dye then immediately irradiating the exposed T10 vertebrae, destroying vascular innervation to the target region and, as a result, causing substantial but localized damage to the dorsal spinal cord. Contusion models apply a targeted blunt force to a region of the

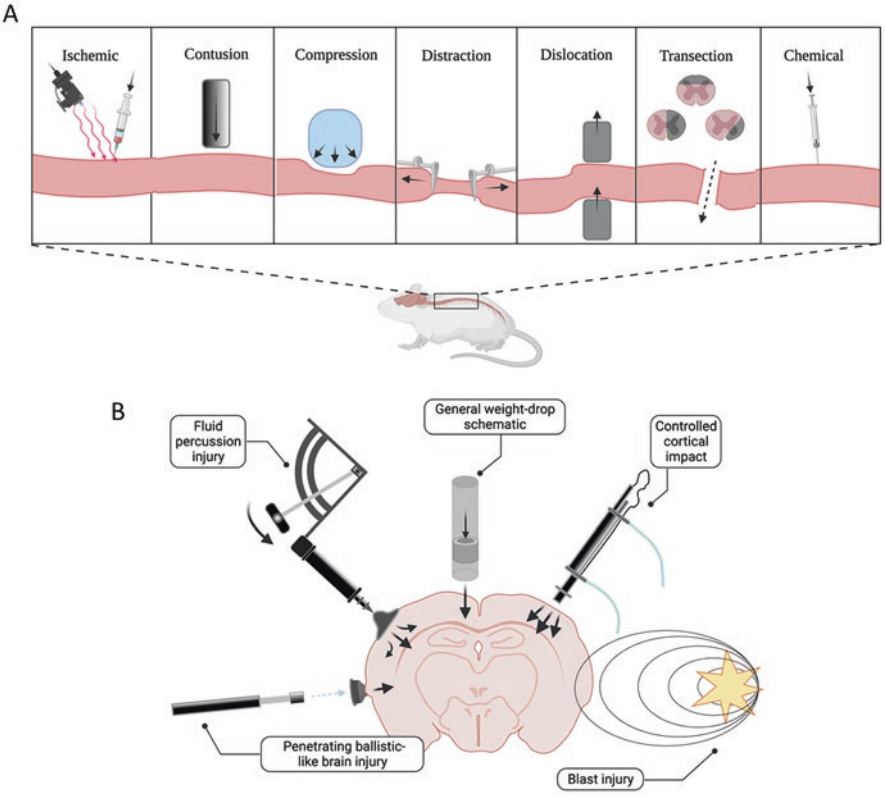

Fig. 3.8 Central neuropathic pain models: (**a**) spinal cord injury (SCI) models; and (**b**) traumatic brain injury (TBI) models

spinal cord, both rupturing blood vessels and directly damaging nervous tissues. The aptly named "weight-drop" model was developed by Allen in 1911; two modernized setups, the "New York University (NYU) impactor" (Gruner 1992) and the "Ohio State injury device" (Bresnahan et al. 1987), remain in use. Pain following contusion SCI was reported in 1995 when it was shown that rats with moderate SCIs caused by shorter weight-drop distance and characterized by a return of motor function after several weeks had more severe mechanical allodynia than rats receiving sham or severe SCI (Siddall et al. 1995). Compression SCIs are modeled by applying a fixed force clip to the exposed spine and causing mechanical allodynia lasting at least 4 weeks (Bruce et al. 2002). Some animals appeared to lose motor function below the site of injury; these animals did not have allodynia in the hindpaws but hypersensitivity was still measurable above the level of the compressed region. The static compression SCI model operates similarly; a 35 g weight placed onto the exposed dura of an anesthetized rat for 5 min causes prolonged mechanical hypersensitivity after a short period (1+ days) of hypoalgesia (Yu et al. 2013). Surgical hemisection (removal of a lateral half) of the spinal cord at the T13 level causes

bilateral pain and unilaterally impacted motor function (Christensen et al. 1996). Motor deficits partially recover over the first month; mechanical and thermal pain persist for at least 50 days. Hindpaw ipsilateral pain appears within days and may be milder than contralateral pain, which onsets almost immediately. Forepaws exhibit comparable symptoms but with a more gradual onset. A complete transection model at T13 (removal of the dorsal half) causes mechanical hypersensitivity in the hindpaws, but not the forepaws. This is in contrast to the multi-level symptoms caused by hemisection (Densmore et al. 2010; Scheifer et al. 2002). SCI can also be induced chemically. Intraspinal injection of the potent α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) agonist quisqualic acid into the dorsal horn causes SCI via excitotoxicity marked by ongoing pain, mechanical allodynia, and thermal hyperalgesia (Yezierski et al. 1998). This pain occurs bilaterally even after unilateral dorsal horn damage and endures without recovery for at least 5 weeks and very likely longer. A host of other models for SCI exist, which are not yet thoroughly characterized in the pain field (Cheriyan et al. 2014).

3.7.8 Traumatic Brain Injury (TBI) Models

Traumatic brain injury (TBI) is defined as brain damage caused by external mechanical forces (Xiong et al. 2013). Common symptoms of TBI include light sensitivity, blurred vision, cognitive deficits, and post-traumatic headache, the last of which often persists well over a year after the injury and can be debilitating (Dikmen et al. 2010). Unlike SCI, many TBI models have been established in mice (Fig. 3.8b). Non-pain assays of the severity of TBI and/or the efficacy of treatment include but are not limited to measures of cerebral edema, of tissue damage (Elliott et al. 2008), and the assessment of blood brain permeability with Evans Blue (Goldim et al. 2019).

In the controlled cortical impact model of TBI, an impactor strikes the exposed brain at a perpendicular angle (Dixon et al. 1991). TBI causes periorbital mechanical allodynia lasting up to 4 weeks and increased photosensitivity (Daiutolo et al. 2016; Elliott et al. 2012), a sign of headache pain (Recober et al. 2009). The fluid percussion injury (FPI) model is established via rapid injections of saline into the brain, substantially increasing intracranial pressure (McIntosh et al. 1987, 1989). Unilateral injections have been shown to induce contralateral mechanical allodynia. Hindpaw allodynia resolves in days to weeks, but periorbital Von Frey testing found mechanical allodynia lingering as late as 60 days after injury (da Silva Fiorin et al. 2018). Repetitive closed-skull TBI uses a stereotaxic impact device similar to the one used for FPI, but delivers blunt force (Shitaka et al. 2011). A direct blow is delivered to the head of the anesthetized animal; the same injury is repeated 24 h later. This model produces mechanical hypersensitivity, increased nocifensive behavior after capsaicin injection, and affects measures of learning and memory; these effects are compounded by peripheral trauma (Sahbaie et al. 2018). Many other TBI models exist, which are yet to be studied in a pain context.

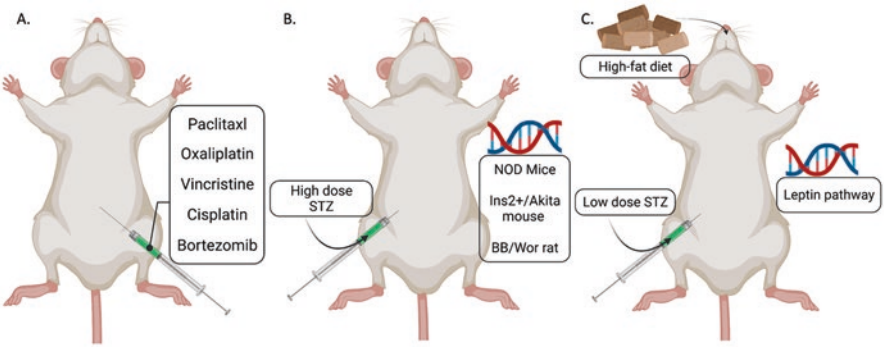

Fig. 3.9 Neuropathic pain models induced by drugs, toxins, and/or transgenic alteration: (**a**) chemotherapy-induced peripheral neuropathy (CIPN) models; (**b**) painful type I diabetic neuropathy models; and (**c**) painful type II diabetic neuropathy models

Apart from physical injuries to the peripheral and central nervous systems, neuropathic pain can also be induced by drugs, toxins, and a high-fat diet (Fig. 3.9).

3.7.9 Chemotherapy-Induced Peripheral Neuropathy (CIPN)

Chemotherapy-induced peripheral neuropathy (CIPN) is a serious side effect faced by 30–40% of patients receiving neurotoxic chemotherapies; for many of these patients, neuropathy persists after treatment cessation (Staff et al. 2017). Several kinds of neurotoxic chemotherapies have been used to model CIPN (Fig. 3.9a). Early studies of CIPN delivered paclitaxel (PTX) sub-epineurally to the sciatic nerve of rats (Röytta and Raine 1985). Intraperitoneal delivery was characterized later (Cavaletti et al. 1995) and remains commonly utilized today as it combines convenience and systemic treatment (Currie et al. 2019). PTX remains the most commonly reported agent, but the platinum-based compounds cisplatin and oxaliplatin, the vinca alkaloid vincristine, and the protease inhibitor bortezomib have also been thoroughly characterized as inducers of CIPN, and further varieties have been reported (Currie et al. 2019). Perhaps the most commonly utilized model is four 2 mg/kg doses of PTX delivered intraperitoneally every other day (Flatters and Bennett 2004). In rats, pain lingers for at least 3 months, with the severity of evoked pain response peaking approximately 3–4 weeks.

After the series of injections conclude, pain typically onsets rapidly. A typical CIPN model causes mechanical, heat, and, when assessed, cold hypersensitivity/hyperalgesia, all in a dose-dependent manner (Currie et al. 2019). Intravenous application better recapitulates chemotherapy treatment and may be underutilized by the field (Höke and Ray 2014).

3.7.10 Type I (T1) Diabetic Peripheral Neuropathy (DPN) Models

In short, type I diabetes mellitus (T1DM) is caused by the loss of insulin-secreting β-cells that reside in the islet of the pancreas. Streptozotocin (STZ), a toxin with homology to glucose, is preferentially taken up by and ablates this population (Furman 2015) (Fig. 3.9b). A single high dose of STZ delivered intraperitoneally recapitulates severe T1DM, including painful neuropathy (Courteix et al. 1993). Mice develop mechanical hypersensitivity and thermal hyperalgesia within 4 weeks (Johnson et al. 2008). A multiple low-dose treatment is less toxic and marked by a more reminiscent T1DM phenotype (Kolb 1987), but many of these rodents never develop DPN (O'Brien et al. 2014). As a result, pain scientists continue to use the single high dose model.

Several genetically altered strains of rodent develop spontaneous T1DM along with varying degrees of DPN. In non-obese diabetes (NOD) aged 4–8 weeks, T- and B-cells attack pancreatic islet cells and diabetes onsets most typically around 3–4 months; 80% of females and 20% of males go on to develop diabetes (Delovitch and Singh 1997). Thermal hyperalgesia is present in these diabetic animals as early as 8 weeks (Gabra and Sirois 2005), but there is little other evidence pointing to a DPN phenotype. Contrastingly, the Ins2+/Akita mouse strain rapidly develops severe neuropathy resulting in mechanical and thermal hypoalgesia (Vastani et al. 2018). BB/Wor rats develop diabetes at ~10 weeks of age, resulting in a neuropathy characterized by slowed nerve conduction velocities (Sima et al. 2001); pain phenotypes in this model are not well characterized.

3.7.11 Type II (T2) Diabetes Diabetic Peripheral Neuropathy (DPN) Models

T2DM develops gradually as a result of declining insulin sensitivity. Ten years after diagnosis with T2DM, 42% of patients in a Dutch cohort developed neuropathy (Partanen et al. 1995). Overfeeding rodents with a high-fat diet leads to T2DM, obesity, and painful neuropathy (O'Brien et al. 2014; Obrosova et al. 2007). Interestingly, a diet rich specifically in omega-6 poly-unsaturated fatty acids caused marked painful neuropathy without causing obesity or the metabolic signatures of T2DM (Boyd et al. 2021). Other models combine a high-fat diet with a low-dose STZ (Fig. 3.9c). Two weekly doses of 30 mg/kg STZ produced painful neuropathy in 85% of rats tested (Zhang et al. 2008); another protocol uses 3 weeks of high-fat diet followed by a single injection (Furman 2021). Transgenic approaches to T2DM center around two mouse strains with mutations in the leptin pathway. Ob/ob mice have inactive leptin while db/db mice have a mutation in the leptin receptor. These mice readily develop obesity, diabetes, and neuropathy (O'Brien et al. 2015; Sullivan et al. 2007).

3.7.12 Responses to Anti-inflammatory Treatments in Neuropathic Models

Unlike inflammatory pain, NSAIDs are understood to be mostly or entirely ineffective against chronic neuropathic pain (Moore et al. 2015) (Table 3.1). In animal models, some benefit can be found early in pain development, but efficacy quickly diminishes. Further, the usage of NSAIDs in centralized pain disorders, especially TBI, must be considered carefully. Some studies have suggested that chronic NSAID treatment can worsen cognition in the TBI setting (Browne et al. 2006). Steroid treatments have been recognized as effective against early-stage neuropathic pain (Scholz et al. 2008). Just as with inflammatory pain, however, new data are calling into question the long-term benefit of this short-term anti-inflammatory-mediated hypoalgesia (Parisien et al. 2022).

Separately, immunomodulatory treatments have been shown to maintain greater efficacy in these models in early phases. Inhibition of spinal p38 MAP kinase is effective against nerve-injury-induced mechanical allodynia in male rodents (Ji and Suter 2007; Taves et al. 2016). Minocycline pretreatment has been shown to greatly reduce mechanical sensitivity after the sciatic nerve was directly treated with zymosan (Ledeboer et al. 2005)—but this was only performed in male rats. The important discovery would later be made that minocycline treatment is only effective against SNI-induced pain in males (Sorge et al. 2015). The extent and origin of sex differences to immunomodulatory treatments is still a matter of scientific discourse.

SPMs have been emerging as promising candidates for the treatment of neuropathic pain. For example, resolvin E1 is able to transiently reverse hypersensitivity-established neuropathic pain models (Xu et al. 2013a), while neuroprotectin D1 potently protects against CCI-induced neuropathic pain and causes transient reversal when given intrathecally after establishment (Xu et al. 2013b).

3.8 Other Animal Models of Pain

3.8.1 Animal Models of Visceral Pain

Visceral pain arises from internal organs innervated by sensory neurons. This pain can be diffuse, referred from other sites, and is often accompanied by other symptoms such as nausea (Cervero and Laird 1999). The study of visceral pain is a field in its own right and of critical clinical importance. Numerous animal models have been used to study visceral pain; only some are covered here (Fig. 3.10a).

The colonic distension model creates visceral bowel pain. Simply, a balloon is inserted into the animal's colon and slowly inflated with water to a pre-specified pressure, causing distension. Animals exhibit measurable pain-like behaviors, but because of the location of the injury, typical pain testing is not well suited for detecting this phenotype. Instead, pain can be tested with visceromotor response (VMR)

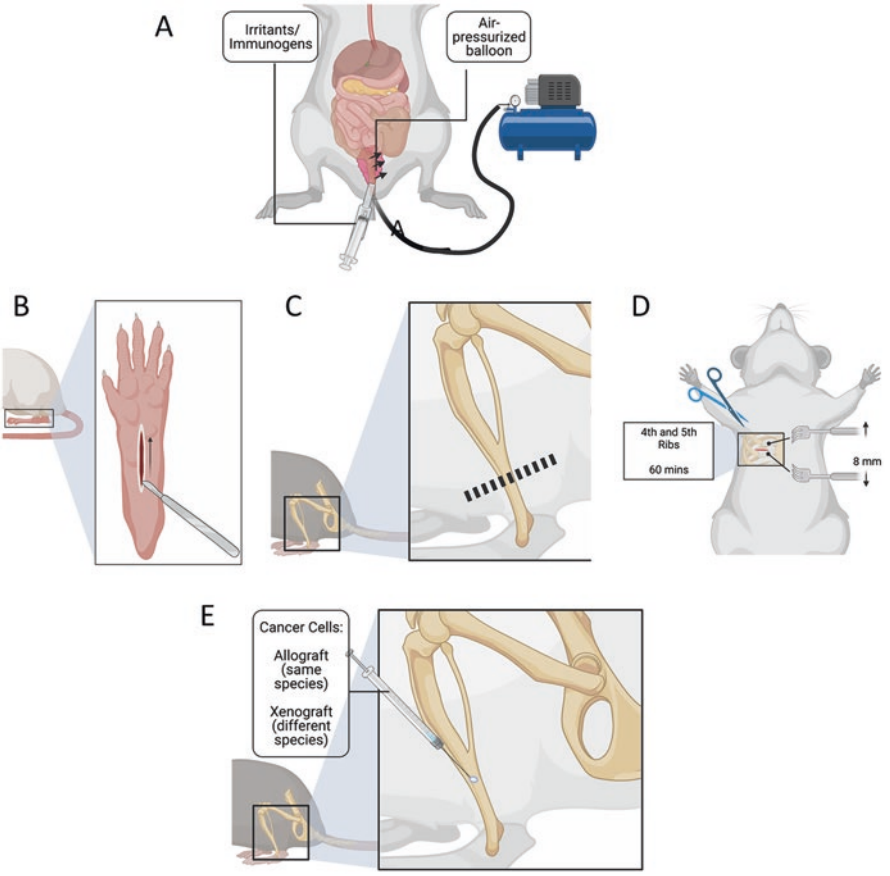

Fig. 3.10 Animal models of visceral pain, postoperative pain, and cancer pain: (**a**) two models of irritable bowel syndrome (IBS) visceral pain; (**b**) plantar incision model for postoperative pain; (**c**) tibial fracture model of postoperative pain; (**d**) thoracotomy model of postoperative pain; and (**e**) bone cancer pain model

(contraction of abdominal musculature) as well as blood pressure, heart rate, and passive avoidance (Moloney et al. 2015). In rats, abdominal withdrawal reflex (AWR) is a well-validated method of testing pain in the injured region. Animals are briefly anesthetized before an inflatable balloon is inserted into the colorectum. The balloon is slowly inflated, during which time the animal's reactions can be recorded (O'Mahony et al. 2012). Other common visceral pain models are established by delivering an irritant such as mustard oil or capsaicin to the colon via anal cannulation (Laird et al. 2001). Like the formalin model of inflammatory pain, animals typically experience a biphasic response. First, receptors such as TRPA1 are bound by the irritant and cause acute pain, then second-order neurons become hypersensitized and cause referred pain in the abdominal region. This referred pain can be powerful and widespread. Many other models, such as stress-induced visceral pain, exist outside the purview of this chapter (Moloney et al. 2015).

3.8.2 Animal Models of Postoperative Pain

Developed in 1996, the incisional pain model (Brennan et al. 1996) (Fig. 3.10b), also referred to as Brennan's model, causes only transient pain in the region surrounding a cut made on the plantar paw. Immediately afterward, the incision itself and a notable radius around it become intensely sensitive to mechanical and thermal stimuli and animals display signs of spontaneous pain. Recovery typically begins within half a week and animals return to baseline sensitivities after a mean of 5 days. Notably, p38 MAP kinase inhibitor is effective in reducing mechanical pain in this model by inhibition of microglial activation in the spinal cord (Wen et al. 2009). Contrastingly, the tibial fracture model produces intense and durable pain (Fig. 3.10c). Animals are anesthetized, and tibia fracture of the left hindleg is conducted using pliers with an adjustable stop (Guo et al. 2004). Mechanical allodynia and hyperalgesia begin immediately and last an average of 20 weeks in rats. Notably, anti-inflammatory treatments, such as anti-IL-1β antibody, are effective in reducing mechanical pain in the late phase in this model (Wei et al. 2016). Intrathecal injection of SPMs (neuroprotectin D1, maresin 1, and resolvins D1 and D5) each can transiently reverse mechanical pain in this fracture model (Zhang et al. 2018). Tibial fracture is also used for the study of chronic regional pain syndrome (CRPS). Thoracotomy, a surgical procedure that grants access to the thoracic cavity by incising an opening between two ribs, is a common cause of postoperative pain (Kehlet et al. 2006). A rodent model causes mechanical and thermal sensitivity in the majority of animals, beginning within 10 days of the procedure and lasting months after symptoms develop (Buvanendran et al. 2004; Wang and Strichartz 2017). An incision is made between the fourth and fifth ribs, the intercostal muscles between these ribs cut, and a retractor placed to hold the ribs apart for 60 minutes (Fig. 3.10d). To test pain in this model, Von Frey filaments, acetone, or other stimuli can be applied to the rib region and the animal's scratching and withdrawal responses recorded.

3.8.3 Cancer Pain Models

Cancer pain animal models can be established in three primary ways (Pineda-Farias et al. 2020). Transplant models, which are by far the most numerous and best studied to date, are established via injection of cancerous cells into a target tissue or the circulatory system. Chemically induced cancer models deliver high doses of carcinogen either locally or systemically; tumors then develop sporadically. Finally, a few transgenic mouse lines have been characterized that reliably develop tumors and tumor-associated pain symptomology. Metastasis to the bone is the most common cause of cancer pain (Mercadante 1997). The first established cancer pain model delivered an allograft of fibrosarcoma cells to the interfemoral cavity of mice (Schwei et al. 1999). In 2002, a similar model was reported in rats using an allograft of mammary gland carcinoma delivered intratibially. A host of similar models using

a range of murine and human cancer cell lines have emerged targeting the femur, tibia humerus, and calcaneus (commonly called the heel bone) (Fig. 3.10e). Taken together, these models cause demonstrable mechanical hypersensitivity, heat hyperalgesia, spontaneous pain, increased movement-evoked pain, and cold pain, although the latter is less frequently reported (Currie et al. 2013).

Transgenic models of cancer pain currently rely on placing SV40 large T-antigen, a known viral oncoprotein, into the genome under the control of a tissue type specific promoter. This strategy was first used to study pancreatic cancer in conjunction with the addition of a rat elastase-1 promoter (Tevethia et al. 1997) to mice. C3TAg mice express SV40 large T-antigen, a known viral oncoprotein, under the control of a promoter active primarily in endothelial cells (Green et al. 2000). In both models, pain reliably occurs after approximately 4 months, but at this point the disease has typically progressed significantly (Lindsay et al. 2005).

3.8.4 Bacterial Infection Models

It is increasingly understood that nociceptors can sense and respond to danger-associated molecular patterns (DAMPs), including common bacterial motifs (Donnelly et al. 2020). Indeed, bacterial infections can cause pain (Chiu 2018). A model of *Staphylococcus aureus* infection via intraplantar injection has been shown to cause mechanical, thermal, and cold pain lasting 2–3 days and peaking in severity at 6 h (Chiu et al. 2013). Mechanical pain resolves more rapidly, but heat hyperalgesia can linger for a week (Blake et al. 2018).

3.8.5 Microbiota-Based Models

The so-called "Microbiota–Brain–Gut Axis" has also been shown to regulate pain sensitivity in rodents (Russo et al. 2018). Germ-free mice have altered perceptions of inflammatory pain (Amaral et al. 2008), and treatment of mice with oral antibiotics has been shown to greatly reduce mechanical hypersensitivity via Von Frey testing after CCI (Ding et al. 2021). On the other hand, gut microbiota is also critical for the induction of chemotherapy-induced pain (Shen et al. 2017). The field of gut microbiota and its relationship with pain is still developing, with few well-characterized models. This is due in part to the complexity and individuality of any single organism's gut microbiome.

3.9 Conclusion

Thanks to the extensive efforts and numerous careers invested into studying some of the above models, we understand the causes, mechanisms, and possible treatment options for inflammatory and neuropathic pain conditions better than ever before. Despite this immense progress, however, relatively little has changed for chronic pain patients. Research must go on, and animal models remain the best tool to address this unmet need. New surprising discoveries are made every year; the finding that anti-inflammatory drugs may worsen chronic pain under some circumstances (Parisien et al. 2022) is only one such example. With so many models and ways to test them now available, more thought than ever ought to be invested into ensuring the validity and translational relevance of animal research.

References

Akintola T, Raver C, Studlack P, Uddin O, Masri R, Keller A. The grimace scale reliably assesses chronic pain in a rodent model of trigeminal neuropathic pain. Neurobiol Pain. 2017;2:13–7.

Amaral F, Sachs D, Costa V, Fagundes C, Cisalpino D, Cunha T, Ferreira SH, Cunha FQ, Silva T, Nicoli J. Commensal microbiota is fundamental for the development of inflammatory pain. Proc Natl Acad Sci. 2008;105(6):2193–7.

Bang S, Xie Y-K, Zhang Z-J, Wang Z, Xu Z-Z, Ji R-R. Gpr37 regulates macrophage phagocytosis and resolution of inflammatory pain. J Clin Invest. 2018;128(8):3568–82.

Baskın V, Bilge SS, Bozkurt A, Akyüz B, Ağrı AE, Güzel H, Ilkaya F. Effect of nonsteroidal anti-inflammatory drugs on colorectal distension-induced visceral pain. Indian J Pharmacol. 2016;48(2):150.

Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma. 1995;12(1):1–21.

Bastos LFS, Merlo LA, Rocha LTS, Coelho MM. Characterization of the antinociceptive and anti-inflammatory activities of doxycycline and minocycline in different experimental models. Eur J Pharmacol. 2007;576(1–3):171–9.

Bennett DL, Woods CG. Painful and painless channelopathies. Lancet Neurol. 2014;13(6):587–99.

Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988;33(1):87–107.

Blackburn-Munro G. Pain-like behaviours in animals – how human are they? Trends Pharmacol Sci. 2004;25(6):299–305.

Blake KJ, Baral P, Voisin T, Lubkin A, Pinho-Ribeiro FA, Adams KL, Roberson DP, Ma YC, Otto M, Woolf CJ. Staphylococcus aureus produces pain through pore-forming toxins and neuronal trpv1 that is silenced by qx-314. Nat Commun. 2018;9(1):1–15.

Bolon B, Stolina M, King C, Middleton S, Gasser J, Zack D, Feige U. Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. J Biomed Biotechnol. 2010;2011:569068.

Bourquin A-F, Süveges M, Pertin M, Gilliard N, Sardy S, Davison AC, Spahn DR, Decosterd I. Assessment and analysis of mechanical allodynia-like behavior induced by spared nerve injury (SNI) in the mouse. Pain. 2006;122(1–2):14.e1–14.

Bove S, Laemont K, Brooker R, Osborn M, Sanchez B, Guzman R, Hook K, Juneau P, Connor J, Kilgore K. Surgically induced osteoarthritis in the rat results in the development of both osteoarthritis-like joint pain and secondary hyperalgesia. Osteoarthr Cartil. 2006;14(10):1041–8.

Boyd JT, LoCoco PM, Furr AR, Bendele MR, Tram M, Li Q, Chang F-M, Colley ME, Samenuk GM, Arris DA. Elevated dietary ω-6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. Nat Metab. 2021;3:1–12.

Bradman MJ, Ferrini F, Salio C, Merighi A. Practical mechanical threshold estimation in rodents using von Frey hairs/Semmes–Weinstein monofilaments: towards a rational method. J Neurosci Methods. 2015;255:92–103.

Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. Nat Protoc. 2007;2(5):1269–75.

Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain. 1996;64(3):493–502

Brenner DS, Golden JP, Gereau RW. A novel behavioral assay for measuring cold sensation in mice. PLoS One. 2012;7(6):e39765.

Bresnahan J, Beattie M, Todd F III, Noyes D. A behavioral and anatomical analysis of spinal cord injury produced by a feedback-controlled impaction device. Exp Neurol. 1987;95(3):548–70.

Brown KK, Heitmeyer SA, Hookfin EB, Hsieh L, Buchalova M, Taiwo YO, Janusz MJ. P38 map kinase inhibitors as potential therapeutics for the treatment of joint degeneration and pain associated with osteoarthritis. J Inflamm. 2008;5(1):1–8.

Browne KD, Iwata A, Putt M, Smith DH. Chronic ibuprofen administration worsens cognitive outcome following traumatic brain injury in rats. Exp Neurol. 2006;201(2):301–7.

Bruce JC, Oatway MA, Weaver LC. Chronic pain after clip-compression injury of the rat spinal cord. Exp Neurol. 2002;178(1):33–48.

Buvanendran A, Kroin JS, Kerns JM, Nagalla SN, Tuman KJ. Characterization of a new animal model for evaluation of persistent postthoracotomy pain. Anesth Analg. 2004;99(5):1453–60.

Carlton SM, Lekan HA, Kim SH, Chung JM. Behavioral manifestations of an experimental model for peripheral neuropathy produced by spinal nerve ligation in the primate. Pain. 1994;56(2):155–66.

Carpenter SE, Lynn B. Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. Br J Pharmacol. 1981;73(3):755–8.

Castro A, Li Y, Raver C, Chandra R, Masri R, Lobo MK, Keller A. Neuropathic pain after chronic nerve constriction may not correlate with chloride dysregulation in mouse trigeminal nucleus caudalis neurons. Pain. 2017;158(7):1366.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 2000;288(5464):306–13.

Cavaletti G, Tredici G, Braga M, Tazzari S. Experimental peripheral neuropathy induced in adult rats by repeated intraperitoneal administration of taxol. Exp Neurol. 1995;133(1):64–72.

Cervero F, Laird JM. Visceral pain. Lancet. 1999;353(9170):2145–8.

Chang W, Berta T, Kim YH, Lee S, Lee SY, Ji RR. Expression and role of voltage-gated sodium channels in human dorsal root ganglion neurons with special focus on nav1.7, species differences, and regulation by paclitaxel. Neurosci Bull. 2018;34(1):4–12.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994;53(1):55–63.

Cheng L, Duan B, Huang T, Zhang Y, Chen Y, Britz O, Garcia-Campmany L, Ren X, Vong L, Lowell BB, et al. Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. Nat Neurosci. 2017;20(6):804–14.

Cheriyan T, Ryan D, Weinreb J, Cheriyan J, Paul J, Lafage V, Kirsch T, Errico T. Spinal cord injury models: a review. Spinal Cord. 2014;52(8):588–95.

Chiu IM. Infection, pain, and itch. Neurosci Bull. 2018;34(1):109–19.

Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR. Bacteria activate sensory neurons that modulate pain and inflammation. Nature. 2013;501(7465):52–7.

Cho I-H, Chung YM, Park C-K, Park S-H, Li HY, Kim D, Piao ZG, Choi S-Y, Lee SJ, Park K. Systemic administration of minocycline inhibits formalin-induced inflammatory pain in rat. Brain Res. 2006;1072(1):208–14.

Christensen MD, Everhart AW, Pickelman JT, Hulsebosch CE. Mechanical and thermal allodynia in chronic central pain following spinal cord injury. Pain. 1996;68(1):97–107.
Christensen SL, Hansen RB, Storm MA, Olesen J, Hansen TF, Ossipov M, Izarzugaza JM, Porreca F, Kristensen DM. Von Frey testing revisited: provision of an online algorithm for improved accuracy of 50% thresholds. Eur J Pain. 2020;24(4):783–90.
Clatworthy A, Illich P, Castro G, Walters E. Role of peri-axonal inflammation in the development of thermal hyperalgesia and guarding behavior in a rat model of neuropathic pain. Neurosci Lett. 1995;184(1):5–8.
Clements KM, Price JS, Chambers MG, Visco DM, Poole AR, Mason RM. Gene deletion of either interleukin-1β, interleukin-1β–converting enzyme, inducible nitric oxide synthase, or stromelysin 1 accelerates the development of knee osteoarthritis in mice after surgical transection of the medial collateral ligament and partial medial meniscectomy. Arthritis Rheum. 2003;48(12):3452–63.
Cobos EJ, Ghasemlou N, Araldi D, Segal D, Duong K, Woolf CJ. Inflammation-induced decrease in voluntary wheel running in mice: a nonreflexive test for evaluating inflammatory pain and analgesia. Pain. 2012;153(4):876–84.
Coderre TJ, Vaccarino AL, Melzack R. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. Brain Res. 1990;535(1):155–8.
Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR, Brandt MR, Liu Y, Flores CM, Qin N. Attenuated cold sensitivity in trpm8 null mice. Neuron. 2007;54(3):379–86.
Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, Freeman R, Truini A, Attal N, Finnerup NB. Neuropathic pain. Nat Rev Dis Primers. 2017;3(1):1–19.
Cope P, Ourradi K, Li Y, Sharif M. Models of osteoarthritis: the good, the bad and the promising. Osteoarthr Cartil. 2019;27(2):230–9.
Cornefjord M, Olmarker K, Otani K, Rydevik B. Nucleus pulposus-induced nerve root injury: effects of diclofenac and ketoprofen. Eur Spine J. 2002;11(1):57–61.
Cottrell JA, Meyenhofer M, Medicherla S, Higgins L, O'Connor JP. Analgesic effects of p38 kinase inhibitor treatment on bone fracture healing. Pain. 2009;142(1–2):116–26.
Courteix C, Eschalier A, Lavarenne J. Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain. 1993;53(1):81–8.
Currie GL, Delaney A, Bennett MI, Dickenson AH, Egan KJ, Vesterinen HM, Sena ES, Macleod MR, Colvin LA, Fallon MT. Animal models of bone cancer pain: systematic review and meta-analyses. Pain. 2013;154(6):917–26.
Currie GL, Angel-Scott HN, Colvin L, Cramond F, Hair K, Khandoker L, Liao J, Macleod M, McCann SK, Morland R. Animal models of chemotherapy-induced peripheral neuropathy: a machine-assisted systematic review and meta-analysis. PLoS Biol. 2019;17(5):e3000243.
D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941;72(1):74–9.
da Silva Fiorin F, do Espirito Santo CC, Santos ARS, Fighera MR, Royes LFF. Implication of surgical procedure in the induction of headache and generalized painful sensation in a fluid percussion injury model in rats. J Neurosci Methods. 2018;307:23–30.
Daiutolo BV, Tyburski A, Clark SW, Elliott MB. Trigeminal pain molecules, allodynia, and photosensitivity are pharmacologically and genetically modulated in a model of traumatic brain injury. J Neurotrauma. 2016;33(8):748–60.
Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain. 2000;87(2):149–58.
Delovitch TL, Singh B. The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the nod. Immunity. 1997;7(6):727–38.
Densmore VS, Kalous A, Keast JR, Osborne PB. Above-level mechanical hyperalgesia in rats develops after incomplete spinal cord injury but not after cord transection, and is reversed by amitriptyline, morphine and gabapentin. Pain. 2010;151(1):184–93.
Dersh J, Polatin PB, Gatchel RJ. Chronic pain and psychopathology: research findings and theoretical considerations. Psychosom Med. 2002;64(5):773–86.

Deseure K, Hans GH. Chronic constriction injury of the rat's infraorbital nerve (ION-CCI) to study trigeminal neuropathic pain. J Vis Exp. 2015;(103). https://doi.org/10.3791/53167.
Dickenson AH, Sullivan AF. Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain. 1987;30(3):349–60.
Dikmen S, Machamer J, Fann JR, Temkin NR. Rates of symptom reporting following traumatic brain injury. J Int Neuropsychol Soc. 2010;16(3):401–11.
Ding W, You Z, Shen S, Yang J, Lim G, Doheny JT, Chen L, Zhu S, Mao J. An improved rodent model of trigeminal neuropathic pain by unilateral chronic constriction injury of distal infraorbital nerve. J Pain. 2017;18(8):899–907.
Ding W, You Z, Chen Q, Yang L, Doheny J, Zhou X, Li N, Wang S, Hu K, Chen L. Gut microbiota influences neuropathic pain through modulating proinflammatory and anti-inflammatory t cells. Anesth Analg. 2021;132(4):1146–55.
Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol. 1980;20:441–62.
Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods. 1991;39(3):253–62.
Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends Neurosci. 2020;43(10):822–38.
Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain. 1977;4:161–74.
Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. J Pharmacol Exp Ther. 1953;107(3):385–93.
Elliott MB, Jallo JJ, Tuma RF. An investigation of cerebral edema and injury volume assessments for controlled cortical impact injury. J Neurosci Methods. 2008;168(2):320–4.
Elliott MB, Oshinsky ML, Amenta PS, Awe OO, Jallo JI. Nociceptive neuropeptide increases and periorbital allodynia in a model of traumatic brain injury. Headache. 2012;52(6):966.
Fan X-C, Fu S, Liu F-Y, Cui S, Yi M, Wan Y. Hypersensitivity of prelimbic cortex neurons contributes to aggravated nociceptive responses in rats with experience of chronic inflammatory pain. Front Mol Neurosci. 2018;11:85.
Fischer BD, Adeyemo A, O'Leary ME, Bottaro A. Animal models of rheumatoid pain: experimental systems and insights. Arthritis Res Ther. 2017;19(1):1–9.
Fishbain DA, Cutler R, Rosomoff HL, Rosomoff RS. Chronic pain-associated depression: antecedent or consequence of chronic pain? A review. Clin J Pain. 1997;13(2):116–37.
Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel-and vincristine-induced painful peripheral neuropathy. Pain. 2004;109(1–2):150–61.
Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc Pharmacol. 2015;70(1):5.47.1–20.
Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr Protoc. 2021;1(4):e78.
Gabra BH, Sirois P. Hyperalgesia in non-obese diabetic (nod) mice: a role for the inducible bradykinin b1 receptor. Eur J Pharmacol. 2005;514(1):61–7.
Gao YJ, Xu ZZ, Liu YC, Wen YR, Decosterd I, Ji RR. The c-Jun N-terminal kinase 1 (JNK1) in spinal astrocytes is required for the maintenance of bilateral mechanical allodynia under a persistent inflammatory pain condition. Pain. 2010;148(2):309–19.
Gilchrist HD, Allard BL, Simone DA. Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. Pain. 1996;67(1):179–88.
Goldim MPS, Della Giustina A, Petronilho F. Using Evans blue dye to determine blood-brain barrier integrity in rodents. Curr Protoc Immunol. 2019;126(1):e83.
Gowler PR, Mapp PI, Burston JJ, Shahtaheri M, Walsh DA, Chapman V. Refining surgical models of osteoarthritis in mice and rats alters pain phenotype but not joint pathology. PLoS One. 2020;15(9):e0239663.
Green JE, Shibata M-A, Yoshidome K, Liu M-l, Jorcyk C, Anver MR, Wigginton J, Wiltrout R, Shibata E, Kaczmarczyk S. The C3 (1)/SV40 T-antigen transgenic mouse model of mammary

cancer: ductal epithelial cell targeting with multistage progression to carcinoma. Oncogene. 2000;19(8):1020–7.
Gruner JA. A monitored contusion model of spinal cord injury in the rat. J Neurotrauma. 1992;9(2):123–8.
Gu X, Wang S, Yang L, Sung B, Lim G, Mao J, Zeng Q, Chang Y, Mao J. Time-dependent effect of epidural steroid on pain behavior induced by chronic compression of dorsal root ganglion in rats. Brain Res. 2007;1174:39–46.
Guan Y, Yuan F, Carteret AF, Raja SN. A partial l5 spinal nerve ligation induces a limited prolongation of mechanical allodynia in rats: an efficient model for studying mechanisms of neuropathic pain. Neurosci Lett. 2010;471(1):43–7.
Guo T-Z, Offley SC, Boyd EA, Jacobs CR, Kingery WS. Substance p signaling contributes to the vascular and nociceptive abnormalities observed in a tibial fracture rat model of complex regional pain syndrome type I. Pain. 2004;108(1–2):95–107.
Hao J-X, Xu X-J, Aldskogius H, Seiger Å, Wiesenfeld-Hallin Z. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. Pain. 1991;45(2):175–85.
Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain. 1988;32(1):77–88.
Helminen HJ, Kiraly K, Pelttari A, Tammi M, Vandenberg P, Pereira R, Dhulipala R, Khillan J, Ala-Kokko L, Hume E. An inbred line of transgenic mice expressing an internally deleted gene for type II procollagen (COL2A1). Young mice have a variable phenotype of a chondrodysplasia and older mice have osteoarthritic changes in joints. J Clin Invest. 1993;92(2):582–95.
Höke A, Ray M. Rodent models of chemotherapy-induced peripheral neuropathy. ILAR J. 2014;54(3):273–81.
Hu S-J, Xing J-L. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. Pain. 1998;77(1):15–23.
Hu K, Xu L, Cao L, Flahiff C, Brussiau J, Ho K, Setton L, Youn I, Guilak F, Olsen B. Pathogenesis of osteoarthritis-like changes in the joints of mice deficient in type ix collagen. Arthritis Rheum. 2006;54(9):2891–900.
Huang W, Calvo M, Karu K, Olausen HR, Bathgate G, Okuse K, Bennett DL, Rice AS. A clinically relevant rodent model of the HIV antiretroviral drug stavudine induced painful peripheral neuropathy. Pain. 2013;154(4):560–75.
Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987;30(1):103–14.
Hutchinson KJ, Gómez-Pinilla F, Crowe MJ, Ying Z, Basso DM. Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. Brain. 2004;127(6):1403–14.
Hylden JL, Thomas DA, Iadarola MJ, Nahin RL, Dubner R. Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms. Eur J Pharmacol. 1991;194(2–3):135–43.
Ibrahim SI, Xie W, Strong JA, Tonello R, Berta T, Zhang JM. Mineralocorticoid antagonist improves glucocorticoid receptor signaling and dexamethasone analgesia in an animal model of low back pain. Front Cell Neurosci. 2018;12:453.
Imamura Y, Kawamoto H, Nakanishi O. Characterization of heat-hyperalgesia in an experimental trigeminal neuropathy in rats. Exp Brain Res. 1997;116(1):97–103.
Ito N, Obata H, Saito S. Spinal microglial expression and mechanical hypersensitivity in a postoperative pain model: comparison with a neuropathic pain model. J Am Soc Anesthesiol. 2009;111(3):640–8.
Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice AS, Treede R-D. A new definition of neuropathic pain. Pain. 2011;152(10):2204–5.
Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain. 2007;3:33.
Ji RR, Zhang Q, Bedecs K, Arvidsson J, Zhang X, Xu XJ, Wiesenfeld-Hallin Z, Bartfai T, Hökfelt T. Galanin antisense oligonucleotides reduce galanin levels in dorsal root ganglia and induce autotomy in rats after axotomy. Proc Natl Acad Sci U S A. 1994;91(26):12540–3.

Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nat Neurosci. 1999;2(12):1114–9.
Ji R-R, Samad TA, Jin S-X, Schmoll R, Woolf CJ. P38 MAPK activation by NGF in primary sensory neurons after inflammation increases trpv1 levels and maintains heat hyperalgesia. Neuron. 2002;36(1):57–68.
Ji R-R, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003;26(12):696–705.
Johnson MS, Ryals JM, Wright DE. Early loss of peptidergic intraepidermal nerve fibers in an STZ-induced mouse model of insensate diabetic neuropathy. Pain. 2008;140(1):35–47.
Joshi S, Hernandez G, Mikusa J, Zhu C, Zhong C, Salyers A, Wismer C, Chandran P, Decker M, Honore P. Comparison of antinociceptive actions of standard analgesics in attenuating capsaicin and nerve-injury-induced mechanical hypersensitivity. Neuroscience. 2006;143(2):587–96.
Kannampalli P, Pochiraju S, Bruckert M, Shaker R, Banerjee B, Sengupta JN. Analgesic effect of minocycline in rat model of inflammation-induced visceral pain. Eur J Pharmacol. 2014;727:87–98.
Kawakami M, Weinstein JN, Spratt KF, Chatani K-I, Traub RJ, Meller ST, Gebhart GF. Immunohistochemical and quantitative demonstrations of pain induced by lumbar nerve root irritation of the rat. Spine. 1994;19(16):1780–94.
Kawakami M, Tamaki T, Weinstein JN, Hashizume H, Nishi H, Meller ST. Pathomechanism of pain-related behavior produced by allografts of intervertebral disc in the rat. Spine. 1996;21(18):2101–7.
Kawasaki Y, Kohno T, Zhuang ZY, Brenner GJ, Wang H, Van Der Meer C, Befort K, Woolf CJ, Ji RR. Ionotropic and metabotropic receptors, protein kinase A, protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding protein phosphorylation in dorsal horn neurons, leading to central sensitization. J Neurosci. 2004;24(38):8310–21.
Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Gao YJ, Roy K, Corfas G, Lo EH, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008;14(3):331–6.
Kayser V. Randall-Selitto paw pressure test. In: Encyclopedia of pain. Berlin: Springer; 2013. p. 3357–60.
Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J. 1991;10(13):4025–31.
Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. Lancet. 2006;367(9522):1618–25.
Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain. 1992;50(3):355–63.
King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. Nat Neurosci. 2009;12(11):1364–6.
Knezevic N, Candido K, Vlaeyen J, Van Zundert J, Cohen S. Low back pain. Lancet. 2021;398:78–92.
Knights CB, Gentry C, Bevan S. Partial medial meniscectomy produces osteoarthritis pain-related behaviour in female C57BL/6 mice. Pain. 2012;153(2):281–92.
Kolb H. Mouse models of insulin dependent diabetes: low-dose streptozocin-induced diabetes and nonobese diabetic (NOD) mice. Diabetes Metab Rev. 1987;3(3):751–78.
Kouskoff V, Korganow A-S, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell. 1996;87(5):811–22.
Kuyinu EL, Narayanan G, Nair LS, Laurencin CT. Animal models of osteoarthritis: classification, update, and measurement of outcomes. J Orthop Surg Res. 2016;11(1):1–27.
Laird J, Martinez-Caro L, Garcia-Nicas E, Cervero F. A new model of visceral pain and referred hyperalgesia in the mouse. Pain. 2001;92(3):335–42.

Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, et al. Coding of facial expressions of pain in the laboratory mouse. Nat Methods. 2010;7(6):447–9.

Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, Watkins LR. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. Pain. 2005;115(1–2):71–83.

Lee JB, Choi SS, Ahn EH, Hahm KD, Suh JH, Leem JG, Shin JW. Effect of perioperative perineural injection of dexamethasone and bupivacaine on a rat spared nerve injury model. Korean J Pain. 2010;23(3):166–71.

Leith JL, Koutsikou S, Lumb BM, Apps R. Spinal processing of noxious and innocuous cold information: differential modulation by the periaqueductal gray. J Neurosci. 2010;30(14):4933–42.

Lew HL, Lin P-H, Fuh J-L, Wang S-J, Clark DJ, Walker WC. Characteristics and treatment of headache after traumatic brain injury: a focused review. Am J Phys Med Rehabil. 2006;85(7):619–27.

Li P, Schwarz EM. The TNF-α transgenic mouse model of inflammatory arthritis. Springer Semin Immunopathol. 2003;25:19–33.

Li H, Xie W, Strong JA, Zhang J-M. Systemic antiinflammatory corticosteroid reduces mechanical pain behavior, sympathetic sprouting, and elevation of proinflammatory cytokines in a rat model of neuropathic pain. Anesthesiologists. 2007;107(3):469–77.

Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ. Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight and disease progression. Pain. 2005;119(1–3):233–46.

Lyu F-J, Cui H, Pan H, Cheung KM, Cao X, Iatridis JC, Zheng Z. Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions. Bone Res. 2021;9(1):1–14.

Ma W, Du W, Eisenach JC. Role for both spinal cord COX-1 and COX-2 in maintenance of mechanical hypersensitivity following peripheral nerve injury. Brain Res. 2002;937(1–2):94–9.

Malmberg AB, Basbaum AI. Partial sciatic nerve injury in the mouse as a model of neuropathic pain: behavioral and neuroanatomical correlates. Pain. 1998;76(1–2):215–22.

Malmberg AB, Chen C, Tonegawa S, Basbaum AI. Preserved acute pain and reduced neuropathic pain in mice lacking PCKgamma. Science. 1997;278(5336):279–83.

McCoy AM. Animal models of osteoarthritis: comparisons and key considerations. Vet Pathol. 2015;52(5):803–18.

McIntosh TK, Noble L, Andrews B, Faden AI. Traumatic brain injury in the rat: characterization of a midline fluid-percussion model. Cent Nerv Syst Trauma. 1987;4(2):119–34.

McIntosh T, Vink R, Noble L, Yamakami I, Fernyak S, Soares H, Faden A. Traumatic brain injury in the rat: characterization of a lateral fluid-percussion model. Neuroscience. 1989;28(1):233–44.

McWilliams LA, Cox BJ, Enns MW. Mood and anxiety disorders associated with chronic pain: an examination in a nationally representative sample. Pain. 2003;106(1–2):127–33.

Medeiros PD, Dos Santos I, Medeiros AC, da Silva J, Ferreira S, de Freitas R, Coimbra NC. Indomethacin attenuates mechanical allodynia during the organization but not the maintenance of the peripheral neuropathic pain induced by nervus ischiadicus chronic constriction injury. Braz J Med Biol Res. 2020;53:e9255.

Meller S, Gebhart G. Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat. Eur J Pain. 1997;1(1):43–52.

Mercadante S. Malignant bone pain: pathophysiology and treatment. Pain. 1997;69(1–2):1–18.

Metz GA, Whishaw IQ. The ladder rung walking task: a scoring system and its practical application. J Vis Exp. 2009;(28):e1204. https://doi.org/10.3791/1204.

Millecamps M, Tajerian M, Sage EH, Stone LS. Behavioural signs of chronic back pain in the SPARC-null mouse. Spine. 2011;36(2):95.

Millecamps M, Tajerian M, Naso L, Sage EH, Stone LS. Lumbar intervertebral disc degeneration associated with axial and radiating low back pain in ageing SPARC-null mice. Pain. 2012;153(6):1167–79.
Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009;10(4):283–94.
Mogil JS, Wilson SG, Bon K, Lee SE, Chung K, Raber P, Pieper JO, Hain HS, Belknap JK, Hubert L. Heritability of nociception I: responses of 11 inbred mouse strains on 12 measures of nociception. Pain. 1999;80(1–2):67–82.
Moloney RD, O'Mahony SM, Dinan TG, Cryan JF. Stress-induced visceral pain: toward animal models of irritable-bowel syndrome and associated comorbidities. Front Psych. 2015;6:15.
Monach P, Hattori K, Huang H, Hyatt E, Morse J, Nguyen L, Ortiz-Lopez A, Wu H-J, Mathis D, Benoist C. The K/BxN mouse model of inflammatory arthritis. In: Arthritis research. Berlin: Springer; 2007. p. 269–82.
Moore RA, Chi CC, Wiffen PJ, Derry S, Rice AS. Oral nonsteroidal anti-inflammatory drugs for neuropathic pain. Cochrane Database Syst Rev. 2015;2015(10):CD010902.
Moriarty O, McGuire BE, Finn DP. The effect of pain on cognitive function: a review of clinical and preclinical research. Prog Neurobiol. 2011;93(3):385–404.
O'Brien PD, Sakowski SA, Feldman EL. Mouse models of diabetic neuropathy. ILAR J. 2014;54(3):259–72.
O'Brien PD, Hur J, Hayes JM, Backus C, Sakowski SA, Feldman EL. BTBR ob/ob mice as a novel diabetic neuropathy model: neurological characterization and gene expression analyses. Neurobiol Dis. 2015;73:348–55.
O'Mahony SM, Tramullas M, Fitzgerald P, Cryan JF. Rodent models of colorectal distension. Curr Protoc Neurosci. 2012;61(1):9.40. 41-49.40. 13
Obrosova IG, Ilnytska O, Lyzogubov VV, Pavlov IA, Mashtalir N, Nadler JL, Drel VR. High-fat diet–induced neuropathy of pre-diabetes and obesity: effects of "healthy" diet and aldose reductase inhibition. Diabetes. 2007;56(10):2598–608.
Pacharinsak C, Beitz A. Animal models of cancer pain. Comp Med. 2008;58(3):220–33.
Parisien M, Lima LV, Dagostino C, El-Hachem N, Drury GL, Grant AV, Huising J, Verma V, Meloto CB, Silva JR. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci Transl Med. 2022;14(644):eabj9954.
Partanen J, Niskanen L, Lehtinen J, Mervaala E, Siitonen O, Uusitupa M. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. N Engl J Med. 1995;333(2):89–94.
Pertin M, Gosselin R-D, Decosterd I. The spared nerve injury model of neuropathic pain. In: Pain research. Berlin: Springer; 2012. p. 205–12.
Pineda-Farias JB, Saloman JL, Scheff NN. Animal models of cancer-related pain: current perspectives in translation. Front Pharmacol. 2020;11:1975.
Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissues. Arch Int Pharmacodyn Ther. 1957;111:409–19.
Recober A, Kuburas A, Zhang Z, Wemmie JA, Anderson MG, Russo AF. Role of calcitonin gene-related peptide in light-aversive behavior: implications for migraine. J Neurosci. 2009;29(27):8798–804.
Ren K, Dubner R. Inflammatory models of pain and hyperalgesia. ILAR J. 1999;40(3):111–8.
Rodriquez E, Sakurai K, Xu J, Chen Y, Toda K, Zhao S, Han B, Ryu D, Yin H, Liedtke W, Wang F. A craniofacial-specific monosynaptic circuit enables heightened affective pain. Nat Neurosci. 2017;20:1734–1743.
Rosenberg MB, Carroll FI, Negus SS. Effects of monoamine reuptake inhibitors in assays of acute pain-stimulated and pain-depressed behavior in rats. J Pain. 2013;14(3):246–59.
Röytta M, Raine CS. Taxol-induced neuropathy: further ultrastructural studies of nerve fibre changes in situ. J Neurocytol. 1985;14(1):157–75.
Russo R, Cristiano C, Avagliano C, De Caro C, La Rana G, Raso GM, Canani RB, Meli R, Calignano A. Gut-brain axis: role of lipids in the regulation of inflammation, pain and CNS diseases. Curr Med Chem. 2018;25(32):3930–52.

Sadler KE, Mogil JS, Stucky CL. Innovations and advances in modelling and measuring pain in animals. Nat Rev Neurosci. 2021;23:1–16.
Sahbaie P, Tajerian M, Yang P, Irvine KA, Huang T-T, Luo J, Wyss-Coray T, Clark JD. Nociceptive and cognitive changes in a murine model of polytrauma. J Pain. 2018;19(12):1392–405.
Scheifer C, Hoheisel U, Trudrung P, Unger T, Mense S. Rats with chronic spinal cord transection as a possible model for the at-level pain of paraplegic patients. Neurosci Lett. 2002;323(2):117–20.
Schmidt EK, Raposo PJ, Torres-Espin A, Fenrich KK, Fouad K. Beyond the lesion site: minocycline augments inflammation and anxiety-like behavior following SCI in rats through action on the gut microbiota. J Neuroinflammation. 2021;18(1):1–16.
Scholz J, Abele A, Marian C, Häussler A, Herbert TA, Woolf CJ, Tegeder I. Low-dose methotrexate reduces peripheral nerve injury-evoked spinal microglial activation and neuropathic pain behavior in rats. Pain. 2008;138(1):130–42.
Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW. Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. J Neurosci. 1999;19(24):10886–97.
Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain. 1990;43(2):205–18.
Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest. 2018;128(7):2657–69.
Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta. 2015;1851(4):397–413.
Shen S, Lim G, You Z, Ding W, Huang P, Ran C, Doheny J, Caravan P, Tate S, Hu K, Kim H, McCabe M, Huang B, Xie Z, Kwon D, Chen L, Mao J. Gut microbiota is critical for the induction of chemotherapy-induced pain. Nat Neurosci. 2017;20(9):1213–6.
Shi C, Qiu S, Riester SM, Das V, Zhu B, Wallace AA, van Wijnen AJ, Mwale F, Iatridis JC, Sakai D. Animal models for studying the etiology and treatment of low back pain. J Orthop Res. 2018;36(5):1305–12.
Shields SD, Eckert WA III, Basbaum AI. Spared nerve injury model of neuropathic pain in the mouse: a behavioral and anatomic analysis. J Pain. 2003;4(8):465–70.
Shiers S, Klein RM, Price TJ. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. Pain. 2020;161(10):2410–24.
Shitaka Y, Tran HT, Bennett RE, Sanchez L, Levy MA, Dikranian K, Brody DL. Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. J Neuropathol Exp Neurol. 2011;70(7):551–67.
Siddall P, Loeser J. Pain following spinal cord injury. Spinal Cord. 2001;39(2):63–73.
Siddall P, Xu CL, Cousins M. Allodynia following traumatic spinal cord injury in the rat. Neuroreport. 1995;6(9):1241–4.
Sima A, Zhang W, Sugimoto K, Henry D, Li Z, Wahren J, Grunberger G. C-peptide prevents and improves chronic type I diabetic polyneuropathy in the BB/Wor rat. Diabetologia. 2001;44(7):889–97.
Simone DA, Ngeow JY, Putterman GJ, LaMotte RH. Hyperalgesia to heat after intradermal injection of capsaicin. Brain Res. 1987;418(1):201–3.
Song Z-P, Xiong B-R, Guan X-H, Cao F, Manyande A, Zhou Y-Q, Zheng H, Tian Y-K. Minocycline attenuates bone cancer pain in rats by inhibiting NF-κB in spinal astrocytes. Acta Pharmacol Sin. 2016;37(6):753–62.
Sorge RE, Mapplebeck J, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin J-S, Sotocinal SG, Chen D. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18(8):1081–3.
Sotocina SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S. The rat grimace scale: a partially automated method for quantifying

pain in the laboratory rat via facial expressions. Mol Pain. 2011;7. https://doi.org/10.1186/1744-8069-7-55.
Staff NP, Grisold A, Grisold W, Windebank AJ. Chemotherapy-induced peripheral neuropathy: a current review. Ann Neurol. 2017;81(6):772–81.
Starobova H, Mueller A, Allavena R, Lohman R-J, Sweet MJ, Vetter I. Minocycline prevents the development of mechanical allodynia in mouse models of vincristine-induced peripheral neuropathy. Front Neurosci. 2019;13:653.
Stein C, Millan M, Herz A. Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: alterations in behavior and nociceptive thresholds. Pharmacol Biochem Behav. 1988;31(2):445–51.
Sullivan KA, Hayes JM, Wiggin TD, Backus C, Oh SS, Lentz SI, Brosius F III, Feldman EL. Mouse models of diabetic neuropathy. Neurobiol Dis. 2007;28(3):276–85.
Szolcsanyi J. A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiol. 1977;73(3):251–9.
Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci USA. 2005;102(16):5856–61.
Tao X, Luo X, Zhang T, Hershey B, Esteller R, Ji RR. Spinal cord stimulation attenuates mechanical allodynia and increases central resolvin D1 levels in rats with spared nerve injury. Front Physiol. 2021;12:687046.
Taves S, Berta T, Liu D-L, Gan S, Chen G, Kim YH, Van de Ven T, Laufer S, Ji R-R. Spinal inhibition of p38 map kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. Brain Behav Immun. 2016;55:70–81.
Tevethia MJ, Bonneau RH, Griffith JW, Mylin L. A simian virus 40 large T-antigen segment containing amino acids 1 to 127 and expressed under the control of the rat elastase-1 promoter produces pancreatic acinar carcinomas in transgenic mice. J Virol. 1997;71(11):8157–66.
Van Gorp S, Kessels A, Joosten E, Van Kleef M, Patijn J. Pain prevalence and its determinants after spinal cord injury: a systematic review. Eur J Pain. 2015;19(1):5–14.
Vastani N, Guenther F, Gentry C, Austin AL, King AJ, Bevan S, Andersson DA. Impaired nociception in the diabetic Ins2+/Akita mouse. Diabetes. 2018;67(8):1650–62.
Vos BP, Strassman AM, Maciewicz RJ. Behavioral evidence of trigeminal neuropathic pain following chronic constriction injury to the rat's infraorbital nerve. J Neurosci. 1994;14(5):2708–23.
Wang JC-F, Strichartz GR. Prevention of chronic post-thoracotomy pain in rats by intrathecal resolvin D1 and D2: effectiveness of perioperative and delayed drug delivery. J Pain. 2017;18(5):535–45.
Wang Z, Jiang C, He Q, Matsuda M, Han Q, Wang K, Bang S, Ding H, Ko MC, Ji RR. Anti-PD-1 treatment impairs opioid antinociception in rodents and nonhuman primates. Sci Transl Med. 2020;12(531):eaaw6471.
Waxman SG, Dib-Hajj SD. Erythromelalgia: a hereditary pain syndrome enters the molecular era. Ann Neurol. 2005;57(6):785–8.
Wei T, Guo TZ, Li WW, Kingery WS, Clark JD. Acute versus chronic phase mechanisms in a rat model of CRPS. J Neuroinflammation. 2016;13:1–15.
Wen YR, Suter MR, Ji RR, Yeh GC, Wu YS, Wang KC, Kohno T, Sun WZ, Wang CC. Activation of p38 mitogen-activated protein kinase in spinal microglia contributes to incision-induced mechanical allodynia. Anesthesiology. 2009;110(1):155–65.
Whiteside GT, Harrison J, Boulet J, Mark L, Pearson M, Gottshall S, Walker K. Pharmacological characterisation of a rat model of incisional pain. Br J Pharmacol. 2004;141(1):85–91.
Williams WO, Riskin DK, Mott KM. Ultrasonic sound as an indicator of acute pain in laboratory mice. J Am Assoc Lab Anim Sci. 2008;47(1):8–10.
Woolf CJ. Evidence for a central component of post-injury pain hypersensitivity. Nature. 1983;306(5944):686–8.
Woolf CJ. What is this thing called pain? J Clin Invest. 2010;120(11):3742–4.
Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet. 1999;353(9168):1959–64.

Xie W-R, Deng H, Li H, Bowen T, Strong J, Zhang J-M. Robust increase of cutaneous sensitivity, cytokine production and sympathetic sprouting in rats with localized inflammatory irritation of the spinal ganglia. Neuroscience. 2006;142(3):809–22.

Xiong Y, Mahmood A, Chopp M. Animal models of traumatic brain injury. Nat Rev Neurosci. 2013;14(2):128–42.

Xu Z-Z, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji R-R. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med. 2010;16(5):592–7.

Xu Z-Z, Berta T, Ji R-R. Resolvin E1 inhibits neuropathic pain and spinal cord microglial activation following peripheral nerve injury. J Neuroimmune Pharmacol. 2013a;8(1):37–41.

Xu ZZ, Liu XJ, Berta T, Park CK, Lu N, Serhan CN, Ji RR. Neuroprotectin/protectin D1 protects against neuropathic pain in mice after nerve trauma. Ann Neurol. 2013b;74(3):490–5.

Yezierski R, Liu S, Ruenes G, Kajander K, Brewer K. Excitotoxic spinal cord injury: behavioral and morphological characteristics of a central pain model. Pain. 1998;75(1):141–55.

Yoon C, Wook YY, Sik NH, Ho KS, Mo CJ. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain. 1994;59(3):369–76.

Yu D, Thakor DK, Han I, Ropper AE, Haragopal H, Sidman RL, Zafonte R, Schachter SC, Teng YD. Alleviation of chronic pain following rat spinal cord compression injury with multimodal actions of huperzine A. Proc Natl Acad Sci. 2013;110(8):E746–55.

Zaninelli TH, Fattori V, Verri WA Jr. Harnessing inflammation resolution in arthritis: current understanding of specialized pro-resolving lipid mediators' contribution to arthritis physiopathology and future perspectives. Front Physiol. 2021;12:1444.

Zhang M, Lv X-Y, Li J, Xu Z-G, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res. 2008;2008:704045.

Zhang L, Terrando N, Xu ZZ, Bang S, Jordt SE, Maixner W, Serhan CN, Ji RR. Distinct analgesic actions of DHA and DHA-derived specialized pro-resolving mediators on post-operative pain after bone fracture in mice. Front Pharmacol. 2018;9:412.

Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain. 2005;114(1–2):149–59.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983;16(2):109–10.

Zychowska M, Rojewska E, Kreiner G, Nalepa I, Przewlocka B, Mika J. Minocycline influences the anti-inflammatory interleukins and enhances the effectiveness of morphine under mice diabetic neuropathy. J Neuroimmunol. 2013;262(1–2):35–45.

Chapter 4
Inflammatory Mediators, Nociceptors, and Their Interactions in Pain

Jasmine Ji, Yul Huh, and Ru-Rong Ji

Abstract Inflammation produces pro-inflammatory mediators for the induction of pain. These mediators include inflammatory cytokines and chemokines, lipids, and microRNAs. These inflammatory mediators bind respective receptors on nociceptor terminals and cell bodies to trigger nociceptor activation and sensitization (peripheral sensitization). Inflammation also generates anti-inflammatory and pro-resolving mediators (SPMs) for the resolution of acute pain. Notably, specialized pro-resolving mediators (SPMs) such as resolvins produce potent inhibition of inflammation and pain. A failure in SPM production and signaling and resolution of acute pain will lead to acute pain to chronic pain transition.

Keywords C-fiber · Chemokines · Cyclooxygenase 2 (COX-2) · Cytokines · Dorsal root ganglion (DRG) · Nociceptors · Nonsteroidal anti-inflammatory drugs (NSAIDs) · Prostaglandin E2 (PGE2) · Specialized pro-resolving mediators (SPMs); Transient receptor potential ion channel subtype A1 (TRPA1) · Transient receptor potential ion channel subtype V1 (TRPV1)

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4.1 Introduction

Anti-inflammatory drugs can be divided into two categories: steroidal and nonsteroidal. Steroidal or corticosteroid drugs include drugs such as dexamethasone, cortisone, hydrocortisone, and betamethasone. These drugs contain corticosteroids, the artificially synthesized counterparts of steroids that are naturally produced by the adrenal cortex. Importantly, corticosteroids can produce glucocorticoid-like effects, which inhibit inflammation and suppress the immune system. When corticosteroids bind to the glucocorticoid receptor, the resulting signal transduction inhibits the translation of certain inflammatory cells, thus reducing the production of pro-inflammatory cytokines and chemokines (Liu et al. 2013). Due to their rapid and broad-ranging effects, steroidal drugs are used to treat a variety of conditions, including allergies, asthma, inflammatory bowel disease, lymphoma, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis (Coutinho and Chapman 2011; Liu et al. 2013). However, steroidal drugs also produce various negative side effects, especially when used at high doses or over long periods of time (60 days or more), which affect several body systems. For example, steroidal drugs can cause immunosuppression, with high doses of corticosteroids resulting in the inhibition of B-cell and T-cell production (Liu et al. 2013). These drugs can also suppress the hypothalamic-pituitary-adrenal (HPA) axis, as the introduction of corticosteroids can result in decreased cortisol production. Moreover, corticosteroids promote osteoclast activity, which can lead to an increase in bone fractures (Ericson-Neilsen and Kaye 2014; Liu et al. 2013). Steroidal drugs can also negatively impact the cardiovascular and gastrointestinal (GI) systems (Liu et al. 2013).

The second category of anti-inflammatory drugs is nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are commonly used to treat conditions such as arthritis, gout, muscle pain, and migraine (Shekelle et al. 2017). NSAIDs inhibit inflammation by inhibiting cyclooxygenase (COX), an enzyme that is essential for the conversion of arachidonic acid into eicosanoids, which include thromboxanes, prostaglandins, and prostacyclins (Vane 1971). COX consists of two isoenzymes, COX-1 and COX-2. While COX-1 is constitutively expressed and important for the maintenance of the gastrointestinal mucosa lining, COX-2 becomes highly inducible following inflammation, and is especially responsible for prostaglandin E2 (PGE2), an important inflammatory mediator that can play a role in pain and inflammation (Brigham et al. 2021; Malmberg and Yaksh 1995; Samad et al. 2001). NSAIDs can be divided into two categories depending on whether they are nonselective or COX-2-selective. The vast majority of NSAIDs, including diclofenac, ibuprofen, ketoprofen, and naproxen, nonselectively inhibit COX-1 and COX-2. Other NSAIDs are specifically COX-2-selective; these include celecoxib, rofecoxib, and valdecoxib. COX-2-selective NSAIDs are preferred over nonselective NSAIDs, as they lower the risk of negative gastrointestinal effects (Chaiamnuay et al. 2006; Sostres et al. 2010). NSAIDs may also be associated with cardiovascular problems such as myocardial infarction and atrial fibrillation. In addition, NSAIDs may exacerbate renal dysfunction, leading to renal problems that include fluid and

electrolyte disorders, acute renal dysfunction, and renal papillary necrosis (Whelton 1999). NSAID toxicity can further cause hypertension, hepatotoxicity, and GI bleeding (Rothenberg and Holcomb 2000).

A useful way to think about the effects of anti-inflammatory drugs is to visualize the flow of inflammatory mediators toward the site of injury as a river. As more immune cells gather downstream, they will release additional inflammatory mediators, which will build up the total level of mediators. This accumulation of mediators can be thought of as forming a reservoir. At a certain concentration, inflammatory mediators will maintain normal levels of pain sensation. Once this concentration is exceeded however, pain sensitization begins. The effects of opioids and local anesthetics can be thought of as a dam placed in the middle of the river. Without a certain concentration of inflammatory mediators, pain sensations will be cut off altogether. As mentioned above, this is a particularly dangerous scenario. The effects of NSAIDs can be thought of as a dam placed at the mouth of the reservoir. It prevents additional inflammatory mediators from joining the "river," which prevents pain sensitization while maintaining normal levels of pain sensation. This scenario is ideal because physiologically beneficial acute pain remains, whereas harmful chronic pain is prevented.

Inflammatory mediators, such as cytokines and chemokines, can induce pain as pro-nociceptive mediators (Fig. 4.1). When pro-nociceptive mediators bind to receptors on nociceptors (see Sect. 4.2), peripheral sensitization can occur. As such, the release of large concentrations of inflammatory mediators may result in chronic inflammation that contributes to chronic pain. Notably, cytokines and chemokines use different types of receptors for pain signaling (Abbadie et al. 2009).

4.2 Nociceptors

Specialized sensory neurons called nociceptors sense pain by detecting noxious stimuli (Gold and Gebhart 2010; Julius and Basbaum 2001; Woolf and Ma 2007). German physiologist von Frey first suggested that pain in the skin originates from nerve endings extending from the original nerve. Sherrington, an English physiologist, through his experiments stimulating the spinal reflex, first proposed the concept of the "noci-receptor" ("noci" meaning injury in Latin), a primary sensory nerve responsible for detecting harmful stimuli. Sherrington's proposal led to the search for the role of the afferent fibers (Aδ- and C-fibers) in the dorsal root ganglion (DRG) and trigeminal ganglion (TG) in regulating primary sensory nerves in the skin, muscles, joints, and GI tract. These fibers were shown to respond to injurious mechanical, thermal, and chemical stimuli. As such, the primary sensory nerves, which convert harmful stimuli to electrical signaling (e.g., action potentials), are referred to as nociceptors. In general, it is believed that thin afferent fibers including myelinated Aδ-fibers and unmyelinated C-fibers are responsible for conducting fast and slow pain responses, respectively. Aδ-fiber-mediated fast pain responses are defined by high-speed transmission, high specificity of location, and sharp pain;

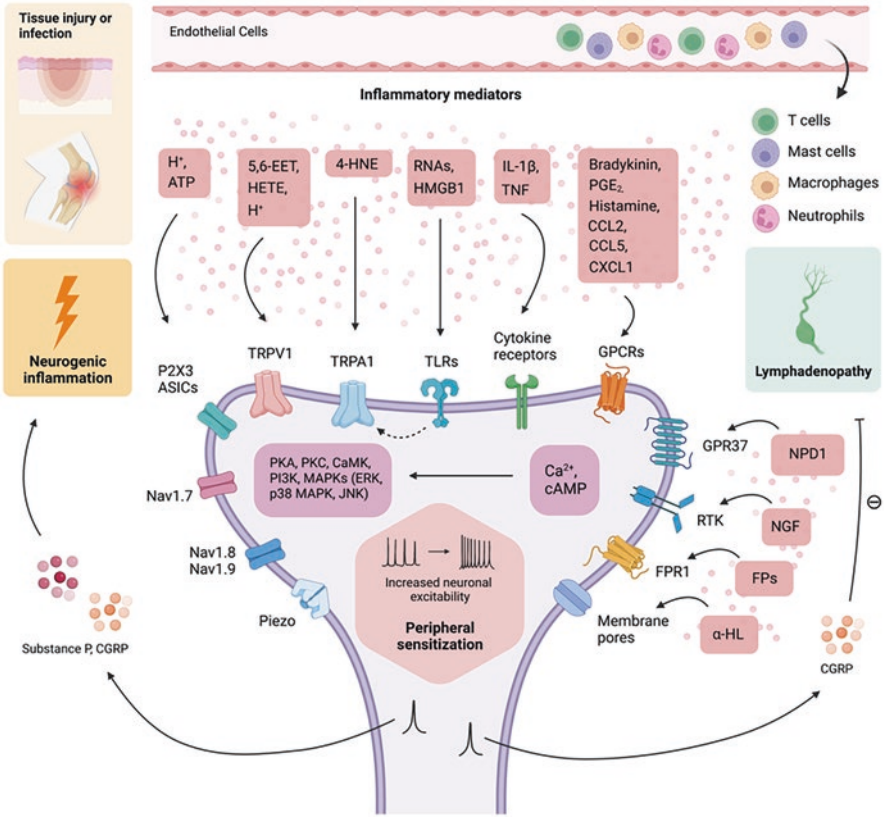

Fig. 4.1 Inflammation evokes pain via inflammatory mediators, their interactions with nociceptor terminals, and peripheral sensitization. Tissue injury and infection cause inflammation, leading to plasma extravasation and infiltration of immune cells to the inflamed tissue. The infiltrated and resident immune cells include mast cells, macrophages, neutrophils, and T-cells. These immune cells and keratinocytes release various inflammatory mediators, such as bradykinin, prostaglandins (PGE2), proton (H^+), adenosine triphosphate (ATP), and nerve growth factors (NGF), as well as pro-inflammatory cytokines (tumor necrosis factor [TNF], interleukin [IL]-1β, IL-6, IL-17, and IL-23) and pro-inflammatory chemokines (CCL2, CXCL1, CXCL5). Importantly, nociceptor neurons express specific receptors for these inflammatory mediators, which activate their respective receptors on nociceptor terminals. These receptors include G-protein-coupled receptors (GPCRs), ionotropic receptors, and tyrosine kinase receptors. Upon activation of these receptors, several second messengers such as Ca^{2+} and cAMP are generated for the subsequent activation of several kinases, such as mitogen-activated protein kinases (MAPKs; including extracellular signal-regulated kinase [ERK], p38, and c-Jun N-terminal kinase [JNK]), protein kinases A and C (PKA, PKC), calmodulin-dependent protein kinase (CaMK), and phosphoinositide-3 kinase (PI3K). Following activation, these kinases cause hypersensitivity and hyperexcitability of nociceptor neurons, termed as peripheral sensitization, through modulation of key ion channels, which include transduction molecules such as transient receptor potential (TRP) ion channels A1 and V1 (TRPA1, TRPV1, TRPV4) and Piezo-2 (a stretch-activated ion channel) and key conduction molecules such as the voltage-gated sodium (Nav) channels Nav1.7, Nav1.8, and Nav1.9. Nociceptor neurons also express functional toll-like receptors (TLRs; e.g., TLR3, TLR4, TLR7/TLR8), which can be activated by exogenous ligands (known as pathogen-activated molecular patterns [PAMPs], which

C-fiber-mediated slow pain responses are defined by delayed transmission, low specificity of location, and burning pain. Nociceptors have a great deal of molecular and functional diversity in primary sensory neurons in dorsal root ganglion (DRG), trigeminal ganglion (TG), and glossopharyngeal ganglion. Recent progress also shows that in addition to noxious thermal, mechanical, and chemical stimuli, blue light can artificially induce pain while green light may suppress pain when nociceptors express light-sensing ion channels (Iyer et al. 2014; Ji et al. 2021).

The best-known marker for C-fiber nociceptors is from their response to capsaicin, an active component in chili peppers. A landmark discovery in the pain research field was the identification of the capsaicin receptor TRPV1 (transient receptor potential ion channel subtype V1) by a group of scientists from the University of California, San Francisco (Caterina et al. 1997). This landmark discovery led to a recent Nobel Prize for Physiology and Medicine (Ji and Lee 2021). TRPV1 is specifically expressed in C-fiber nociceptors and responsible for nociception, especially heat pain and inflammatory pain (Caterina et al. 2000). TRPA1 (transient receptor potential ion channel subtype A1) is also expressed by nociceptors and activated by mustard oil (wasabi) and mediates pain by inflammatory mediators and very cold temperature, while TRPM8 is activated by mint and mediates cooling sensation (Patapoutian et al. 2009; Bautista et al. 2007). Another ion channel, called Piezo, discovered by Dr. Ardem Patapoutian's group, mediates mechanical pain in nociceptors (Kim et al. 2012) (Fig. 4.1). Dr. Patapoutian and Dr. David Julius shared the 2021 Nobel Prize for Physiology and Medicine for their discovery of TRPV1 and Piezo proteins (Ji and Lee 2021).

Voltage-gated sodium (Nav) channels are critical for the initiation and propagation of action potentials in nociceptors, and, therefore, are the major targets of analgesics, such as local anesthetics (Cummins et al. 2000; Bennett and Woods 2014; Jiang et al. 2014; Brigham et al. 2021). So far, nine subtypes of sodium channels (Nav1.1–Nav1.9) have been identified; each subtype plays a distinct physiological role and exhibits distinct expression patterns in DRG neurons. Nav1.7–Nav1.9 are mainly expressed in DRG nociceptors and contribute to the genesis of pathological pain (Cummins et al. 2000; Waxman et al. 1999; Bao 2015; Pan et al. 2016; Amaya et al. 2006) (Fig. 4.1).

Nociceptor classification has been greatly improved by single-cell RNA-sequencing (RNA-seq) (Usoskin et al. 2015). Using transcriptional profiling analysis at whole population and single-cell levels, Chiu et al. initially revealed molecular

Fig. 4.1 (continued) include viral and bacterial components) and endogenous ligands (known as danger-activated molecular patterns [DAMPs], such as RNAs/DNAs). Certain miRNAs (e.g., let-7b) serve as novel pain mediators to activate nociceptors via TLR7, which is coupled with TRPA1 (Donnelly et al. 2020a). Bacterial infection (e.g., *Staphylococcus aureus*) can also directly activate nociceptors and increases neuronal excitability via releasing bacterial *N*-formylated peptides (FPs) and the formation of pore-forming toxin α-hemolysin (α-HL) (Chiu et al. 2013). Activation of nociceptors also releases neuropeptide substances P and CGRP, which are involved in the generation of neurogenic inflammation. CGRP also negatively regulates lymphadenopathy after inflammation. Notably, some cytokines such as IL-23 and CXCL5 activate nociceptors via indirect mechanisms. (Modified from fig. 1 of Ji et al. 2014; *Springer Nature*, authors copyright)

diversity for six distinct groups of mouse DRG neurons (Chiu et al. 2014). Usoskin et al. employed unbiased single-cell RNA sequencing (RNA-seq) and identified 11 types of mouse DRG neurons (~3600 genes per cell), which includes 6 principal types of nociceptive neurons (Usoskin et al. 2015). Using high-coverage single-cell RNA-seq (~11,000 genes per neuron), together with functional characterization, Li et al. identified 10 main types and 14 subtypes of mouse DRG neurons (Li et al. 2016). Deep sequencing of eight DRG neuron subtypes using individual mouse genetic lines revealed differentially expressed and functionally distinct genes in nociceptor subtypes (Zheng et al. 2019). This approach demonstrates that nociceptor neurons also express immune genes such as toll-like receptors (TLRs) (Donnelly et al. 2020a, b).

4.3 Nociceptor Sensitization: Peripheral Sensitization

Some inflammatory mediators, such as bradykinin and endothelin, may be sufficient to activate the peripheral nerve endings of nociceptors, which express the appropriate receptors for these mediators to generate action potentials, leading to spontaneous pain. Most inflammatory mediators (e.g., PGE2), however, only change sensory neuron sensitivity and excitability, without evoking action potentials. These changes include early posttranslational changes in nociceptors termed "peripheral sensitization." Voltage-gated sodium (Nav) ion channels play a critical role in the initiation and propagation of action potentials in excitable cells such as neurons and are the major targets of analgesics (Cummins et al. 2000; Bennett and Woods 2014).

4.3.1 Role of Ion Channels

So far, nine subtypes of Nav (Nav1.1–Nav1.9) have been characterized; each subtype has a distinct physiological role in sensory neurons and displays distinct and overlapping expression patterns in DRG neurons. Importantly, Nav1.7–Nav1.9 are expressed by small nociceptive neurons in DRGs (Cummins et al. 2000; Waxman et al. 1999; Amaya et al. 2006). Human genetic studies have revealed a pivotal role of Nav1.7 in human pain sensation. Loss-of-function mutations in SCN9A, the gene that codes for Nav1.7 in humans, result in a congenital inability to sense pain and anosmia but have no effects on sensations of touch and temperature (Cox et al. 2006; Weiss et al. 2011). Conversely, gain-of-function mutations cause episodic pain including primary erythromelalgia and paroxysmal extreme pain disorder (Drenth et al. 2001; Fertleman et al. 2006). TRP channels, such as TRPV1, TRPA1, and TRPV4 are expressed by nociceptor neurons and play crucial roles in peripheral sensitization and the generation of pain (Moore et al. 2018).

Nociceptor sensitization (peripheral sensitization) may be the most direct cause of pain hypersensitivity and the most obvious target for pain therapeutics (Hucho and Levine 2007). A unique feature of nociceptors is their high threshold for activation under the normal conditions. However, following inflammation or repeated exposure to noxious stimuli, the nociceptor's activation threshold dramatically drops such that lower-intensity stimuli can now initiate activation (e.g., discharges) in the nociceptors. This peripheral sensitization, which can be detected within a very short period, is the result of changes either in the transduction receptor molecules (e.g., TRP channels) themselves or in sodium channels in the nociceptor terminal (Fig. 4.1). A change in the transducer molecule is best exemplified by the capsaicin receptor TRPV1, where repeated heat stimuli cause a progressively augmenting inward current through the ion channel, which can also be induced by exposure to protons. The other key event of peripheral sensitization is phosphorylation of membrane-bound receptors/ion channels by enzymes. For example, many inflammatory mediators activate either protein kinase A (PKA) or protein kinase C (PKC); both have been shown to phosphorylate the tetrodotoxin-resistant (TTXr) sodium channels (Nav1.8–1.9) and contribute to a greater sodium current in the terminal and axon (Gold and Gebhart 2010; Woolf and Costigan 1999).

4.3.2 Transcriptional and Translational Regulation

Transcription (mRNA expression) and translation (protein expression) are highly regulated in the cell soma of sensory neurons. Inflammation results in an increase in peripheral nerve growth factor (NGF) levels, and NGF is a key signal molecule for many of the transcriptional and translational changes in sensory neurons (Ji et al. 2002; Nicol and Vasko 2007). Although transcription is not required for peripheral sensitization, an increase in the substrate for such sensitization (e.g., TRPV1 and Nav1.7–1.9) is very likely to amplify the phenomenon. Because of the delay (hours to days for gene transcription) in the initiation of transcriptional changes in the expression and transport of signaling proteins (NGF, TRPV1, and Nav1.7–1.9), such transcription-dependent augmentation can only occur many hours after inflammation (Woolf and Costigan 1999).

While nociceptor sensitization typically refers to peripheral sensitization, it is important to point out that nociceptors also project to the spinal cord and the trigeminal nucleus in the brain stem, and all the machinery for inducting peripheral sensitization in the peripheral nociceptor terminals are also present in the central terminals of nociceptors (Gold and Gebhart 2010). Thus, nociceptor terminals may also contribute to central sensitization via presynaptic modulation in spinal cord neurons. A recent study suggests that the Nav1.7 subtype of sodium ion channels in the central terminal of a nociceptor may play a more important role in pain sensitization (MacDonald et al. 2021).

4.3.3 Protein Kinase Signal Transduction Pathways

Protein kinase A and protein kinase C (PKA and PKC) play important roles in nociceptor activation and peripheral sensitization by posttranslational regulation of sodium channels (Nav1.8) and TRPV1 (Aley and Levine 1999; Bhave et al. 2003; Gold and Gebhart 2010; Gold et al. 1998; Julius and Basbaum 2001). Mitogen-activated protein kinases (MAPKs) are crucial for several functions, including signal transduction, neural plasticity regulation, and inflammatory response. This family of kinases has three primary members: extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinase (JNK), and p38 (Ji et al. 2009a). These kinases represent three different signaling pathways. Increasingly, studies point to the involvement of these three pathways in pain sensitization following tissue and nerve injury, through both molecular and cellular mechanisms. Under a variety of chronic pain conditions, phosphorylation (and thus activation) of MAPKs results in persistent pain hypersensitivity through both transcriptional and nontranscriptional regulation. ERK activation in the spinal cord dorsal horn neurons regulates the activity of glutamate receptors and potassium channels and induces gene transcription, thus playing an indispensable role in central sensitization (Ji et al. 2009a, b). Following nerve injury, ERK, JNK, and p38 are separately activated in spinal glial cells, resulting in pro-inflammatory/pro-nociceptive mediator synthesis, which enhances and lengthens pain (Ji et al. 2009a, b). Inhibiting the MAPK pathways has been demonstrated to diminish inflammatory and neuropathic pain in a variety of animal models, making MAPK pathways notable research targets for developing pain therapies. However, while MAPK inhibitors can diminish allodynia and hyperalgesia in neuropathic and inflammatory pain models, they do not diminish pain in basal conditions. These results imply that MAPKs play a specific role in the production of pathological pain after tissue and nerve injury. Early MAPK regulation studies focusing on neuronal mechanisms after severe noxious stimulation and peripheral tissue inflammation revealed that neuronal activation of MAPKs in both nociceptive primary sensory neurons and spinal cord dorsal horn neurons is critical to the production of lasting peripheral and central sensitization (Ji et al. 1999, 2002; Dai et al. 2002). Furthermore, nerve injury and spinal cord injury (SCI) resulted in significant MAPK activation in spinal cord glial cells (e.g., injuries in peripheral nerves and spinal cord activated both p38 and ERK in spinal microglia) (Jin et al. 2003; Zhuang et al. 2005). Nerve injury can also activate JNK in astrocytes (Zhuang et al. 2006). Intriguingly, while nerve injury activates ERK in microglia within days (early-phase), it takes weeks to activate ERK in astrocytes (late-phase) (Zhuang et al. 2005). Combined with increasing evidence that points to the crucial involvement of glial cells in pain pathogenesis, it follows that the activation of MAPKs in glial cells is essential to the development of lasting neuropathic pain.

4.4 Human Nociceptors

Recent progress in pain research has generated human nociceptors by reprogramming fibroblasts; this amazing technique can recapitulate many aspects of human disease phenotypes in vitro to model "pain in a dish," which can accelerate the translation of pain research from animals to humans (Wainger et al. 2015; Chang et al. 2018). It is also worthwhile to mention that human and mouse nociceptors have key differences in terms of gene expression and sensory function (Davidson et al. 2014; Zhang et al. 2019; Tavares-Ferreira et al. 2022). Human genetic studies have revealed a crucial role of Nav1.7 (coded by SCN9A) in human sensation of pain. Loss-of-function mutations in SCN9A lead to a congenital inability to sense pain and anosmia without affecting other sensations such as touch and temperature (Cox et al. 2006; Weiss et al. 2011). By contrast, gain-of-function mutations result in episodic pain such as primary erythromelalgia and paroxysmal extreme pain disorder (Drenth et al. 2001; Fertleman et al. 2006). It was found that compared to mouse DRGs, human DRGs display higher expression ratio of Nav1.7 (Chang et al. 2018). Quantitative polymerase chain reaction (PCR) study of seven Nav subtypes expressed by DRG revealed that the human DRG has higher expression ratio of Nav1.7 (~50% of total Nav expression) but lower expression ratio of Nav1.8 (~12% of total Nav expression). In sharp contrast, the mouse DRG has higher expression ratio of Nav1.8 (~45%) and lower expression ratio of Nav1.7 (~18%) (Chang et al. 2018). Consistently, Nav1.7 expression and function are enhanced in both rodent and human DRG neurons by the chemotherapy drug paclitaxel (Chang et al. 2018; Li et al. 2018), as well as in nociceptor neurons of neuropathic pain patients (Li et al. 2018). Recent progress also demonstrates the expression of autism gene SHANK3 in human sensory neurons including nociceptors, which has been implicated in pain and touch dysregulation in autism patients (Han et al. 2016; Orefice et al. 2019). Notably, as a scaffold protein, SHANK3 regulates the surface expression of TRPV1 in nociceptors. Partial knockdown of SHANK3 with siRNA blocked the TRPV1 function in human DRG neurons (Han et al. 2016). Another example is programmed death protein-1 (PD-1, encoded by PDCD1), which is typically expressed by immune cells. PD-1 is a notable target of immune therapy for treating cancers (Topalian et al. 2012). Electrophysiological studies revealed that functional PD-1 is present in human DRG neurons, and activation of PD-1 by its ligand PD-L1 can powerfully silence human nociceptor firing. Thus, the PD-L1/PD-1 axis may act as a novel pain modulatory system in humans (Chen et al. 2017). However, the distinction of the peptidergic and nonpeptidergic nociceptors in human DRG is not as obvious as in mouse DRG (Shiers et al. 2020). The same is true for opioid receptor subtypes in mouse and human nociceptors (Moy et al. 2020; Scherrer et al. 2009).

4.5 Classic Inflammatory Mediators

The eicosanoids include arachidonic acid, which is the main component of membrane phospholipids and one of the key substrates for eicosanoid synthesis. Eicosanoids are biologically active mediators of inflammation and processed by several enzymes, including 5-lipoxygenase (5-LOX for leukotriene and 5-hydroxyeicosatetraenoic acid), cyclooxygenases (COX for prostaglandins and thromboxanes), and 12-1ipoxygenase (12-LOX for 12-hydroxyeicosatetraenoic acid). The 5-LOX is mainly created in myeloid immune cells, including mononuclear cells (e.g., lymphocytes) and polymorphonuclear leukocytes (e.g., neutrophils and eosinophils), cells that play indispensable roles in immune responses during inflammatory reactions. Notably, however, 5-LOX may not be present in other immune cells and components such as erythrocytes, platelets, endothelial cells, and T-cells. COX is an enzyme that participates in the synthesis of proteinoids, such as powerful pro-inflammatory prostaglandins and metabolism of arachidonic acid. Prostanoids, produced by cyclooxygenase-l (COX-1), are important in many physiological functions including regulation of platelet aggregation. In contrast, prostaglandin E2 has many regulatory functions important to inflammation including increasing vascular permeability and strengthening the effects of other inflammatory mediators such as kinin, serotonin, and histamine, which is exemplified in the redness, increased blood flow, and edema, which are characteristic signs of inflammation. Prostaglandin E2 (PGE2) also acts on neurons in the thermoregulatory network of the hypothalamus, causing an increase in body temperature.

PGE2 is the primary pro-inflammatory prostanoid and plays a critical role in the genesis of inflammatory pain. At the site of inflammation, PGE2 sensitizes peripheral nociceptors through GPCRs, PKA, and PKC (Gold et al. 1998). Four subtypes of GPCRs are identified as PGE2 receptors (EP1, EP2, EP3, and EP4). EP4 expression in nociceptors regulates peripheral sensitization and drives inflammatory pain (Lin et al. 2006). PGE2 is also produced in the spinal cord after tissue injury and inflammation and contributes to acute and persistent inflammatory pain (Samad et al. 2001; Malmberg and Yaksh 1995).

Additional important mediators include the vasoactive amines and peptides and adenosine triphosphate (ATP). Vasoactive amines and peptides include histamine, a few picograms of which are released by basophils to maintain acute-phase response during inflammation events. Serotonin, another type of amine, is produced via decarboxylation of tryptophan and stored in granules. Serotonin is found in basophilic granules in mice and in platelets in humans and is mediated by four serotonin receptors (5-$HT_{l\text{-}4}$). Another member of this category of mediators is bradykinin, a nanopeptide created from plasma from the Kinin–Kallikrein system. Bradykinin has two receptors, B1 and B2, which can increase synthesis of prostaglandins and produce pain locally. Recently, it was found that sensitization of nociceptors by bradykinin requires TRPV1 and TRPA1 association (Patil et al. 2020). ATP can activate and sensitize nociceptors via specific P2X and P2Y receptors. P2X3 is expressed by nociceptors and plays an important role in nociceptor sensitization (Chen et al. 1995).

4.6 Cytokines

Cytokines are small proteins secreted by a variety of cells, including leukocytes and certain cells of the nervous system. Cytokines are produced as the body requires, generally at low concentrations (ranging from a couple picograms to nanograms/mL), and act locally (as they are unable to travel long distances). Their low concentrations are not a problem; the activation of a few dozen receptors per cell can be enough for some cytokines to generate an effect. Cytokines serve many different functions, including affecting immune cells and controlling inflammatory responses. Often many different cytokines will serve the same functions. Cytokine involvement has been identified in almost every disease condition, including Crohn's disease, multiple sclerosis, rheumatoid arthritis, and sepsis. Scientists have long suspected cytokine involvement in the production of pain and hyperalgesia (an irregular increased sensitivity to pain), as cytokines play an enormous role in connecting the immune and nervous system (Sommer and Kress 2004; Kalpachidou et al. 2022).

Inflammatory pain, to recap, is defined by a heightened sensitivity to heat or mechanical pain. After tissue injury, resident immune cells produce the initial inflammatory response, the effects of which are then magnified by blood cells that have been attracted to the area. The effect of cytokines on hyperalgesia has been well demonstrated. In 1988, Ferreira et al. reported that IL-1 beta (IL-1β) is a potent hyperalgesic agent after systemic injection due to a peripheral action (Ferreira et al. 1988). Rats administered intraplantar injections of the cytokines IL-1 beta (IL-1β) and TNF were observed to have lower mechanical pain thresholds (Sommer et al. 1997; Homma et al. 2002).

4.6.1 Pro-inflammatory Cytokines

Pro-inflammatory cytokines can be released by various cells, including stromal cells, fibroblasts, endothelial cells, and glial cells, as well as neurons. In general, cytokines play crucial roles in cellular activity. In inflammation, they are especially important as they regulate the immune system. Some of the best-known pro-inflammatory cytokines that contribute to the inflammatory response include IL-1β, tumor necrosis factor alpha (TNF-α), IL-6, and IL-17, IL-18, and IL-23 (Fig. 4.1). An initial inflammatory response eliminates the cause of infection, limiting the area of tissue damage. This action causes an increase in macrophage-derived cytokine concentrations in the plasma. In turn, these cytokines affect other organs, especially the brain and liver, leading to the acute-phase response, or a systemic immune response.

TNF-α has been called the "prototypic pro-inflammatory cytokine" due to its important role in initiating the activation cascade of other cytokines and growth factors during an inflammatory response. This cytokine is produced by several types of

cells following injury, including immune cells and glial cells (e.g., Schwann cells, microglia). Local injection, such as intraplantar, intramuscular, and intraneural injections of TNF-α, results in mechanical allodynia and thermal hyperalgesia, while subcutaneous injection decreases the threshold of mechanical activation in C-fiber nociceptors in the rat sural nerve (Schäfers et al. 2003a). TNF-α involvement has been identified in the production and continuation of neuropathic pain, and conversely blocking TNF-α significantly decreases hyperalgesia in rodent models of painful neuropathy (Sommer and Kress 2004). TNF-α induces the expression and activation of various pro-inflammatory cytokines, including IL-1β, IL-6, and IL-8; this cascade of cytokine activation also leads to the release of COX-2-dependent prostanoids, which mediate inflammatory reactions. However, TNF-α is not limited to indirect effects. Perfusion of TNF-α on DRGs in vitro caused neuronal discharges in both A- and C-fibers (Homma et al. 2002; Schäfers et al. 2003b). This result demonstrates TNF-α's ability to sensitize injured sensory neurons. Moreover, DRG neurons attached to injured afferents along the same peripheral nerve also became more sensitive. Additionally, directly injecting TNF-α into rat DRGs in vivo resulted in allodynia at far lower doses than perfusion, causing rapid induction of allodynia. This finding in turn confirms the ability of TNF-α to sensitize injured nerve fibers due to its potent excitatory effects (Schäfers et al. 2003b). It is likely that these sensitizing effects result from activation of protein kinases mediated by TNF-α receptors, including TNFR1 and TNFR2, that are expressed by sensory neurons (Constantin et al. 2008; Jin and Gereau 2006; Park et al. 2011a).

IL-1β is shown to produce hyperalgesia, or hypersensitivity to pain, in rodents. Its release is also correlated with increased neuropathic pain following nerve injury. The activation and signaling of this cytokine can be attributed to caspase-1, as well as matrix metalloproteinases (MMP-9 and MMP-2), enzymes that usually function in breaking down the extracellular matrix but are also involved in disease conditions such as arthritis (Ji et al. 2009a, b). Meanwhile, the release of IL-1β can be credited to the activation of the NLRP3 inflammasome, which is responsible for a form of inflammatory cellular apoptosis and the release of pro-inflammatory cytokines (Donnelly et al. 2020a, b). Upon release, IL-1β potently controls both excitatory and inhibitory synaptic transmission in the spinal dorsal horn (Kawasaki et al. 2008b). Such control allows it to induce pain on several fronts. First, it increases the excitability of nociceptors, meaning stimuli are more likely to activate nociceptors than before and thus more likely to induce pain. Secondly, IL-1β increases the activity of NMDAR (N-methyl-D-aspartate receptor) receptors, proteins crucial to synaptic plasticity. And thirdly, IL-1β decreases inhibitory synaptic transmission (inhibitory postsynaptic currents, IPSC) and currents induced by GABA (gamma aminobutyric-acid) and glycine, whose actions are associated with pain inhibition, which in sum further lowers the body's ability to inhibit pain signals. IL-1β's actions are blocked by IL-1 receptor antagonists (IL1-Ra); however, IL1-Ra is frequently downregulated in chronic pain conditions. Spinal IL-1β has been shown to play a role in both inflammatory pain hypersensitivity and central sensitization (Watkins et al. 2001). IL-1β can cause transcriptional regulation that upregulates COX-2, an enzyme that plays a significant role in inflammation and pain. For example,

inflammation in the hind paw of rats can result in the widespread increase of COX-2 in the CNS; this may be due to the increase of IL-1β in the cerebrospinal fluid (CSF) (Samad et al. 2001). As such, IL-1β, along with the pro-inflammatory cytokines TNF (produced by microglia, astrocytes, and even DRG primary sensory neurons) and IL-6, can directly lead to central sensitization. All three can rapidly (within a minute) increase excitatory synaptic transmission in spinal cord neurons by acting on neurotransmitter receptors, including AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor), NMDAR (NMDA receptor), GlyR (glycine receptor), and GABAR (GABA receptor). These actions enhance excitatory synaptic transmission and prevent spinal pain circuit inhibitory synaptic transmission. Additionally, the intrathecal injection of IL-1β, TNF, and IL-6 can quickly produce pain hypersensitivity in otherwise normal animals. Thus, it is possible that increased levels of cytokine circulation in the CSF can mediate widespread central sensitization (Ji et al. 2018).

IL-18 is a cytokine related to IL-1β, and it is also activated by caspase-1. IL-18 drives neuropathic pain and cancer via neuron–glial and glia–glial interactions (Miyoshi et al. 2008; Yang et al. 2015). Nerve injury induces significant increases in spinal cord expression of IL-18 and IL-18R in different types of glial cells: IL-18 is induced in microglia while IL-18R is upregulated in astrocytes. The functional inhibition of IL-18 signaling pathways has been found to suppress injury-induced tactile allodynia in rats (Miyoshi et al. 2008). Microglia have been found to maintain advanced-stage bone cancer pain through IL-18 signaling, which increases the transmission of pain signals to spinal cord nociceptive neurons. Bone cancer was found to increase ATP levels in cerebrospinal fluid, leading to the activation of the P2X7 receptor and subsequent phosphorylation of p38 MAP kinase. p38 activation further results in IL-18 production in spinal microglia. Inhibition of the P2X7/p-38/IL-18 in the spinal cord, however, diminishes advanced-stage bone cancer pain and suppresses the hyperactivity of spinal wide dynamic range (WDR) neurons (Yang et al. 2015).

IL-6 is upregulated during various disease conditions connected with heightened levels of hyperalgesia and pain (Kalpachidou et al. 2022). Higher serum levels of IL-6 have been seen in patients with autoimmune and chronic inflammatory conditions, burn injuries, malignant tumors, musculoskeletal disorders, and neuropathies. Additionally, treatment with IL-6, via intrathecal, intracerebroventricular, or intraplantar injection, has been shown to result in allodynia or hyperalgesia in rats. Research points to IL-6 being both a pro-inflammatory and pro-nociceptive cytokine. The cytokine induces persistent nociceptor sensitization by increasing protein synthesis in primary sensory neurons (Melemedjian et al. 2010). Furthermore, IL-6 relies on the presence of its receptor, soluble IL-6 receptor (sIL-6R), to exert its effects on neurons. The complex between IL-6 and sIL-6R binds to and activates cells expressing gp130 (also known as IL-6β), which makes up a part of the IL-6 receptor and is crucial for signal transduction (Kummer et al. 2021). Notably, brief exposure of the IL-6/sIL-6R complex is sufficient to modulate nociceptor release of calcitonin gene-related peptide (CGRP). CGRP, when released in the brain, results

in tremendous inflammation of the meninges, leading to severe migraines in females (Avona et al. 2019).

IL-17, especially IL-17A, has been shown to promote inflammatory and neuropathic pain. IL-17 receptor (IL-17R) is expressed by sensory neurons and a single injection of IL-17A into the rat knee joint elicited long-lasting sensitization of C-fiber nociceptors of the joint, through ERK activation in nociceptors, leading to arthritic pain (Richter et al. 2012; Luo et al. 2021). In the spinal cord, IL-17 is produced by astrocytes, which drives neuropathic pain arising from chemotherapy treatments, through suppression of inhibitory synaptic transmission. After knockdown of IL-17R in spinal cord interneurons expressing somatostatin, mechanical hypersensitivity arising from paclitaxel was decreased. Moreover, the overexpression of IL-17 was enough to cause mechanical allodynia in otherwise untreated animals (Luo et al. 2019). IL-17 drives mechanical pain in a female-dominant manner (Luo et al. 2021). Notably, IL-17 can directly sensitize nociceptor neurons in different species, including mice, nonhuman primates, and humans, via TRPV1 activation. The IL-17-induced nociceptor sensitization is also female dominant (Luo et al. 2021).

IL-23 is an upstream cytokine of IL-17, as it can induce IL-17 expression in T-cells and macrophages. IL-23 has been implicated in psoriatic arthritis and inflammatory pain (Luo et al. 2021; Lee et al. 2022). Interestingly, IL-23-evoked mechanical pain is sex-dependent following intraplantar administration: IL-23 only produces mechanical pain (mechanical allodynia) in female mice but not male mice (Luo et al. 2021). Furthermore, chemotherapy-induced mechanical pain is selectively impaired in female knockout mice lacking *Il23* or *Il23r*. IL-23-induced pain can be promoted by estrogen but suppressed by androgen, suggesting that the sex hormones of both males and females are involved. Deletion of estrogen receptor subunit α (ERα) in TRPV1+ nociceptors could abolish IL-23- and IL-17-induced pain in females (Luo et al. 2021). It was found that IL-23 induces mechanical pain via macrophage–neuron interaction: IL-23-evoked pain is abolished after ablation of C-fiber nociceptors. Surprisingly, IL-23 does not directly activate nociceptor neurons. Instead, IL-23 requires IL-17A release from macrophages to evoke mechanical pain in females (Luo et al. 2021). A follow-up study used an optogenetic approach in transgenic mice expressing channelrhodopsin-2 (ChR2) in TRPV1-positive nociceptive neurons and demonstrated that IL-23 enhanced blue-light-induced pain in female mice. IL-23 also induced greater p38 MAPK activation in sensory neurons, a marker for nociceptor activation, in female mice compared to male mice (Ji et al. 2021).

4.6.2 Anti-inflammatory Cytokines

TGF-β1 and IL-10 are two prominent anti-inflammatory cytokines and have exhibited anti-hyperalgesia and anti-allodynia in animal models of chronic pain (Buchheit et al. 2020). TGF-β1 powerfully inhibits neuropathic pain through inhibiting glial

activation in the spinal cord and neuroinflammation following nerve injury (Echeverry et al. 2009). Interestingly, it can also activate its own receptors on neurons. Such an ability proves especially powerful, allowing TGF-β1 to suppress the hyperexcitability caused by nerve injury in the DRG and spinal cord in mere minutes (Chen et al. 2015). TGF-β1 is downregulated by nerve injury and negatively regulated by miR-30c-5p; nerve-injury-induced upregulation of miR-30c-5p in cerebrospinal fluid, DRG, plasma, and spinal cord makes it especially difficult for TGF-β1 to exert its anti-nociceptive effects. Inhibitors of miR-30c-5p have been demonstrated to upregulate TGF-β1 and attenuate neuropathic pain following nerve injury (Tramullas et al. 2018).

IL-10 is probably the most-studied anti-inflammatory cytokine in pain research. This is unsurprising, considering its numerous intriguing effects. Notably, nerve injury fails to induce pain in young animals (<1 month). It was found that IL-10 essentially inhibits nerve-injury-induced neuropathic pain in the early life of mice via mediating anti-inflammatory neuroimmune regulation in the spinal cord (McKelvey et al. 2015). IL-10-based gene therapy via intrathecal delivery successfully produced lasting pain relief by increasing the endogenous production of this cytokine (Ledeboer et al. 2007). Furthermore, endogenous IL-10 involvement was shown in various pain-relieving activities, including inflammatory pain, neuropathic pain, acupuncture, exercise, and transplantation of CD8+ T-cells. IL-10 further inhibits nociceptor excitability following paclitaxel treatment in vitro (Buchheit et al. 2020; Krukowski et al. 2016).

IL-4, like IL-10, inhibits nerve-injury-induced neuropathic pain in the early life of mice. Nerve injuries in infants have been shown to upregulate this cytokine, which could mask pain in early life (McKelvey et al. 2015). However, nerve injury in adult mice was shown to decrease IL-4 concentrations in the spinal cord, offering some insight into why adults have more difficulty recovering from injuries. Notably, analgesia mediated by IL-4 can also be induced by low-intensity exercise. Such a mechanism makes low-intensity exercise another good option for alternative pain treatment (Bobinski et al. 2018).

4.6.3 Interferons

Interferons (IFNs) are cytokines that possess antiviral, antiproliferative, and immunomodulatory properties (Tan et al. 2021). IFNs can be divided into three classes: type-I, type-II, and type-III IFNs. IFN-α and IFN-β are two major family members of type-I IFNs and have been used to treat human diseases such as hepatitis and multiple sclerosis. IFN-γ is the only family member of type-II IFN family. Type-III IFNs are also known as lambda-IFNs. IFN receptors are expressed by immune cells and glial cells. Emerging evidence suggests that type-I IFN receptors (IFNARs) are also robustly expressed in the peripheral nervous systems including DRG neurons (Donnelly et al. 2021; Tan et al. 2022). It is generally believed that IFNs regulate biological actions by transcriptional regulation. Apart from these canonical

regulations, low doses of IFN-α and IFN-β suppress pain under the physiological and pathological conditions. Type-I IFN can rapidly suppress nociceptor neuronal activity and synaptic transmission via nongenomic regulation, leading to potent analgesia (Tan et al. 2021). It is also possible that at high doses, type-I IFN may promote pain via translational regulation or IFNAR-independent actions (Barragán-Iglesias et al. 2020). In contrast to type-I IFNs, type-II IFN is pro-nociceptive. IFN-γ drives neuropathic pain through microglial activation (Tsuda et al. 2009). Recently, it was found that activation of STING (stimulator of IFN gene) produces long-term pain relief via induction of type-I IFNs in DRG sensory neurons and spinal cord glial cells (Donnelly et al. 2021).

4.7 Chemokines

Chemokines are small cytokines that cause inflammatory cells to migrate in certain directions, both in vitro and in vivo. Chemokines consist of four main subfamilies: C, CC, CXC, and CX3C. Chemokines can be produced by immune and glial cells, as well as neurons including sensory neurons. These molecules function by GPCRs. Increasing evidence suggests that chemokines play critical roles in the pathogenesis of pain via neuron–immune and neuron–glial interactions (Gao and Ji 2010) and as integrators of pain and inflammation (White et al. 2005).

CCL2, also named monocyte chemoattractant protein-1 (MCP-1), has been strongly implicated in peripheral sensitization via activation of CCR2. CCR2 is expressed by nociceptors, and the activation of CCR2 drives inflammatory pain, neuropathic pain, and cancer pain, in part via MAP kinase-mediated activation of TRPV1 (White et al. 2005). It was found that CCL2 is upregulated within the local tumor microenvironment in a mouse model of bone cancer pain. Bone cancer also resulted in upregulation of CCR2 in primary sensory neurons, and, furthermore, CCR2 antagonism effectively reduced bone cancer pain (Wang et al. 2020). CCR2 is also expressed by macrophages in DRG, which causes macrophage activation and infiltration, in response to neuron-released CCL2 in chronic pain conditions, such as nerve trauma, inflammation, and chemotherapy-induced peripheral neuropathy (CIPN) (Liu et al. 2016; Zhang et al. 2016). Meanwhile, CCL2 is involved in both signaling between neurons and microglia, and signaling between astroglia and neurons, following nerve injury (Gao and Ji 2010). In the spinal cord, CCL2 is induced in astrocytes by nerve injury. By amplifying NMDA receptor activity in dorsal horn neurons, CCL2 can speedily produce central sensitization, which is critical to the maintenance of chronic pain (Gao et al. 2009). Nerve-injury-induced CCL2 release from primary sensory neurons was also implicated in the activation of spinal microglia in neuropathic pain (Zhang et al. 2007).

The chemokine receptor CX3CR1 is selectively expressed by microglia and upregulated after nerve injury. CX3CR1 signaling in microglia promotes neuropathic pain via p38 activation. CX3CL1 (fractalkine), the ligand of CX3CR1, is expressed by neurons (Milligan et al. 2004; Zhuang et al. 2007). Intrathecal

injection of a neutralizing antibody against CX3CR1 suppresses mechanical allodynia. Conversely, intrathecal infusion of CXCL1 (fractalkine) is sufficient to activate microglia and mechanical allodynia (Zhuang et al. 2007). CX3CR1 signaling in microglia also contributes to other chronic pain conditions such as arthritic pain. It is noteworthy that the CX3CL1/CX3CR1 signaling cascade also mediates critical physiological functions necessary for immune regulation and microglial homeostasis. The soluble form CX3CL1 mediates chemotaxis of immune cells, while the membrane-bound form of CX3CL1 acts as an adhesion molecule (Clark et al. 2007, 2011).

CXCL1, also known as keratinocyte-derived chemokine (KC), plays similar biological roles in rodents as IL-8 in humans. CXCL1 is expressed in macrophages and astrocytes after chemotherapy and nerve injury. CXCR2 is the major receptor of CXCL1 and present in primary sensory and spinal cord neurons. The CXCL1/CXCR2 pathway contributes to inflammatory pain and neuropathic pain via neuron–immune interactions in the DRG and neuron–glial interactions in the spinal cord (Ji et al. 2014; Luo et al. 2019; Zhang et al. 2013). Thus, both peripheral sensitization and central sensitization have been implicated in CXCL1/CXCR2-mediated pathological pain (Silva et al. 2017).

CXCL12, also known as stromal cell-derived factor-1 (SDF-1), appears to contribute to both analgesia and pain. Bone marrow stromal cells (BMSCs) have been found to produce analgesic properties. Interestingly, when BMSCs were transplanted into a mouse that had undergone chronic constriction injury (CCI), the cells targeted only lumbar DRGs that had sustained injury; these DRGs then produced CXCL12, which attracted BMSCs expressing CXCR4, the corresponding receptor for CXCL12 (Chen et al. 2015). CCI produced significantly higher CXCL12 levels in DRG than sham surgery did, showing that neuropathic pain leads to upregulation of CXCL12. Additionally, a transwell migration assay demonstrated that CXCL12 was able to cause BMSC migration in vitro, while AMD1300, a CXCR4 antagonist, prevented such migration. When BMSCs were implanted into mice that had suffered CCI, the cells were able to migrate to either injured lumbar DRGs or tissues of the spinal cord via the CXCL12/CXCR4 axis. Such an action allowed the BMSCs to survive for long periods of time in the tissues bearing upregulated CXCL12, producing long-term analgesia and neuroprotection. Improving the ability of the CXCL12/CXCR4 axis to deliver BMSCs may increase the effectiveness of the analgesia. However, CXCL12 and CXCR4 have also been shown to be involved in causing neuropathic pain via neuron–immune interaction in DRG (Bhangoo et al. 2007). Thus, the CXCL12/CXCR4 axis differentially regulates pain in different cell types.

CXCL13 is a B-lymphocyte chemoattractant that was shown to sustain neuropathic pain via CXCR5 activation in astrocytes (Jiang et al. 2016). After nerve injury, CXCL13 is persistently upregulated in spinal cord neurons, leading to the activation of spinal astrocytes via CXCR5. Furthermore, peripheral nerve injury increased CXCR5 expression in spinal astrocytes; and nerve-injury-induced astrocyte reaction and neuropathic pain is reduced in knockout mice lacking *Cxcr5*. Interestingly, spinal overexpression of miR-186-5p can decrease CXCL13 expression and alleviate neuropathic pain. Thus, targeting miR-186-5p or CXCL13/

CXCR5-mediated astrocyte signaling may be therapeutic targets for neuropathic pain. In addition to spinal cord mechanism, CXCL13 may also promote pain via peripheral sensitization of DRG and trigeminal sensory neurons. It was found that zinc finger protein 382 (ZNF382) in injured DRG neurons regulates CXCL13 expression in DRG neurons to promote neuropathic pain (Ma et al. 2021).

4.8 Specialized Pro-resolving Mediators (SPMs)

Specialized pro-resolving mediators (SPMs) are lipid mediators such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) derived from omega-3 unsaturated fatty acids (Fig. 4.2). Increasing studies have demonstrated potent

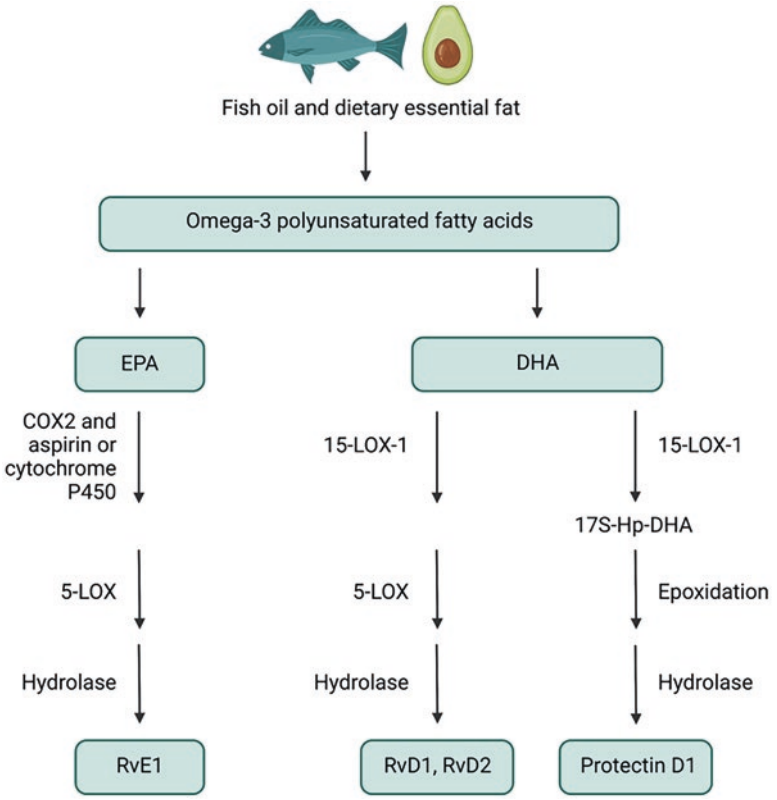

Fig. 4.2 Biosynthetic pathways of specialized pro-resolving mediators (SPMs) resolvins and protectin D1. Omega-3 polyunsaturated fatty acids include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), enriched in healthy diet and fish oil. Resolvins D1 and D2 (RvD1, RvD2) and protectin D1 (PD1, also known as neuroprotectin D1) are derived from DHA. Resolvin E1 (RvE1) is derived from EPA. The biosynthesis of these SPMs depends on several enzymes, including cyclooxygenase 2 (COX-2), cytochrome P450 (P450), and 5- and 15-lipoxygenase (5-LOX and 12-LOX)

analgesic actions of exogenous SPMs (Ji 2023). Work by Dr. Charles Serhan at Harvard Medical School and his colleagues and collaborators has demonstrated potent anti-inflammatory and pro-resolving effects of SPMs (Serhan 2014). SPMs belong to a rapidly expanding family of lipid molecules and could be generated during the resolution phase of inflammation. The SPM family includes D-series resolvins (RvD1-RvD6), E-series resolvins (RvE1-RvE5), protectin D1 (PD1), also called neuroprotectin D1 (NPD1), and maresins (MaR1, MaR2R), as well as cysteinyl-SPMs and n-3 docosapentaenoic acid (DPA)-derived SPMs (PD1n-3 DPA). Additionally, SPMs consist of ω-6 arachidonic-acid-derived lipoxins, such as lipoxin A4 and lipoxin B4 (LXA4 and LXB4). SPMs have demonstrated potent protective actions in rodent models of certain human diseases, including but not restricted to Alzheimer's diseases, arthritis, cancer, dry eye disease, infections, kidney injury, sepsis, and periodontal disease (Chiang and Serhan 2020).

4.8.1 Resolvins

Resolvins refer to a class of SPMs that come from omega-3 polyunsaturated fatty acids (PUFAs), which can be found in a variety of health products, including fish oil, krill oil, and algal oil. In this section, we will focus on the resolvin D series and E series, which are derived from docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), respectively.

Exogenous resolvins have powerful analgesic effects on inflammatory pain (Xu et al. 2010). These effects are in part directly anti-inflammatory and in part reliant on the modulation of neuron–immune interactions. Interestingly, RvE1 reduced inflammatory pain more than reducing the infiltration of leukocytes.

It was found that D series and E series resolvins contribute to the resolution of inflammatory pain through the regulation of TRP channels. TRP channels, especially the TRPV1 and TRPA1 channels, are important regulators of inflammatory pain (Julius and Basbaum 2001). The TRPV1 channel controls body temperature and produces a burning and painful sensation in response to capsaicin, a substance found in many spicy foods, as well as inflammation. The TRPA1 channel is responsible for sensing noxious and inflammatory stimuli that cause sensations of pain and itch and result in protective responses, like tear secretion, coughing, and scratching (Bautista et al. 2013).

In laboratory experiments using rodent models, it was found that an intraplantar injection of RvE1 prevented TRPV1-mediated pain caused by capsaicin but not by TRPA1-mediated pain caused by mustard oil, showing that RvE1 can inhibit TRPV1 but not TRPA1. Meanwhile, an intraplantar injection of RvD1 could block pain arising from TRPA1 but not TRPV1, showing that RvD1 can inhibit TRPA1 but not TRPV1. Additionally, RvD2 was shown to inhibit the actions of both TRPA1 and TRPV1 (Park et al. 2011a, b).

Resolvins have also exhibited powerful analgesic effects in rodent models with postoperative pain. Generally, postoperative pain after extended periods of muscle

retraction lasts 3–4 weeks in people and rodents. However, treatment with RvE1 and RvD1 was able to prevent this pain. Postoperative pain in the skin–muscle retraction model can result in mechanical hypersensitivity that lasts up to 4 weeks; intriguingly, a single RvD1 treatment was able to prevent this in rat models. Furthermore, intrathecal injection of resolvins was able to slow and prevent the development of chronic thoracotomy (a chest-opening surgery) pain. Treatment with RvD1 in animal models that already had chronic thoracotomy pain could only produce temporary pain relief, however. This suggests that the effects of resolvins change over time in such a model. Finally, posttreatment with intrathecal injections of RvD1 and RvD5 was also able to decrease mechanical and cold allodynia (Wang and Strichartz 2017; Ji 2023).

The analgesic effects of resolvins are not just limited to inflammatory pain and postoperative pain, however (Ji 2023). Several SPMs can also decrease neuropathic pain induced by nerve trauma and chemotherapy. For example, an intrathecal injection of RvE1 successfully decreased both neuropathic pain induced by chronic constriction injury (CCI) to the sciatic nerve and the reaction of microglia in the spinal cord. Some SPMs were also able to provide pain relief in chemotherapy-induced peripheral neuropathy (CIPN) models, where paclitaxel, a common chemotherapy drug, was used to induce neuropathic pain. It was found that intrathecal injection of RvD1 and RvD2 at 2 weeks could completely reverse paclitaxel-induced neuropathic pain (Luo et al. 2019a, b). RvD2 also displayed anti-cancer and pain-relief effects in mouse models with oral squamous cell carcinoma, with reduced tumor size by inhibiting pro-inflammatory cytokines (e.g., IL-6), reduced tumor necrosis, and temporary analgesia in xenograft (using outside tissue) squamous cell cancer models (Ye et al. 2018).

4.8.2 Protectin/Neuroprotectin D1 (PD1/NPD1)

Protectin D1 (PD1), also known as neuroprotectin D1 (NPD1), like its name implies, has powerful neuroprotective capabilities. It has been shown to preserve neural function and structure even in injured brains, oxidative-stressed retinal pigment cells, and human brain cells exposed to beta-amyloid peptides (a substance present in patients with Alzheimer's disease) (Lukiw et al. 2005).

In dissociated mouse DRG neurons, NPD1 can also inhibit TRPV1 currents without affecting TRPA1 currents. Its inhibition of TRPV1 is mediated by GPCRs, proven by the fact that its effects are blocked when pertussis toxin, a protein produced by Bordetella pertussis that causes whooping cough and can mediate G-protein actions, is applied. Furthermore, intrathecal injection of NPD1 can inhibit spinal long-term potentiation (LTP) (Park et al. 2011a). Notably, LTP is a form of synaptic plasticity wherein synaptic strength is increased when intense noxious stimuli are applied, resulting in increased pain levels, and serves as a driving force behind chronic pain (Sandkühler 2007).

NPD1 even plays a role in reducing neuropathic pain. It can reduce pain hypersensitivity arising from TNF. In a CCI model of neuropathic pain, an intrathecal injection of NPD1 effectively reduced mechanical allodynia, despite being applied at a dose 500 times lower than that of gabapentin (a commonly used medication for treating clinical neuropathic pain) (Xu et al. 2013). When NPD1 was applied to the injured sciatic nerve around the time of surgery, it prevented nerve-injury-induced mechanical allodynia, ongoing pain, and neuroinflammation in the spinal cord. Finally, repeated injections of NPD1 also exhibited no signs of analgesic tolerance. Overall, NPD1 shows promise as a treatment option with its ability to block nerve-injury-induced LTP, glial cell activation, as well as inflammatory responses, all of which are key drivers of chronic pain in the spinal cord (Xu et al. 2013).

4.8.3 Maresins

Maresins are a type of macrophage SPM derived from DHA. Maresin 1 (MaR1) has several functions. In peritonitis, MaR1 prevents the infiltration of polymorphonuclear (generally referring to eosinophils and basophils) neutrophils. Astoundingly, MaR1 could induce tissue regeneration, accelerating head regeneration in planaria after the head was postpharyngeally resectioned. In addition, MaR1 could also inhibit TRPV1 currents in neurons in a dose-dependent way, blocking currents induced by capsaicin and reducing inflammatory pain in mice (Serhan et al. 2012). Moreover, intrathecal injection with MaR1 in mice 2 weeks after surgery, decreased neuropathic pain induced by chemotherapy and postoperative pain induced by bone fracture (Reviewed in Ji 2023). Interestingly, in a mouse model of rheumatoid arthritis, chronic pain was correlated with reduced MaR1 concentrations. Systematically administering MaR1 was even able to reverse mechanical hypersensitivity and decrease levels of inflammatory macrophages in DRG (Allen et al. 2020). Macrophages are a key player in the formation of two-way communication with nociceptors (Chen et al. 2020); however, the exact extent to which macrophage-derived maresins contribute to chronic pain remains unclear.

4.8.4 Lipoxins

Unlike the majority of SPMs that are derived from omega-3 unsaturated fatty acids, lipoxins, most notably lipoxin A4 (LXA4) and lipoxin B4 (LXB4), are synthesized from arachidonic acid (an omega-6 fatty acid found in phospholipids of cell membranes) and requires lipoxygenase (an enzyme that helps break down fatty acids) (Serhan 2014). It was found that LXA4 and LXB4, when delivered either intravenously or intrathecally, effectively decreased inflammatory pain induced by carrageenan, a food-thickening agent derived from red seaweeds known for its inflammatory properties, in rats. Additionally, ALX, a lipoxin receptor, was

discovered to be expressed in astrocytes in the spinal cord. C-Jun N-terminal kinase (JNK), an enzyme belonging to the MAPK family, when activated in spinal cord astrocytes, plays a role in both inflammatory and neuropathic pain. LXA4 was able to prevent the ATP-mediated phosphorylation of JNK, a crucial step in the activation of JNK, thus helping to inhibit inflammatory pain (Svensson et al. 2007). Furthermore, LXA4 has also been demonstrated to decrease neuroinflammation and neuropathic pain after spinal cord injury (SCI) by hemisection. LXA4 treatment has been shown to decrease pain hypersensitivity, spinal microglial markers, and pro-inflammatory cytokines, which can all occur after SCI. When injected intrathecally, LXA4 was also shown to be capable of relieving neuropathic pain in a rat model of low back pain, by preventing the upregulation of the pro-inflammatory cytokines TNF-α and IL-1β and increasing the expression of the anti-inflammatory cytokines TGF-β1 and 1L-10 (Ji 2023; Martini et al. 2016).

4.8.5 Endogenous Production of SPMs by Neuromodulation and Mild Sham Surgery

Neuromodulation is a treatment method for chronic pain that uses electricity to affect nerve activity. It can be applied to the spinal cord, dorsal root ganglia, and various nerves, including the vagus nerve. Neuromodulation has shown promising results, effectively controlling pain in both laboratory and clinical settings (Tao et al. 2020).

Neuromodulation can regulate both inflammation and neuroinflammation through neuron–immune interactions—in part through the production of SPMs. SPMs can control pain by modulating immune cells, glial cells, and neurons.

A particularly popular form of neuromodulation is vagus nerve neuromodulation (VNS). The vagus nerve, the longest cranial nerve, spanning from the brain all the way to the abdomen, joins the brainstem to the body. The nerve includes two nerve ganglia (clusters of nerves) and several “branches” that join it to the ear, pharynx, larynx (voice box), esophagus, bronchi, and heart. Not only does the vagus nerve send information to the brain about the various body parts it is connected to, but also it can deliver impulses from the brain to the pharynx and larynx, slow heart rate, and control a number of involuntary muscles in various parts of the digestive system. VNS has been used to treat Alzheimer’s disease, depression, and epilepsy. Tracey and his coworkers discovered that VNS can effectively attenuate the systemic inflammatory response to endotoxin lipopolysaccharide (LPS). It was found that acetylcholine, the principal vagal neurotransmitter, significantly attenuated the release of pro-inflammatory cytokines (TNF, IL-1β, IL-6, and IL-18) but not the anti-inflammatory cytokine IL-10 in LPS-stimulated macrophage cultures. VNS not only inhibited TNF synthesis in the liver and reduced serum TNF amounts, but also prevented the development of LPS-induced septic shock (Borovikova et al. 2000).

What interests pain scientists about the vagus nerve, however, is its ability to produce SPMs (Serhan et al. 2019). VNS can prompt the production of SPMs

in vitro and in vivo. These SPMs can then modulate neuron–immune interactions, thus reducing pain (Tao et al. 2020). VNS through auricular electroacupuncture—stimulating acupuncture points in the ear connected to the vagus nerve by using acupuncture needles with electrical currents running through them—has been found to lessen chemotherapy-induced neuropathic pain. Auricular stimulation increases resolvin levels in mice, again highlighting the role of SPMs, which control inflammation and neuroinflammation, in neuromodulation (Tao et al. 2020).

Sham surgery is typically included in pain research for the control of pain models such as nerve injury. Interestingly, sham surgery resulted in acute pain (transient mechanical allodynia) and increased RvD1 levels, suggesting that SPM production in sham surgery appears to be able to resolve acute pain (Zhang et al. 2018).

4.8.6 Fish Oil and SPMs

EPA and DHA are known precursors for producing powerful SPMs, such as resolvins and protectins (Fig. 4.2). EPA and DHA were proven to have anti-inflammatory effects through an experiment utilizing transgenic mice, engineered to express excess fat-1, an enzyme that helps break down omega-3 fatty acids. The increased breakdown of omega-3 fatty acids facilitated by the extra fat-1 allowed the mice to produce more protectins and resolvins from omega-3 polyunsaturated fatty acids (PUFAs). As a result, these mice were protected from dextran-sulfate-induced colitis (inflammation of the inner lining of the colon and a model of inflammation). Additionally, EPA and DHA can prevent transcription nuclear factor-κB activation and IL-1β and TNF-α release, effectively intercepting the primary regulators of inflammation (Zhang et al. 2021).

In a recent study that analyzed 17 trials measuring the effectiveness of omega-3 PUFAs to relieve joint pain in patients with inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, and dysmenorrhea (menstrual cramps), omega-3 PUFAs were found to be a promising treatment (Goldberg and Katz 2007). Due to their lack of side effects, omega-3 PUFAs may serve as an effective alternative for NSAIDS, which are known to cause damage to the stomach lining that can lead to gastric ulcers.

Fish oil contains omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that can be broken down to create SPMs. Because the body produces SPMs using the body's PUFA intake, diets rich in omega-3 may lead to higher levels of SPM production. Despite this overlap, there are still subtle distinctions between the effects of omega-3 fatty acids and SPMs (Ji 2023). DHA is only effective in preventing and delaying neuropathic and postoperative pain after nerve injury and bone fracture but ineffective in reversing chronic pain. In contrast, SPMs show efficacy in reducing chronic pain (Xu et al. 2013; Zhang et al. 2018). Notably, DHA and EPA may not serve the same functions. Compared to EPA, DHA is richer in nervous system membrane phospholipids; as such, its role in neuroprotection is much more significant than EPA in neurological conditions.

4.9 Other Mediators

MicroRNAs (miRNAs) are small single-stranded RNAs that do not code for amino acids or proteins; rather they are recognized to control gene expression after transcription inside cells. MicroRNAs can also be secreted by cells, in which case they may serve as damage-associated molecular patterns (DAMPs). Crucially, miRNAs are dysregulated in human diseases such as cancer; in these cases, the miRNAs can be identified as biomarkers of disease (McDonald and Ajit 2015). Let-7b, for example, is released from damaged cells including neurons, alerting immune cells in the tumor microenvironment. Let-7b binds to TLR7 on immune cells. This interaction results in the activation of NF-kB and secretion of IL-6 and TNF-alpha, which promotes inflammatory responses. Recent studies demonstrated that let-7b is a potent pain inducer by activating TLR7 on nociceptor neurons. Activation of TLR7 on nociceptors results in further activation of TRPA1 to trigger acute pain (Park et al. 2014). It was also found that sensory neurons secrete miR-21 following painful insult and uptake of miR-21 by macrophages can induce the pro-inflammatory M1-macrophage phenotype to facilitate chronic pain development (Simeoli et al. 2017). Thus, miRNAs may serve as novel pain mediators via extracellular actions.

Accumulating evidence demonstrated that lipid mediators of lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), and linoleic acid also contribute to the pathogenesis of pain (Ueda 2021). LPA induces neuropathic pain via specific LPA receptor (LPAR). The LPAR1-mediated peripheral mechanisms include demyelination of the dorsal root, while the LPAR3 mediates glial activation in the spinal cord (Ueda 2021). LPC (e.g., LPC 18:1 and LPC 16:0) contributes to chemotherapy-induced acute pain via activation of TRP channels (e.g., TRPV1 and TRPM8) in sensory nerve terminals (Rimola et al. 2020). Of particular interest is the Western-style diet, enriched with omega-6 polyunsaturated fatty acids that accumulate in membrane phospholipids and oxidize into pro-nociceptive oxylipins (Boyd et al. 2021). Elevated dietary omgea-6 polyunsaturated fatty acids are sufficient to induce neuropathy and pain. However, diet-induced peripheral nerve dysfunction is reversible and can be rescued by a healthy diet containing a greater proportion of omega-3 polyunsaturated fatty acids (McGinnis and Ji 2021).

Multiple proteases such as cathepsin, caspases, and matrix metalloproteases (MMPs) are involved in chronic pain sensitization by regulating neuron–immune interactions in the peripheral and central nervous systems (Ji et al. 2014). Tissue injury and inflammatory stimuli activate proteases (e.g., trypsin) in the circulation and in immune, epithelial and neuronal tissues that cleave protease-activated receptors (PARs), a family of four GPCRs, including PAR1-4. PAR2 is expressed in nociceptors that can sensitize TRPV1 to promote inflammatory pain (Cattaruzza et al. 2014). Several proteases can degrade both neuronal cells and the extracellular matrix in the vicinity of the activated microglia. Nerve injury was shown to induce a rapid but transient upregulation of MMP-9 in mouse DRG neurons, which is required for the onset—but not the maintenance—of neuropathic pain. MMP-9 does so by inducing microglial activation in the spinal cord following secretion

from primary sensory neurons. It was found in rodents that MMP-9 is sufficient and required for generating persistent mechanical pain; and furthermore this action of MMP-9 depended on the activation of IL-1β by MMP-9 (Kawasaki et al. 2008a, b). Caspase-6 is expressed by axons of primary sensory neurons and has been implicated in axonal degeneration. Nerve injury induces caspase-6 expression in primary sensory neurons, which is involved in microglial activation and neuropathic pain (Berta et al. 2014). Cathepsin S is induced in microglial cells after nerve injury and contributes to the genesis of neuropathic pain via microglial activation (Clark et al. 2007).

4.10 Concluding Remarks

Inflammation not only produces pro-inflammatory mediators for the induction of pain but also generates anti-inflammatory and pro-resolving mediators such as SPMs for the resolution of acute pain. A failure in the resolution of acute pain will lead to transition from acute pain to chronic pain (Ji et al. 2011). A recent study shows that acute inflammatory response via neutrophil activation protects against the development of chronic low back pain. Strikingly, in mouse pain assays, early treatment with a steroid or NSAID also led to prolonged pain despite being analgesic in the short term (Parisien et al. 2022). Thus, inflammation is a double-edged sword and must be tightly controlled for the benefits of pain control. Notably, immune suppression is dangerous during bacterial and virus infections (e.g., coronavirus disease-2019 [COVID-19]). We highlight pro-resolution approaches in this book for safe and effective control of inflammation (see Chaps. 10, 11, 12, 13, 14, and 15).

References

Abbadie C, Bhangoo S, De Koninck Y, Malcangio M, Melik-Parsadaniantz S, White FA. Chemokines and pain mechanisms. Brain Res Rev. 2009;60(1):125–34.

Aley KO, Levine JD. Role of protein kinase a in the maintenance of inflammatory pain. J Neurosci. 1999;19(6):2181–6.

Allen BL, Montague-Cardoso K, Simeoli R, Colas RA, Oggero S, Vilar B, McNaughton PA, Dalli J, Perretti M, Sher E, Malcangio M. Imbalance of proresolving lipid mediators in persistent allodynia dissociated from signs of clinical arthritis. Pain. 2020;161(9):2155–66.

Amaya F, Wang H, Costigan M, Allchorne AJ, Hatcher JP, Egerton J, Stean T, Morisset V, Grose D, Gunthorpe MJ, Chessell IP, Tate S, Green PJ, Woolf CJ. The voltage-gated sodium channel Na(v)1.9 is an effector of peripheral inflammatory pain hypersensitivity. J Neurosci. 2006;26(50):12852–60.

Avona A, Burgos-Vega C, Burton MD, Akopian AN, Price TJ, Dussor G. Dural calcitonin gene-related peptide produces female-specific responses in rodent migraine models. J Neurosci. 2019;39(22):4323–31.

Bao L. Trafficking regulates the subcellular distribution of voltage-gated sodium channels in primary sensory neurons. Mol Pain. 2015;11:61.
Barragán-Iglesias P, Franco-Enzástiga Ú, Jeevakumar V, Shiers S, Wangzhou A, Granados-Soto V, Campbell ZT, Dussor G, Price TJ. Type I interferons act directly on nociceptors to produce pain sensitization: implications for viral infection-induced pain. J Neurosci. 2020;40(18):3517–32.
Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL, Jordt SE, Julius D. The menthol receptor TRPM8 is the principal detector of environmental cold. Nature. 2007;448(7150):204–8.
Bautista DM, Pellegrino M, Tsunozaki M. TRPA1: a gatekeeper for inflammation. Annu Rev Physiol. 2013;75:181–200.
Bennett DL, Woods CG. Painful and painless channelopathies. Lancet Neurol. 2014;13(6):587–99.
Berta T, Park CK, Xu ZZ, Xie RG, Liu T, Lü N, Liu YC, Ji RR. Extracellular caspase-6 drives murine inflammatory pain via microglial TNF-α secretion. J Clin Invest. 2014;124(3):1173–86.
Bhangoo SK, Ren D, Miller RJ, Chan DM, Ripsch MS, Weiss C, McGinnis C, White FA. CXCR4 chemokine receptor signaling mediates pain hypersensitivity in association with antiretroviral toxic neuropathy. Brain Behav Immun. 2007;21(5):581–91.
Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW. Protein kinase c phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci U S A. 2003;100(21):12480–5.
Bobinski F, Teixeira JM, Sluka KA, Santos ARS. Interleukin-4 mediates the analgesia produced by low-intensity exercise in mice with neuropathic pain. Pain. 2018;159(3):437–50.
Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405:458–62.
Boyd JT, LoCoco PM, Furr AR, Bendele MR, Tram M, Li Q, Chang FM, Colley ME, Samenuk GM, Arris DA, Locke EE, Bach SBH, Tobon A, Ruparel SB, Hargreaves KM. Elevated dietary ω-6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. Nat Metab. 2021;3(6):762–73.
Brigham NC, Ji RR, Becker ML. Degradable polymeric vehicles for postoperative pain management. Nat Commun. 2021;12(1):1367.
Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and regenerative pain medicine. J Clin Invest. 2020;130(5):2164–76.
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389(6653):816–24.
Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 2000;288(5464):306–13.
Cattaruzza F, Amadesi S, Carlsson JF, Murphy JE, Lyo V, Kirkwood K, Cottrell GS, Bogyo M, Knecht W, Bunnett NW. Serine proteases and protease-activated receptor 2 mediate the proinflammatory and algesic actions of diverse stimulants. Br J Pharmacol. 2014;171(16):3814–26.
Chaiamnuay S, Allison JJ, Curtis JR. Risks versus benefits of cyclooxygenase-2-selective nonsteroidal antiinflammatory drugs. Am J Health Syst Pharm. 2006;63(19):1837–51.
Chang W, Berta T, Kim YH, Lee S, Lee SY, Ji RR. Expression and role of voltage-gated sodium channels in human dorsal root ganglion neurons with special focus on Nav1.7, species differences, and regulation by paclitaxel. Neurosci Bull. 2018;34(1):4–12.
Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN. A P2X purinoceptor expressed by a subset of sensory neurons. Nature. 1995;377(6548):428–31.
Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion. J Clin Invest. 2015;125(8):3226–40.
Chen G, Kim YH, Li H, Luo H, Liu DL, Zhang ZJ, Lay M, Chang W, Zhang YQ, Ji RR. PD-L1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via PD-1. Nat Neurosci. 2017;20(7):917–26.
Chen O, Donnelly CR, Ji RR. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020;62:17–25.

Chiang N, Serhan CN. Specialized pro-resolving mediator network: an update on production and actions. Essays Biochem. 2020;64(3):443–62.
Chiu IM, Heesters BA, Ghasemlou N, Von Hehn CA, Zhao F, Tran J, Wainger B, Strominger A, Muralidharan S, Horswill AR, Bubeck Wardenburg J, Hwang SW, Carroll MC, Woolf CJ. Bacteria activate sensory neurons that modulate pain and inflammation. Nature. 2013;501(7465):52–7.
Chiu IM, Barrett LB, Williams EK, Strochlic DE, Lee S, Weyer AD, Lou S, Bryman GS, Roberson DP, Ghasemlou N, Piccoli C, Ahat E, Wang V, Cobos EJ, Stucky CL, Ma Q, Liberles SD, Woolf CJ. Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. elife. 2014;3:e04660.
Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, Dehvari M, Wotherspoon G, Winter J, Ullah J, Bevan S, Malcangio M. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. Proc Natl Acad Sci U S A. 2007;104(25):10655–60.
Clark AK, Staniland AA, Malcangio M. Fractalkine/CX3CR1 signalling in chronic pain and inflammation. Curr Pharm Biotechnol. 2011;12(10):1707–14.
Constantin CE, Mair N, Sailer CA, Andratsch M, Xu ZZ, Blumer MJ, Scherbakov N, Davis JB, Bluethmann H, Ji RR, Kress M. Endogenous tumor necrosis factor alpha (TNFalpha) requires TNF receptor type 2 to generate heat hyperalgesia in a mouse cancer model. J Neurosci. 2008;28(19):5072–81.
Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 2011;335(1):2–13.
Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, Woods CG. An SCN9A channelopathy causes congenital inability to experience pain. Nature. 2006;444(7121):894–8.
Cummins TR, Dib-Hajj SD, Black JA, Waxman SG. Sodium channels and the molecular pathophysiology of pain. Prog Brain Res. 2000;129:3–19.
Dai Y, Iwata K, Fukuoka T, Kondo E, Tokunaga A, Yamanaka H, Tachibana T, Liu Y, Noguchi K. Phosphorylation of extracellular signal-regulated kinase in primary afferent neurons by noxious stimuli and its involvement in peripheral sensitization. J Neurosci. 2002;22(17):7737–45.
Davidson S, Copits BA, Zhang J, Page G, Ghetti A, Gereau RW 4th. Human sensory neurons: membrane properties and sensitization by inflammatory mediators. Pain. 2014;155(9):1861–70.
Donnelly CR, Andriessen AS, Chen G, Wang K, Jiang C, Maixner W, Ji RR. Central nervous system targets: glial cell mechanisms in chronic pain. Neurotherapeutics. 2020a;17(3):846–60.
Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends Neurosci. 2020b;43(10):822–38.
Donnelly CR, Jiang C, Andriessen AS, Wang K, Wang Z, Ding H, Zhao J, Luo X, Lee MS, Lei YL, Maixner W, Ko MC, Ji RR. STING controls nociception via type I interferon signalling in sensory neurons. Nature. 2021;591(7849):275–80.
Drenth JP, Finley WH, Breedveld GJ, Testers L, Michiels JJ, Guillet G, Taieb A, Kirby RL, Heutink P. The primary erythermalgia-susceptibility gene is located on chromosome 2q31-32. Am J Hum Genet. 2001;68(5):1277–82.
Echeverry S, Shi XQ, Haw A, Liu H, Zhang ZW, Zhang J. Transforming growth factor-beta1 impairs neuropathic pain through pleiotropic effects. Mol Pain. 2009;5:16. https://doi.org/10.1186/1744-8069-5-16.
Ericson-Neilsen W, Kaye AD. Steroids: pharmacology, complications, and practice delivery issues. Ochsner J. 2014;14(2):203–7.
Ferreira SH, Lorenzetti BB, Bristow AF, Poole S. Interleukin-1 beta as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature. 1988;334(6184):698–700.
Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM, Rees M. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron. 2006;52(5):767–74.
Gao YJ, Ji RR. Chemokines, neuronal-glial interactions, and central processing of neuropathic pain. Pharmacol Ther. 2010;126(1):56–68.

Gao YJ, Zhang L, Samad OA, Suter MR, Yasuhiko K, Xu ZZ, Park JY, Lind AL, Ma Q, Ji RR. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. J Neurosci. 2009;29(13):4096–108.

Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. Nat Med. 2010;16(11):1248–57.

Gold MS, Levine JD, Correa AM. Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. J Neurosci. 1998;18(24):10345–55.

Goldberg RJ, Katz J. A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. Pain. 2007;129(1–2):210–23.

Han Q, Kim YH, Wang X, Liu D, Zhang ZJ, Bey AL, Lay M, Chang W, Berta T, Zhang Y, Jiang YH, Ji RR. SHANK3 deficiency impairs heat hyperalgesia and TRPV1 signaling in primary sensory neurons. Neuron. 2016;92(6):1279–93.

Homma Y, Brull SJ, Zhang JM. A comparison of chronic pain behavior following local application of tumor necrosis factor alpha to the normal and mechanically compressed lumbar ganglia in the rat. Pain. 2002 Feb;95(3):239–46.

Hucho T, Levine JD. Signaling pathways in sensitization: toward a nociceptor cell biology. Neuron. 2007;55(3):365–76.

Iyer SM, Montgomery KL, Towne C, Lee SY, Ramakrishnan C, Deisseroth K, Delp SL. Virally mediated optogenetic excitation and inhibition of pain in freely moving nontransgenic mice. Nat Biotechnol. 2014;32(3):274–8.

Ji RR. Specialized pro-resolving mediators as resolution pharmacology for the control of pain and itch. Annua Rev Pharmacol Toxicol. 2023;63(1):273–293. https://doi.org/10.1146/pharmtox.2023.63.issue-1. https://doi.org/10.1146/annurev-pharmtox-051921-084047.

Ji RR, Lee SY. Molecular sensors of temperature, pressure, and pain with special focus on TRPV1, TRPM8, and PIEZO2 ion channels. Neurosci Bull. 2021;37(12):1745–9.

Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nat Neurosci. 1999;2(12):1114–9.

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron. 2002;36:57–68.

Ji RR, Gereau RW 4th, Malcangio M, Strichartz GR. MAP kinase and pain. Brain Res Rev. 2009a;60(1):135–48.

Ji RR, Xu ZZ, Wang X, Lo EH. Matrix metalloprotease regulation of neuropathic pain. Trends Pharmacol Sci. 2009b;30(7):336–40.

Ji RR, Xu ZZ, Strichartz G, Serhan CN. Emerging roles of resolvins in the resolution of inflammation and pain. Trends Neurosci. 2011;34(11):599–609.

Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533–48.

Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology. 2018;129(2):343–66.

Ji J, He Q, Luo X, Bang S, Matsuoka Y, McGinnis A, Nackley AG, Ji RR. IL-23 enhances C-fiber-mediated and blue light-induced spontaneous pain in female mice. Front Immunol. 2021;12:787565.

Jiang F, Hua LM, Jiao YL, Ye P, Fu J, Cheng ZJ, Ding G, Ji YH. Activation of mammalian target of rapamycin contributes to pain nociception induced in rats by BmK I, a sodium channel-specific modulator. Neurosci Bull. 2014;30(1):21–32.

Jiang BC, Cao DL, Zhang X, Zhang ZJ, He LN, Li CH, Zhang WW, Wu XB, Berta T, Ji RR, Gao YJ. CXCL13 drives spinal astrocyte activation and neuropathic pain via CXCR5. J Clin Invest. 2016;126(2):745–61.

Jin X, Gereau RW 4th. Acute p38-mediated modulation of tetrodotoxin-resistant sodium channels in mouse sensory neurons by tumor necrosis factor-alpha. J Neurosci. 2006;26(1):246–55.

Jin SX, Zhuang ZY, Woolf CJ, Ji RR. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. J Neurosci. 2003;23(10):4017–22.

Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature. 2001;413(6852):203–10.
Kalpachidou T, Riehl L, Schöpf CL, Ucar B, Kress M. Proinflammatory cytokines and their receptors as druggable targets to alleviate pathological pain. Pain. 2022 Nov 1;163(Suppl 1):S79–98.
Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Gao YJ, Roy K, Corfas G, Lo EH, Ji RR. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008a;14(3):331–6.
Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci. 2008b;28(20):5189–94.
Kim SE, Coste B, Chadha A, Cook B, Patapoutian A. The role of drosophila piezo in mechanical nociception. Nature. 2012;483(7388):209–12.
Krukowski K, Eijkelkamp N, Laumet G, Hack CE, Li Y, Dougherty PM, Heijnen CJ, Kavelaars A. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. J Neurosci. 2016;36(43):11074–83.
Kummer KK, Zeidler M, Kalpachidou T, Kress M. Role of IL-6 in the regulation of neuronal development, survival and function. Cytokine. 2021;144:155582.
Ledeboer A, Jekich BM, Sloane EM, Mahoney JH, Langer SJ, Milligan ED, Martin D, Maier SF, Johnson KW, Leinwand LA, Chavez RA, Watkins LR. Intrathecal interleukin-10 gene therapy attenuates paclitaxel-induced mechanical allodynia and proinflammatory cytokine expression in dorsal root ganglia in rats. Brain Behav Immun. 2007;21(5):686–98.
Lee KM, Sherlock JP, Hamilton JA. The role of interleukin (IL)-23 in regulating pain in arthritis. Arthritis Res Ther. 2022;24(1):89.
Li CL, Li KC, Wu D, Chen Y, Luo H, Zhao JR, Wang SS, Sun MM, Lu YJ, Zhong YQ, Hu XY, Hou R, Zhou BB, Bao L, Xiao HS, Zhang X. Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. Cell Res. 2016;26(8):967.
Li Y, North RY, Rhines LD, Tatsui CE, Rao G, Edwards DD, Cassidy RM, Harrison DS, Johansson CA, Zhang H, Dougherty PM. DRG voltage-gated sodium channel 1.7 is upregulated in paclitaxel-induced neuropathy in rats and in humans with neuropathic pain. J Neurosci. 2018;38(5):1124–36.
Lin CR, Amaya F, Barrett L, Wang H, Takada J, Samad TA, Woolf CJ. Prostaglandin E2 receptor EP4 contributes to inflammatory pain hypersensitivity. J Pharmacol Exp Ther. 2006;319(3):1096–103.
Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A, Kim H. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma Clin Immunol. 2013;9(1):30.
Liu XJ, Liu T, Chen G, Wang B, Yu XL, Yin C, Ji RR. TLR signaling adaptor protein MyD88 in primary sensory neurons contributes to persistent inflammatory and neuropathic pain and neuroinflammation. Sci Rep. 2016;6:28188.
Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest. 2005;115(10):2774–83.
Luo H, Liu HZ, Zhang WW, Matsuda M, Lv N, Chen G, Xu ZZ, Zhang YQ. Interleukin-17 regulates neuron-glial communications, synaptic transmission, and neuropathic pain after chemotherapy. Cell Rep. 2019;29(8):2384–97.e5.
Luo X, Gu Y, Tao X, Serhan CN, Ji RR. Resolvin D5 inhibits neuropathic and inflammatory pain in male but not female mice: distinct actions of D-series resolvins in chemotherapy-induced peripheral neuropathy. Front Pharmacol. 2019a Jul 5;10:745. https://doi.org/10.3389/fphar.2019.00745.
Luo X, Huh Y, Bang S, He Q, Zhang L, Matsuda M, Ji RR. Macrophage toll-like receptor 9 contributes to chemotherapy-induced neuropathic pain in male mice. J Neurosci. 2019b;39(35):6848–64.

Luo X, Chen O, Wang Z, Bang S, Ji J, Lee SH, Huh Y, Furutani K, He Q, Tao X, Ko MC, Bortsov A, Donnelly CR, Chen Y, Nackley A, Berta T, Ji RR. IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice. Neuron. 2021;109(17):2691–706.e5.

Ma L, Yu L, Jiang BC, Wang J, Guo X, Huang Y, Ren J, Sun N, Gao DS, Ding H, Lu J, Zhou H, Zou L, Gao Y, Wang L, Sun K, Ming Y, Meng Z, Tao YX, Yan M. ZNF382 controls mouse neuropathic pain via silencer-based epigenetic inhibition of Cxcl13 in DRG neurons. J Exp Med. 2021;218(12):e20210920.

MacDonald DI, Sikandar S, Weiss J, Pyrski M, Luiz AP, Millet Q, Emery EC, Mancini F, Iannetti GD, Alles SRA, Arcangeletti M, Zhao J, Cox JJ, Brownstone RM, Zufall F, Wood JN. A central mechanism of analgesia in mice and humans lacking the sodium channel NaV1.7. Neuron. 2021;109(9):1497–512.e6.

Malmberg AB, Yaksh TL. Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: a microdialysis study in unanesthetized rats. J Neurosci. 1995;15(4):2768–76.

Martini AC, Berta T, Forner S, Chen G, Bento AF, Ji RR, Rae GA. Lipoxin A4 inhibits microglial activation and reduces neuroinflammation and neuropathic pain after spinal cord hemisection. J Neuroinflammation. 2016;13(1):75.

McDonald MK, Ajit SK. MicroRNA biology and pain. Prog Mol Biol Transl Sci. 2015;131:215–49. https://doi.org/10.1016/bs.pmbts.2014.11.015.

McGinnis A, Ji RR. Can a Western high-fat diet lead to painful neuropathy? Nat Metab. 2021;3(6):735–6.

McKelvey R, Berta T, Old E, Ji RR, Fitzgerald M. Neuropathic pain is constitutively suppressed in early life by anti-inflammatory neuroimmune regulation. J Neurosci. 2015;35(2):457–66.

Melemedjian OK, Asiedu MN, Tillu DV, Peebles KA, Yan J, Ertz N, Dussor GO, Price TJ. IL-6- and NGF-induced rapid control of protein synthesis and nociceptive plasticity via convergent signaling to the eIF4F complex. J Neurosci. 2010;30(45):15113–23.

Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, Verge GM, Chapman G, Green P, Foster AC, Naeve GS, Maier SF, Watkins LR. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. Eur J Neurosci. 2004;20(9):2294–302.

Miyoshi K, Obata K, Kondo T, Okamura H, Noguchi K. Interleukin-18-mediated microglia/astrocyte interaction in the spinal cord enhances neuropathic pain processing after nerve injury. J Neurosci. 2008;28(48):12775–87.

Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. Regulation of pain and itch by TRP channels. Neurosci Bull. 2018;34(1):120–42.

Moy JK, Hartung JE, Duque MG, Friedman R, Nagarajan V, Loeza-Alcocer E, Koerber HR, Christoph T, Schröder W, Gold MS. Distribution of functional opioid receptors in human dorsal root ganglion neurons. Pain. 2020;161(7):1636–49.

Nicol GD, Vasko MR. Unraveling the story of NGF-mediated sensitization of nociceptive sensory neurons: ON or OFF the Trks? Mol Interv. 2007;7(1):26–41.

Orefice LL, Mosko JR, Morency DT, Wells MF, Tasnim A, Mozeika SM, Ye M, Chirila AM, Emanuel AJ, Rankin G, Fame RM, Lehtinen MK, Feng G, Ginty DD. Targeting peripheral somatosensory neurons to improve tactile-related phenotypes in ASD models. Cell. 2019;178(4):867–86.e24.

Pan HL, Liu BL, Lin W, Zhang YQ. Modulation of Nav1.8 by lysophosphatidic acid in the induction of bone cancer pain. Neurosci Bull. 2016;32(5):445–54.

Parisien M, Lima LV, Dagostino C, El-Hachem N, Drury GL, Grant AV, Huising J, Verma V, Meloto CB, Silva JR, Dutra GGS, Markova T, Dang H, Tessier PA, Slade GD, Nackley AG, Ghasemlou N, Mogil JS, Allegri M, Diatchenko L. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci Transl Med. 2022;14(644):eabj9954.

Park CK, Lü N, Xu ZZ, Liu T, Serhan CN, Ji RR. Resolving TRPV1- and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. J Neurosci. 2011a;31(42):15072–85.

Park CK, Xu ZZ, Liu T, Lü N, Serhan CN, Ji RR. Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. J Neurosci. 2011b;31(50):18433–8.

Park CK, Xu ZZ, Berta T, Han Q, Chen G, Liu XJ, Ji RR. Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron. 2014;82(1):47–54.

Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov. 2009;8(1):55–68.

Patil MJ, Salas M, Bialuhin S, Boyd JT, Jeske NA, Akopian AN. Sensitization of small-diameter sensory neurons is controlled by TRPV1 and TRPA1 association. FASEB J. 2020;34(1):287–302.

Richter F, Natura G, Ebbinghaus M, von Banchet GS, Hensellek S, König C, Bräuer R, Schaible HG. Interleukin-17 sensitizes joint nociceptors to mechanical stimuli and contributes to arthritic pain through neuronal interleukin-17 receptors in rodents. Arthritis Rheum. 2012;64(12):4125–34.

Rimola V, Hahnefeld L, Zhao J, Jiang C, Angioni C, Schreiber Y, Osthues T, Pierre S, Geisslinger G, Ji RR, Scholich K, Sisignano M. Lysophospholipids contribute to oxaliplatin-induced acute peripheral pain. J Neurosci. 2020;40(49):9519–32.

Rothenberg RJ, Holcomb JP. Guidelines for monitoring of NSAIDS: who listened? J Clin Rheumatol. 2000;6(5):258–65.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature. 2001;410(6827):471–5.

Sandkühler J. Understanding LTP in pain pathways. Mol Pain. 2007 Apr;3(3):9. https://doi.org/10.1186/1744-8069-3-9.

Schäfers M, Geis C, Svensson CI, Luo ZD, Sommer C. Selective increase of tumour necrosis factor-alpha in injured and spared myelinated primary afferents after chronic constrictive injury of rat sciatic nerve. Eur J Neurosci. 2003a;17(4):791–804.

Schäfers M, Sorkin LS, Sommer C. Intramuscular injection of tumor necrosis factor-alpha induces muscle hyperalgesia in rats. Pain. 2003b;104(3):579–88.

Scherrer G, Imamachi N, Cao YQ, Contet C, Mennicken F, O'Donnell D, Kieffer BL, Basbaum AI. Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. Cell. 2009;137(6):1148–59.

Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510(7503):92–101.

Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB J. 2012;26(4):1755–65.

Serhan CN, de la Rosa X, Jouvene C. Novel mediators and mechanisms in the resolution of infectious inflammation: evidence for vagus regulation. J Intern Med. 2019;286(3):240–58.

Shekelle PG, Newberry SJ, FitzGerald JD, Motala A, O'Hanlon CE, Tariq A, Okunogbe A, Han D, Shanman R. Management of Gout: a systematic review in support of an American College of Physicians clinical practice guideline. Ann Intern Med. 2017;166(1):37–51.

Shiers S, Klein RM, Price TJ. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. Pain. 2020;161(10):2410–24.

Silva RL, Lopes AH, Guimarães RM, Cunha TM. CXCL1/CXCR2 signaling in pathological pain: role in peripheral and central sensitization. Neurobiol Dis. 2017;105:109–16.

Simeoli R, Montague K, Jones HR, Castaldi L, Chambers D, Kelleher JH, Vacca V, Pitcher T, Grist J, Al-Ahdal H, Wong LF, Perretti M, Lai J, Mouritzen P, Heppenstall P, Malcangio M. Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma. Nat Commun. 2017;8(1):1778. https://doi.org/10.1038/s41467-017-01841-5.

Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett. 2004;361(1–3):184–7.

Sommer C, Schmidt C, George A, Toyka KV. A metalloprotease-inhibitor reduces pain associated behavior in mice with experimental neuropathy. Neurosci Lett. 1997 Nov 14;237(1):45–8.

Sostres C, Gargallo CJ, Arroyo MT, Lanas A. Adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs, aspirin and coxibs) on upper gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2010;24(2):121–32.

Svensson CI, Zattoni M, Serhan CN. Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. J Exp Med. 2007;204(2):245–52.

Tan PH, Ji J, Yeh CC, Ji RR. Interferons in pain and infections: emerging roles in neuro-immune and neuro-glial interactions. Front Immunol. 2021;12:783725.

Tan PH, Ji J, Hsing CH, Tan R, Ji RR. Emerging roles of type-I interferons in neuroinflammation, neurological diseases, and long-haul COVID. Int J Mol Sci. 2022;23(22):14394. https://doi.org/10.3390/ijms232214394.

Tao X, Lee MS, Donnelly CR, Ji RR. Neuromodulation, specialized proresolving mediators, and resolution of pain. Neurotherapeutics. 2020;17(3):886–99.

Tavares-Ferreira D, Shiers S, Ray PR, Wangzhou A, Jeevakumar V, Sankaranarayanan I, Cervantes AM, Reese JC, Chamessian A, Copits BA, Dougherty PM, Gereau RW 4th, Burton MD, Dussor G, Price TJ. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci Transl Med. 2022;14(632):eabj8186.

Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.

Tramullas M, Francés R, de la Fuente R, Velategui S, Carcelén M, García R, Llorca J, Hurlé MA. MicroRNA-30c-5p modulates neuropathic pain in rodents. Sci Transl Med. 2018;10(453):eaao6299.

Tsuda M, Masuda T, Kitano J, Shimoyama H, Tozaki-Saitoh H, Inoue K. IFN-gamma receptor signaling mediates spinal microglia activation driving neuropathic pain. Proc Natl Acad Sci U S A. 2009;106(19):8032–7.

Ueda H. Pathogenic mechanisms of lipid mediator lysophosphatidic acid in chronic pain. Prog Lipid Res. 2021;81:101079.

Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, Hjerling-Leffler J, Haeggström J, Kharchenko O, Kharchenko PV, Linnarsson S, Ernfors P. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci. 2015;18(1):145–53.

Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231(25):232–5.

Wainger BJ, Buttermore ED, Oliveira JT, Mellin C, Lee S, Saber WA, Wang AJ, Ichida JK, Chiu IM, Barrett L, Huebner EA, Bilgin C, Tsujimoto N, Brenneis C, Kapur K, Rubin LL, Eggan K, Woolf CJ. Modeling pain in vitro using nociceptor neurons reprogrammed from fibroblasts. Nat Neurosci. 2015;18(1):17–24.

Wang JC, Strichartz GR. Prevention of chronic post-thoracotomy pain in rats by intrathecal resolvin D1 and D2: effectiveness of perioperative and delayed drug delivery. J Pain. 2017;18(5):535–45.

Wang K, Gu Y, Liao Y, Bang S, Donnelly CR, Chen O, Tao X, Mirando AJ, Hilton MJ, Ji RR. PD-1 blockade inhibits osteoclast formation and murine bone cancer pain. J Clin Invest. 2020;130(7):3603–20.

Watkins LR, Milligan ED, Maier SF. Glial activation: a driving force for pathological pain. Trends Neurosci. 2001;24(8):450–5.

Waxman SG, Dib-Hajj S, Cummins TR, Black JA. Sodium channels and pain. Proc Natl Acad Sci U S A. 1999;96(14):7635–9.

Weiss J, Pyrski M, Jacobi E, Bufe B, Willnecker V, Schick B, Zizzari P, Gossage SJ, Greer CA, Leinders-Zufall T, Woods CG, Wood JN, Zufall F. Loss-of-function mutations in sodium channel Nav1.7 cause anosmia. Nature. 2011;472(7342):186–90.

Whelton A. Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiologic foundations and clinical implications. Am J Med. 1999;106(5B):13S–24S.

White FA, Bhangoo SK, Miller RJ. Chemokines: integrators of pain and inflammation. Nat Rev Drug Discov. 2005;4(10):834–44.

Woolf CJ, Costigan M. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. Proc Natl Acad Sci U S A. 1999;96(14):7723–30.

Woolf CJ, Ma Q. Nociceptors – noxious stimulus detectors. Neuron. 2007;55:353–64.

Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji RR. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med. 2010;16:592–7.

Xu ZZ, Liu XJ, Berta T, Park CK, Lü N, Serhan CN, Ji RR. Neuroprotectin/protectin D1 protects against neuropathic pain in mice after nerve trauma. Ann Neurol. 2013;74(3):490–5.

Yang Y, Li H, Li TT, Luo H, Gu XY, Lü N, Ji RR, Zhang YQ. Delayed activation of spinal microglia contributes to the maintenance of bone cancer pain in female Wistar rats via P2X7 receptor and IL-18. J Neurosci. 2015;35(20):7950–63.

Ye Y, Scheff NN, Bernabé D, Salvo E, Ono K, Liu C, Veeramachaneni R, Viet CT, Viet DT, Dolan JC, Schmidt BL. Anti-cancer and analgesic effects of resolvin D2 in oral squamous cell carcinoma. Neuropharmacology. 2018;139:182–93.

Zhang J, Shi XQ, Echeverry S, Mogil JS, De Koninck Y, Rivest S. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. J Neurosci. 2007;27(45):12396–406.

Zhang ZJ, Cao DL, Zhang X, Ji RR, Gao YJ. Chemokine contribution to neuropathic pain: respective induction of CXCL1 and CXCR2 in spinal cord astrocytes and neurons. Pain. 2013;154(10):2185–97.

Zhang H, Li Y, de Carvalho-Barbosa M, Kavelaars A, Heijnen CJ, Albrecht PJ, Dougherty PM. Dorsal root ganglion infiltration by macrophages contributes to paclitaxel chemotherapy-induced peripheral neuropathy. J Pain. 2016;17(7):775–86.

Zhang L, Terrando N, Xu ZZ, Bang S, Jordt SE, Maixner W, Serhan CN, Ji RR. Distinct analgesic actions of DHA and DHA-derived specialized pro-resolving mediators on post-operative pain after bone fracture in mice. Front Pharmacol. 2018;9:412.

Zhang X, Hartung JE, Friedman RL, Koerber HR, Belfer I, Gold MS. Nicotine evoked currents in human primary sensory neurons. J Pain. 2019;20(7):810–8.

Zhang Z, Xue Z, Yang H, Zhao F, Liu C, Chen J, Lu S, Zou Z, Zhou Y, Zhang X. Differential effects of EPA and DHA on DSS-induced colitis in mice and possible mechanisms involved. Food Funct. 2021;12(4):1803–17.

Zheng Y, Liu P, Bai L, Trimmer JS, Bean BP, Ginty DD. Deep sequencing of somatosensory neurons reveals molecular determinants of intrinsic physiological properties. Neuron. 2019;103(4):598–616.e7.

Zhuang ZY, Gerner P, Woolf CJ, Ji RR. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain. 2005;114(1–2):149–59.

Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, Decosterd I, Ji RR. A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance. J Neurosci. 2006;26(13):355.

Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR. Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. Brain Behav Immun. 2007;21(5):642–51.

Chapter 5
Immune and Glial Cells in Pain and Their Interactions with Nociceptive Neurons

Jasmine Ji, Yul Huh, and Ru-Rong Ji

Abstract While pain is sensed and conducted by neurons, including primary sensory neurons (nociceptors) and spinal cord pain transmission neurons, mounting evidence suggests that non-neuronal cells such as immune cells and glial cells in the peripheral nervous system (PNS) and central nervous system (CNS) play active roles in the pathogenesis and resolution of pain. We review how immune cells and glial cells interact with peripheral and central nociceptive neurons by secreting neuroactive signaling molecules (neuromodulators), leading to altered pain sensitivity. It is generally believed that chronic pain is maintained by central sensitization, that is, increased synaptic and neuronal responsiveness (synaptic or neural plasticity) in central pain pathways, after painful injuries and insults. Recent studies also suggest that central sensitization is driven by neuroinflammation. We also discuss how immune cells and glial cells regulate central sensitization and neuroinflammation in the context of chronic pain.

Keywords Astrocytes · B cells · DRG · Fibroblasts · Macrophages · Mast cells · Microglia · Neutrophils · Oligodendrocytes · Satellite glial cells · Schwann's cells · Spinal cord · T cells

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5.1 Introduction

Pain research in the past decade has demonstrated numerous non-neuronal cell types influence pain sensation, including immune, glial, epithelial, mesenchymal, cancer, and bacterial cells (Ji et al. 2016). In this chapter, we focus on immune cells and glial cells that interact with nociceptors and spinal cord nociceptive neurons in distinct anatomical compartments in the peripheral nervous system (PNS) and central nervous system (CNS), especially under pathological conditions. Despite the diversity of these cells, these non-neuronal cells modulate pain in surprisingly similar ways. In response to an injury or insult, immune and glial cells produce and release neuromodulatory substances (neuromodulators) in close proximity to nociceptive neurons, which either promote or dampen pain depending on the specific identities of the mediators involved (Fig. 5.1). Additionally, some inflammatory mediators such as IL-23 indirectly activate nociceptors by releasing another inflammatory mediator (e.g., IL-17, Luo et al. 2021).

5.2 Immune Cells in Pain

Leukocytes are white blood cells. They are responsible for defending the body against foreign substances and invaders. They are found throughout the body and play an indispensable role in inflammation. Leukocytes include neutrophils, eosinophils, basophils, lymphocytes, monocytes, and natural killer cells. In addition, mast cells and dendritic cells are resident immune cells. We discuss how these immune cells regulate pain via interactions with nociceptors (Fig. 5.1).

5.2.1 Neutrophils

Neutrophils are by far the largest category of leukocytes, making up around 60% of the white blood cells. Neutrophils are the body's first line of defense and kill bacteria and fungi. They have an extremely short lifespan, living for only 5–135 h. They are also highly mobile, thanks to their ability to secrete proteolytic enzymes, which erode intercellular connections. These enzymes are also used in phagocytosis, wherein neutrophils consume foreign substances by trapping the foreign substances in a small compartment known as a vacuole, which then merges with the neutrophil as the neutrophil consumes the foreign substance. During inflammation, neutrophils are the first to react; indeed, they are considered the defining sign of acute inflammation. They are generally located in the bloodstream; to travel to the site of damage, they will move through blood vessels and tissue located just outside blood vessels, known as interstitial tissue. They are guided to the site of injury via chemical signals, in a process known as chemotaxis. Earlier studies showed an essential

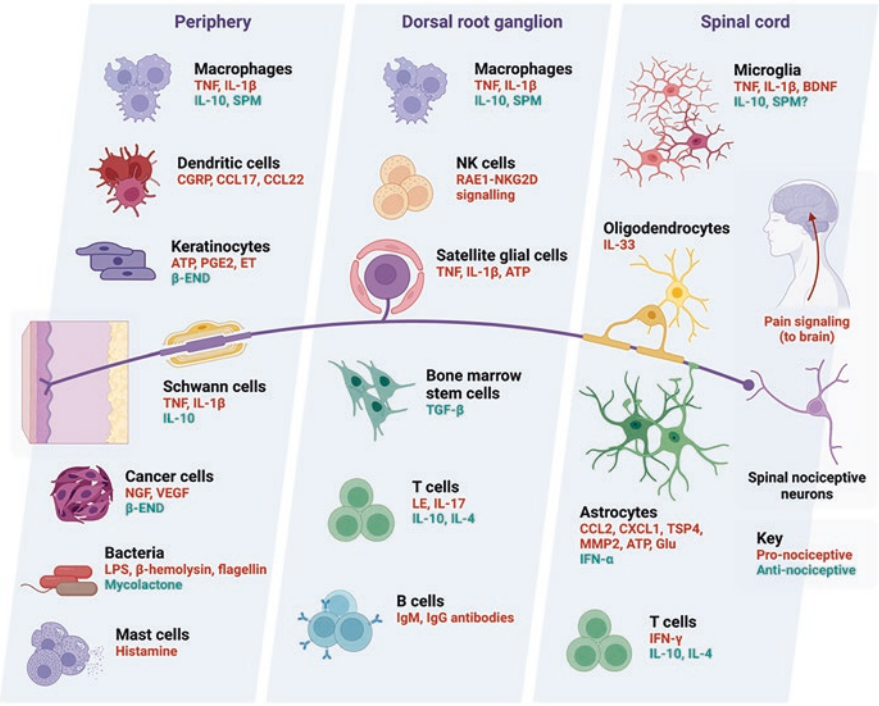

Fig. 5.1 Interactions between distinct parts of a nociceptor with different types of non-neuron cells Nociceptor neurons are characterized by distinct morphology and anatomy, with their cell bodies localized in the dorsal root ganglion (DRG), peripheral axons in the skin and muscle, and central axons in the spinal cord and brain stem. At these distinct locations, nociceptors interact with various types of non-neuronal cells, including keratinocytes in the skin, Schwann cells in the peripheral nerve, satellite glial cells in the DRG, as well as microglia, oligodendrocytes, and astrocytes in the spinal cord. Nociceptor neurons also interact with immune cells (e.g., macrophages, T cells, dendritic cells, mast cells, B cells, NK cells), cancer cells, stem cells, and bacteria. These non-neuronal cells produce both pronociceptive (highlighted in red) and antinociceptive (highlighted in blue) mediators, which can bind their respective receptors on the nociceptor to modulate its sensitivity and excitability. The central terminals of the nociceptor form nociceptive synapses with postsynaptic neurons in the spinal cord dorsal horn to mediate pain transmission in the CNS. *END* endorphin, *ET* endothelin, *Glu* glutamate, *IFN-α* interferon-α, *LE* leukocyte elastase, *SPM* specialized proresolving mediators. (Modified from Ji et al., Science, Ji et al. 2016 with permission)

role of neutrophils in the development of inflammatory, postoperative, and neuropathic pain. Planar surgery results in increases in both neutrophil accumulation and the CXCL1 level in the incised paws. The chemokine CXCL1 can recruit neutrophils and induce pain via CXCR2 receptor. The depletion of the mouse neutrophils by antineutrophil antibody treatment reduced the mechanical hyperalgesia after paw incision (Carreira et al. 2013). Neutrophils release leukocyte elastase, and inhibitors of leucocyte elastase were shown to inhibit neuropathic pain in rodents (Bali and Kuner 2017).

However, a recent study demonstrated that neutrophils also contribute to the resolution of pain by inducing acute inflammatory response. Depletion of neutrophils delayed resolution of pain in mice following complete Freund's adjuvant (CFA) induced inflammation. Strikingly, CFA-induced inflammatory pain is prolonged for several months in animals treated with steroid (dexamethasone). Furthermore, peripheral adoptive transfer of neutrophils themselves was sufficient to prevent the development of long-lasting pain induced by steroids (Parisien et al. 2022). Thus, neutrophils play distinct roles in regulating the induction and resolution of pain.

5.2.2 Eosinophils

Eosinophils make up about 2–4% of all white blood cells, although this percentage increases in response to allergies, parasitic infections, collagen diseases, spleen diseases, and CNS diseases. They are often found in mucous membranes, including those in the digestive, respiratory, and lower urinary tracts. They mainly deal with parasitic infections. They are also the most prominent inflammatory cells in allergic reactions. Eosinophilia becomes most active during allergic reactions, including during asthma attacks, hay fever, hives, and of course, parasitic infections. They produce and secrete chemicals that break up parasites too large for phagocytizing (e.g., hookworms and tapeworms). Eosinophils are known for having two lobes, which are linked with a thin strand. The cytoplasm contains numerous granules. With eosin staining, the granules appear a distinct pink-orange. The role of eosinophils in pain is not well studied, although a history of allergy doubles the risk of vulvodynia, a chronic female genital pain (Arriaga-Gomez et al. 2019).

5.2.3 Basophils

Basophils are the rarest of white blood cells, making up less than 0.5% of the cells. They are mainly in charge of allergic and antigen response. During such events, they release either histamine or heparin. Histamines can widen blood vessels in order to increase blood flow to injured tissue. It also increases blood vessel permeability, facilitating neutrophils and clotting protein entry into connective tissue. Heparin serves as an anticoagulant, preventing blood clotting, thus encouraging white blood cells to move into the area. Additionally, basophils can secrete chemical signals to attract eosinophils and neutrophils to an infected site. Generally, basophils have two or three lobes. Their granules are generally coarse, making the lobes difficult to see. When dyed, the dark violet granules will appear blue. The specific role of basophils in pain remains to be investigated, although mediators (e.g., histamine) secreted from basophils could induce pain and itch.

5.2.4 *Monocytes and Macrophages*

Monocytes make for 2–8% of white blood cell count. Like neutrophils, they can perform phagocytosis, "consuming" parasites and other foreign substances; unlike neutrophils; however, they last much longer. In addition, they can present pathogen pieces to T cells; this function allows T cells to recognize pathogens to kill, leading to an antigen response. Monocytes will leave the bloodstream, becoming tissue macrophages that remove dead cells debris and defend the body against microorganisms, neither of which neutrophils alone can deal with effectively. Monocytes have the ability to replace the contents of their lysosomes, granting them a longer lifespan than their neutrophil counterparts. Monocytes can be recognized by their kidney-shaped nucleus, lack of granules, and abundant cytoplasm.

Monocytes can differentiate into macrophages. Interestingly, macrophages can both cause and relieve pain. Macrophages play a chief role in the pathogenesis of pain and have bilateral communications with nociceptors, the specialized primary sensory neurons that sense pain (Chen et al. 2020). It is increasingly appreciated that macrophages display different functional states or phenotypes: M1-like macrophages produce proinflammatory cytokines and chemokines (e.g., IL-1β, TNF-α) to promote pain; M2-like macrophages are immunosuppressive cells and secrete anti-inflammatory cytokines (IL-10 and TGF-β1) and growth factors to promote tissue repair and resolution of pain. However, macrophages are a heterogeneous population of immune cells and may exhibit additional functional states. It is well established that activation of macrophages can induce and promote pain. Adoptive transfer of chemotherapy-treated macrophages is sufficient to induce mechanical pain (Luo et al. 2019). Macrophages "talk to" nociceptor neurons by releasing pronociceptive mediators (e.g., proinflammatory cytokines and chemokines IL-1β, TNF-α, IL-17, and CXCL1). These inflammatory mediators induce pain via direct activation or sensitization of nociceptors. Macrophages also "listen to" nociceptor neurons, as nociceptors secrete neuropeptides (e.g., CGRP) and chemokines (e.g., CCL2/MCP-1) that act on macrophages. Activation of toll-like receptors (TLRs) results in release of CCL2 from nociceptors, leading to macrophage infiltration to DRG neurons and potentiating pathological pain (Chen et al. 2020). In addition to cytokines, chemokines, and neuropeptides, macrophage–nociceptor interactions are also mediated by microRNAs. For example, nociceptor-released miR-21-5p could trigger gene expression in macrophages to promote a proinflammatory phenotype of macrophages that can drive neuropathic pain (Simeoli et al. 2017).

Accumulating evidence also points to a proresolution role of macrophages in inflammatory and neuropathic pain. The orphan receptor GPR37 is expressed by macrophages and activated by neuroprotectin D1, a specialized proresolving mediator (SPM) derived from omega-3 unsaturated fatty acids. Activation of GPR37 in macrophages resolves inflammatory pain via phagocytosis of apoptotic neutrophils and production of the anti-inflammatory cytokines IL-10 that can inhibit nociceptors (Bang et al. 2018). Furthermore, macrophages can biosynthesize SPMs such as maresins (MaR1 and MaR2), which is able to resolve inflammation. They are also

able to aid in the regeneration of tissue and relieve pain (Serhan et al. 2012). A recent study reported that M2-like macrophages in the spinal cord play an important role in the resolution of neuropathic pain after peripheral injury (Niehaus et al. 2021). Furthermore, it was also found that macrophages can transfer mitochondria to sensory neurons to resolve inflammatory pain (van der Vlist et al. 2022).

Osteoclasts are specialized cells in bone tissue and act to resorb bone. They develop from the monocyte/macrophage lineage. Osteoclasts play an important role in bone cancer pain, one of the most painful conditions (Mantyh 2006). Common cancers such as prostate, breast, and lung cancer can metastasize to bones at advanced stages, producing severe pain. In the tumor microenvironment, there are reciprocal interactions between tumor cells, bone-resorbing osteoclasts, and nociceptors, which drive bone cancer pain. Osteoclast overactivation contributes to bone cancer pain through (1) direct mechanisms by production of pronociceptive mediators (e.g., proton, CCL2) and (2) by indirect mechanisms by which osteoclasts cause bone resorption, leading to bone fracture and breaking through pain (Andriessen et al. 2021; Mantyh 2006). Interestingly, the PD-L1 (programed death protein ligand 1) and PD-1 (programed death protein 1) immune checkpoint pathway was shown to regulate osteogenesis in bone cancer. Thus, cancer-produced PD-L1 can bind their PD-1 receptor to induce osteogenesis. It was found that Nivolumab, an FDA-approved anti-PD-1 monoclonal antibody immunotherapy can not only suppress osteogenesis but also inhibit bone destruction and cancer pain in a mouse model of bone cancer (Wang et al. 2020).

Together, these studies suggest that macrophages have both detrimental and beneficial roles in the pathogenesis and resolution of pain.

5.2.5 T Lymphocytes (T Cells)

T lymphocytes, also known as T cells, are white blood cells that originate from stem cells found in bone marrow. They later migrate to the thyroid, where they differentiate into different types of T cells. T cells are crucial for a second type of immunity—adaptive immunity. In contrast to the mechanisms of innate immunity, the mechanisms of adaptive immunity target specific foreign invaders in the body. Categories of note are the “killer” T cells and the “helper” T cells. As their name implies, killer T cells kill infected or damaged cells (including cancer cells) through cytotoxic methods. They can also release cytokines to recruit other immune cells to help fight invaders. If killer T cells are the soldiers, helper T cells can be seen as the strategists; they communicate with other immune cells and let them know how to respond to particular invaders.

T cells have been found to participate in the development of pain. The evidence of T cell involvement is particularly plentiful for neuropathic pain. After nerve injury, T cells will infiltrate the damaged nerve and ipsilateral DRG and release pain-causing mediators such as leukocyte elastase (LE), resulting in mechanical allodynia. In the spinal cord, T cells will also infiltrate after nerve injury; this

infiltration is necessary for mechanical hypersensitivity to develop. T cells produce IFN-γ to enhance nociceptive synaptic transmission and activate microglial cells. Interestingly, sex seems to play a role in how T cells participate in the development of pain. One experiment found that spinal T cells account for neuropathic pain after nerve injury in only female mice; microglial signaling accounts for such pain in male mice (Sorge et al. 2015).

T cells have also been found to inhibit and resolve neuropathic and inflammatory pain. Intrathecal injection of anti-inflammatory T-regulatory cells (Treg) was able to reverse nerve trauma-induced mechanical pain (Liu et al. 2014). CD8+ T cells produce IL-10 to resolve chemotherapy-induced neuropathic pain (Krukowski et al. 2016). In inflamed hind paw, memory T cells could produce endogenous opioid peptides such as beta-endorphin to counteract inflammatory pain (Mousa et al. 2001). T cells may also promote the differentiation of macrophages into the M2 phenotype to resolve pain.

5.2.6 B Lymphocytes (B Cells)

B cells, also known as B lymphocytes, are a type of white blood cell that mediates adaptive immunity. B cells develop from hematopoietic stem cells that originate from bone marrow. Emerging evidence suggests that B-cell-produced autoantibodies play a critical role in autoimmunity and chronic pain. Complex regional pain syndrome (CRPS) is a posttraumatic autoimmune disease, and B cells are required for CRPS-like changes in a mouse CRPS model of tibia fracture (Guo et al. 2017b). It was postulated that fracture induces expression of neoantigens in the fracture limb to trigger B cells to secrete IgM antibodies. Other studies have identified IgG as a major cause of chronic pain in CRPS patients. Serum IgG, transferred from patients to mice, was shown to induce pain hypersensitivity. Moreover, in fibromyalgia and rheumatoid arthritis, passive transfer experiments have shown that either IgG or IgM antibodies from patient donors cause pain-like symptoms and other clinical symptoms that mimic clinical disorders (Goebel et al. 2022). IgG may promote microglia and astrocyte activation in the spinal cord. It is noteworthy that FcγRI, an immune receptor for IgG immune complex (IgG-IC), is expressed by DRG sensory neurons and neuronal FcγRI mediates acute and chronic joint pain in rheumatoid arthritis (Wang et al. 2019).

5.2.7 Natural Killer Cells (NK Cells)

Natural killer cells, also known as NK cells, are cytotoxic lymphocytes and analogous to the function of cytotoxic T cells. Following nerve injury, NK cells are involved in the degeneration of intact sensory afferents. Interestingly, endogenous ligand (Retinoic Acid Early 1, RAE1) for the NK cell receptor NKG2D is induced

in DRG neurons, leading to selective degeneration of injured axons. This neuroimmune interaction allows for selective NK cell-mediated degeneration of damaged sensory axons for Wallerian degeneration. Like macrophage phagocytosis of apoptotic cells, clearance of partially damaged nerves by NK cells may help to resolve painful neuropathy (Davies et al. 2019). Unbiased immune profiling also reveals a role of NK cells in fibromyalgia. Chronic activation and redistribution of circulating NK cells to the peripheral nerves may contribute to the immunopathology associated with FMS (Verma et al. 2022).

Pain research in the past two decades has demonstrated critical roles of glial cells in the pathogenesis of pain. Research also suggests a beneficial and preresolving role of glial cells, such as microglia (Chen et al. 2018).

5.2.8 Mast Cells

Mast cells are very similar to basophil granulocytes that contain many granules rich in histamine and heparin. Mast cells are resident cells of connective tissue. Mast cells are best known for their role in allergy, a chronic inflammatory disease. It is also well established that mast cells play an important role in itch (pruritus) by producing histamine. A subset of nociceptor neurons (nociceptors) express histamine receptors (e.g., H1 receptor) and respond to histamine, therefore, termed "pruriceptors" (itch-sensing sensory neurons). Mast cell activation was implicated in several pain syndromes, such as migraine, chronic pelvic pain, endometriosis, and vulvodynia. Mast cell hyperplasia was observed bladder pain syndrome and vulvodynia in patients with vulvodynia (Regauer 2016). Depletion of mast cells alleviated pain in a mouse model of vulvodynia (Arriaga-Gomez et al. 2019). A recent study reported that local immune response to food antigens drives meal-induced abdominal pain. This pain is triggered by bacterial infection and bacterial toxins, leading to the production of dietary-antigen-specific IgE antibodies in mice in the intestine. Subsequent oral ingestion of the dietary antigen will elicit visceral pain through an IgE- and mast-cell-dependent mechanism. Mechanistically, histamine released by mast cells can activate the H1 receptor to sensitize TRPV1-expressing visceral afferents. This finding is also clinically relevant to patients with irritable bowel syndrome (IBS), as injection of food antigens (gluten, wheat, soy and milk) into the rectosigmoid mucosa of IBS patients could induce local oedema and mast cell activation (Aguilera-Lizarraga et al. 2021).

5.2.9 Dendritic Cells

Dendritic cells are resident immune cells present in the skin of a specialized dendritic cell type called the Langerhans cell. A pioneer study reported that sensory neuron-derived neuropeptide CGRP is present in epidermal nerves and is associated

with Langerhans cells. Furthermore, CGRP inhibited LC antigen presentation, providing early evidence of local interaction between the nervous system and immunological function (Hosoi et al. 1993). Dendritic cells are also present in tissues that are in contact with the external environment, including the inner lining of the nose, lungs, stomach, and intestines. Upon activation, dendritic cells migrate to the lymph nodes where they interact with T cells and B cells to regulate adaptive immune response. Dendritic cells are antigen-presenting cells and functions to process antigen material and present it on the cell surface to the T cells. Unlike neutrophils/monocytes and T cells, dendritic cells serve as messengers between the innate and the adaptive immune systems. The role of dendritic cells in pain is not well understood. A recent study showed skin-resident dendritic cells promote postoperative pain. Skin incision upregulates the chemokines CCL17 and CCL22 in skin-resident dendritic and Langerhans cells, where their cognate receptor CCR4 is expressed by sensory neurons. CCL17 and CCL22 are sufficient to induce pain and nociceptor activation via CCR4 (Silva et al. 2022). However, Langerhans cell-deficient mice showed normal nociceptive sensitization and postoperative pain after fracture, despite an essential role of CGRP signaling in this process (Li et al. 2018). Dendritic cells also expressed Toll-like receptor 7 (TLR7) and activation of TLR7 produces Type-I interferons (e.g., IFN-α) to mediate antiviral effects, meanwhile changing pain sensitivity (Tan et al. 2021).

5.3 Keratinocytes, Fibroblasts, and Bone Marrow Stromal Cells in Pain

5.3.1 Keratinocytes

Keratinocytes are the cells that make up most of the epidermis, the outermost layer of skin. These cells produce keratin, a protein that is a major constituent of hair and nails. Keratinocytes are located near the peripheral terminals of nociceptors, where painful stimuli are initially sensed. The cells produce several mediators, including adenosine triphosphate (ATP), IL-1β, prostaglandin E2, endothelin (which can raise blood pressure), and nerve growth factor (NGF), which can promote the growth of pain-sensing nerve fibers.

Channelrhodopsin is a type of light-sensitive protein commonly used in a lab technique known as optogenetics, wherein channelrhodopsin is incorporated into a particular kind of cell, allowing those cells to be activated via light. The activation of those cells then causes animals to behave in a certain way, which researchers can observe. For example, expression of channelrhodopsin 2 (ChR2) in nociceptors will make it possible that blue light stimulation in a hindpaw can elicit painful behaviors in mice (e.g., lifting and licking) (Ji et al. 2021). Researchers also tried to put ChR2 in keratinocytes. It has been found that light stimulation of keratinocytes in a hind paw causes mice to behave in a nocifensive way, or as if in response to pain (Baumbauer et al. 2015).

Notably, keratinocytes can produce both pleasure and pain. Consider going out for a walk on a summer day. At first, the warmth of the sun on your skin feels gentle and pleasing. At this time, your keratinocytes are producing endogenous opioid peptide beta-endorphin, a type of protein that can relieve pain and produce a "reward" sensation. After 2 h in the sun with insufficient sun protection, however, you develop a sunburn. Now, your keratinocytes are no longer producing endogenous opioid peptide beta-endorphin. Instead, the excessive light exposure results in the activation of a protein called TRPV4, which reacts to changes in temperature. This results in the release of endothelin, a peptide that can increase blood pressure by constricting blood vessels, onto nociceptors in your skin, causing you to feel pain—a lot of it (Moore et al. 2013). Intriguingly, keratinocytes are able to release inflammatory mediators like cytokines despite not being immune cells, showing how immune cells are not the only non-neuronal cells involved in pain.

5.3.2 Fibroblasts

Fibroblasts are the most common cells of connective tissue in animals. They produce the extracellular matrix and collagen and play a crucial role in wound healing. Fibroblasts maintain homeostasis in tissues. In inflammatory diseases, however, fibroblasts can become inflammatory cells or recruit leukocytes to promote angiogenesis, in turn promoting chronic inflammation. Single-cell profiling techniques have allowed fibroblast states to be observed in greater detail, showing that fibroblasts may play a pathological role in many diseases. Fibroblasts are especially known to be involved in rheumatoid arthritis and inflammatory bowel disease. In a recent study, single-cell RNA sequence (scRNA-Seq) on colonic mesenchymal cells from patients with ulcerative colitis revealed that among the fibroblast subtypes, S1 fibroblasts were distributed throughout the lamina propria and exhibited elevated expression of TNF-responsive genes, while S2 fibroblasts were restricted to areas close to the epithelium and displayed high expression of BMPs (BMP2 and BMP5) and noncanonical Wnt ligands (WNT5a and WNT5b) (Wei et al. 2021).

Increasing evidence suggests a role of fibroblasts in the pathogenesis of arthritic pain. Within the knee joint, distal endings of DRG neurons communicate with fibroblast-like synoviocytes (FLS), and these FLS secrete inflammatory mediators to promote peripheral sensitization of sensory neurons innervating the knee. RNA sequencing has demonstrated detectable levels of proinflammatory genes in FLS derived from arthritis patients (Chakrabarti et al. 2020). The fibroblast-derived protein PI16 (Peptidase inhibitor 16) was shown to promote neuropathic pain in mice. Nerve injury increases PI16 protein levels in fibroblasts in DRG meninges and in the epi/perineurium of the sciatic nerve (Singhmar et al. 2020).

5.3.3 *Bone Marrow Stromal (Stem) Cells*

Regenerative pain medicine refers to using the body's own recovery mechanisms to heal pain (Buchheit et al. 2020). This field of medicine is particularly exciting for pain scientists due to the pain-relieving potential such methods have shown in degenerative arthritis (where the cartilage in joints slowly breaks down) and neurologic diseases. Generally, the products used in regenerative pain medicine can be split into two categories: cellular products and blood-derived products. Cellular products refer to those products taken from bone marrow, lipids (i.e., fats), and the umbilical cord. Blood-derived products refer to those products taken from components of the blood, such as plasma rich in platelets (which are critical for blood clotting) and autologous conditioned serum.

Mesenchymal stem cells, also known as mesenchymal stromal cells (MSCs), are cells found in nearly all organs in a space around the blood vessels called the perivascular space. They are most prominent in the bone marrow but can also be taken from tissue like the umbilical cord, peripheral blood, and fat. These cells are important because of their ability to differentiate, or develop, into a variety of other mesodermal cells, including cartilage, fat, muscle, and bone.

Most laboratory studies used bone marrow-derived stromal/stem cells (BMSC). These cells can be delivered into the body through either intravenous injection (where the cells are delivered to the body's circulatory system via a vein) or localized injection (where the cells are delivered straight to the site of injury). A number of studies have described the powerful pain-relieving effects of BMSCs when used in models of mice, rats, and other rodents with neuropathic pain induced by nerve injury, spinal cord injury, neuropathy from diabetes induced by streptozotocin (which is toxic to insulin-producing cells found in the pancreas), and arthritis. These effects were seen with BMSCs taken from mice, rats, and human bone marrow (Huh et al. 2017).

Both intravenous and local injection of rat BMSCs were powerful enough to reverse mechanical allodynia (where even a light touch can cause pain) in rats after they had suffered a tendon injury (Guo et al. 2011). Researchers found that this antiallodynic effect could be blocked by naloxone, an opioid receptor antagonist with the ability to inhibit opioids from binding with their receptors. This result seems to suggest that endogenous opioids, or opioids produced by the body itself, have some role in the antiallodynic effect of BMSCs. Later on, a follow-up study showed that immune interactions and the activation of monocytes also play a part in this antiallodynic effect (Guo et al. 2017a, b).

Another study found that just one intrathecal lumbar injection (in which the needle is inserted between bones low on your spinal cord) of 250,000 mouse BMSCs resulted in effective pain-relief for more than 6 weeks in mouse models of neuropathic pain. Further analysis showed that the injected BMSCs moved to the dorsal root ganglia and the spinal cord meninges (membranes around the spinal cord and part of DRG that contain cerebrospinal fluid), where they proceeded to survive for 3 months (Chen et al. 2015).

Intrathecal injections performed as described above also reduced neuroinflammation in the spinal cord that arose from nerve injury, particularly decreasing or even reversing the activation of microglia and astrocytes via a type of cell communication called paracrine communication, in which cells communicate directly with nearby cells (Chen et al. 2015).

MSC treatment has likewise been shown to be promising in treating chronic neuropathic conditions, with the ability to reverse opioid tolerance and hyperalgesia induced by chronic opioid exposure (Hua et al. 2016). Please see more details in Chaps. 10 and 11.

5.4 Microglia in Pain

5.4.1 Microglia in Health

Microglia are a type of glial cell found through the central nervous system and play an important physiological role in the CNS (Kettenmann et al. 2011). They make up 10–15% of all cells in the brain, where they act as resident macrophages, and thus the primary form of active immune defense in the CNS. Microglia are spread through the CNS in specific, nonoverlapping areas. They are indispensable in immune surveillance and the maintenance of brain homeostasis: they search for waste products such as damaged or unnecessary neurons and synapses, infectious agents, and plaques. These processes need to be maximally efficient to prevent lethal brain damage following injury and infection. As a result, microglia are extraordinarily sensitive to all pathological changes in the CNS. Such sensitivity is possible due to changes in extracellular potassium, ATP, or neurotransmitter. Recent studies have also demonstrated microglia to be crucial players in the brain under nonpathological conditions, allowing for the maintenance of normal brain functions. By overseeing neuronal functions using direct somatic contacts, microglia are also able to provide neuroprotective effects as necessary.

The components of the CNS, the brain and the spinal cord, normally cannot be accessed by pathogens due the blood–brain barrier (BBB), a series of endothelial cells that is capable of preventing most infections from reaching the susceptible tissue of the nervous system. However, when infectious agents do make it across the BBB (e.g., during inflammation, when the BBB becomes more permeable), microglia mobilize rapidly to diminish inflammation and dispose of pathogens before any damage can be dealt to neural tissue. Because most antibodies from the rest of the body are unable to cross the BBB (very few are small enough to do so), microglia must also be capable of foreign agent recognition. Once they recognize foreign agents, they will swallow them and present the pieces of said agent, effectively acting like an antigen-presenting cell, which then allows them to activate T cells.

Microglia can change their structure to suit the particular needs of the area in which they are located. The form microglia adopt, known as a phenotype, depends

on both local conditions as well as any chemical signals they may receive. Such extraordinary plasticity allows microglia to achieve a wide variety of functions. This plasticity also sets microglia apart from macrophages, which need to be constantly replaced, and gives microglia the ability to react rapidly to foreign agents without causing disturbances in the immune system (Kreutzberg 1996).

After inflammation, microglia undertake a couple of steps to promote neural tissue regrowth. These include synaptic stripping (Salter and Stevens 2017), as well as secretion of anti-inflammatory cytokines, recruitment of neurons and astrocytes to the damaged area, and formation of gitter cells. These steps speed up and make possible regrowth and remapping in resident CNS areas and vascular systems around the brain, respectively. Recent studies have demonstrated that microglial processes continuously oversee the neuronal functions through specialized somatic junctions and sense nerve cell "well-being" (Liu et al. 2019). This intercellular communication pathway allows microglia to exert notable neuroprotective effects, contributing robustly to repair following brain injury.

5.4.2 Microglia in Disease

When microglia are activated, they become amoeboid in shape and change their gene expression. This new, altered gene expression can cause the production of a number of potentially neurotoxic mediators. These mediators are important in the normal functions of microglia and their production; they are usually reduced once the microglia complete their task. In chronic neuroinflammation; however, microglia remain activated for an extended period during, which means the production of mediators is also sustained. This increase in mediators plays a role in neuronal degeneration including loss of synapses and neuronal death.

Neuroinflammation is not the same as inflammation in other organs but does include several similar mechanisms (e.g., the localized production of chemoattractant molecules to the site of inflammation) (Ji et al. 2018). The following are a few of the many substances microglia secrete upon activation (Heneka et al. 2015).

Microglia produce many proinflammatory cytokines such as IFN-γ, IL-1α, IL-1β, and TNF-α in the CNS (Hanisch 2002). Direct injection of the cytokines IL-1β and TNF-α into the CNS can produce local inflammatory responses and neuronal degradation. As such, it is possible that cytokines may play a role in neurodegeneration when microglia remain in a sustained activated state. Pro- and anti-inflammatory cytokines contribute differently to the neuroinflammatory process following acute brain injury. Microglia can produce certain chemokines such as MCP-1 (CCL2), MIP-1α, and MIP-1β. Microglia also express chemokine receptors such as CX3CR1, CXCR2, CCR3, CCR5, and CXCR4. Multiple proteases are also implicated in microglial signaling. When microglia are activated, they cause proteolytic enzymes to be produced and secreted. A number of proteases can degrade both neuronal cells and the extracellular matrix in the vicinity of the activated microglia. These proteases include cathepsins (B, K, L, and S) and matrix

metalloproteinases (MMP-1, MMP-2, MMP-3, and MMP-9; and ADAM8 or plasminogen). They form outside microglia and degrade the extracellular matrix.

Microglia play a critical role in neurodegenerative disorders (disorders involving progressive cell loss in particular populations of neurons). Many normal functions of glia can be lost or damaged when the cells become chronically activated in progressive neurodegenerative disorders. A significant body of evidence has demonstrated that activated glial cells play destructive roles via direct and indirect inflammatory attacks in neurodegenerative disorders. The following list includes the role of microglia in some well-known neurodegenerative disorders (Salter and Stevens 2017).

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, in which the brain develops abnormal clumps (amyloid plaques) and tangled fiber bundles (neurofibrillary tangles). Notably, microglia prominently express many of the genes associated with heightened AD risk. Triggering receptor expressed on myeloid cell 2 (TREM2) is a cell surface receptor on microglia that notably interacts Apolipoprotein E. TREM2 has been shown to be associated with heightened AD risk. Soluble TREM2 mediates the clearance of pathological amyloid-β (Aβ) in AD (Zhao et al. 2022). Studies have shown high levels of activated microglia overexpressing IL-1 in the brains of Alzheimer patients. These microglia are distributed with both Aβ plaques and neurofibrillary tangles. Such overexpression of IL-1 results in excessive tau phosphorylation, which is related to tangle development in Alzheimer's disease. Parkinson's disease is a movement disorder involving abnormal functioning of dopamine-producing neurons in the brain. The neurons of the substantia nigra become dysfunctional and eventually die, resulting in a lack of dopamine input into the striatum. Studies suggest that glial cell line-derived neurotrophic factor (GDNF) may be able to chemoprotect the cells of the substantia nigra. Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of CNS, characterized by focal lesions of inflammation, axonal loss, gliosis, and demyelination that affect both white and gray matter. In MS patients, destruction of myelin in the CNS is associated with activated macrophages and microglia, which are suspected to be involved in the pathogenesis of the disease. While most of the findings of microglial activation are non-MS specific, the M1 activation (CD40, CD86) appears to be specific for this disease.

5.4.3 Microglia in Chronic Pain

A major research progress in pain research in the past two decades is to demonstrate critical roles of microglia in the pathogenesis of pain, especially neuropathic pain. Many review articles have been published to discuss this important topic in pain research (Chen et al. 2018; Inoue and Tsuda 2018; Ji et al. 2013; Tsuda et al. 2005). Peripheral nerve injury results in microgliosis, which causes the cells to increase in

size, shorten cellular processes, and change from a branching shape (ramified) to a blobby shape (ameboid). Nerve injury also causes rapid proliferation of microglia, an increase in the number of microglia in the first week of injury. Furthermore, nerve injury results in rapid activation of intracellular signaling pathways, such as activation of MAP kinases (p38 and ERK) by phosphorylation. Cellular markers for microglia such as IBA1, CD11b, and CX3CR1 are increased after nerve injury and other painful injuries such as arthritis and cancer. Microglial activation is broad term and can include all the changes in morphology, gene expression, and signal transduction pathways. Following painful injuries, microglia can be activated by many mediators that are known to cause pain. These microglial activators include ATP, cytokines and chemokines, CSF-1 (colony-stimulating factor), proteases, and ligands of TLRs (HMGB1), which can activate multiple receptors present in microglia, including CX3CR1, CSFR1, TLR4, P2X4, P2X7, and P2Y12 (Chen et al. 2018; Guan et al. 2016). Activation of each of these microglial receptors will result in phosphorylation of p38 MAP kinase, which can trigger downstream signaling in gene expression and protein synthesis, leading to increased production and secretion of TNF-α, IL-1β, IL-18, and BDNF (brain-derived neurotrophic factor) (Coull et al. 2005; Ji and Suter 2007) (Fig. 5.2).

Importantly, these neuromodulators produced by microglia, however, have the ability to rapidly change synaptic plasticity in the spinal cord pain circuit. Often, synaptic plasticity can be induced by tissue or nerve injury known to cause central sensitization. This process can maintain chronic pain and cause pain to spread far beyond the initial site of injury. The process can also result in mechanical allodynia, wherein even a light touch can generate intense pain. Strikingly, these synaptic plastic changes can be induced by microglial mediators (TNF-α, IL-1β, IL-18, and BDNF). For example, IL-1β and BDNF not only increase excitatory synaptic transmission but also suppress inhibitory synaptic transmission (Coull et al. 2005; Kawasaki et al. 2008b). Numerous studies from many labs in the world have shown that inhibition of microglial function by various pharmacological and genetic manipulations (including chemogenetic and optogenetic manipulations) can alleviate pain (Grace et al. 2018; Jin et al. 2003; Parusel et al. 2022; Raghavendra et al. 2003; Tsuda et al. 2003; Yao et al. 2016).

While most studies support a pronociceptive role of microglia in driving chronic pain, microglia also have a protective role in maintaining homeostasis of the CNS (Chen et al. 2018). Recently, it was found that CD11c-expressing spinal microglia contribute to the resolution of neuropathic pain. Interestingly, these cells appear in the late-phase (3 weeks) following nerve injury. In pain-recovered mice, the depletion of CD11c+ microglia resulted in a relapse of neuropathic pain hypersensitivity (Kohno et al. 2022).

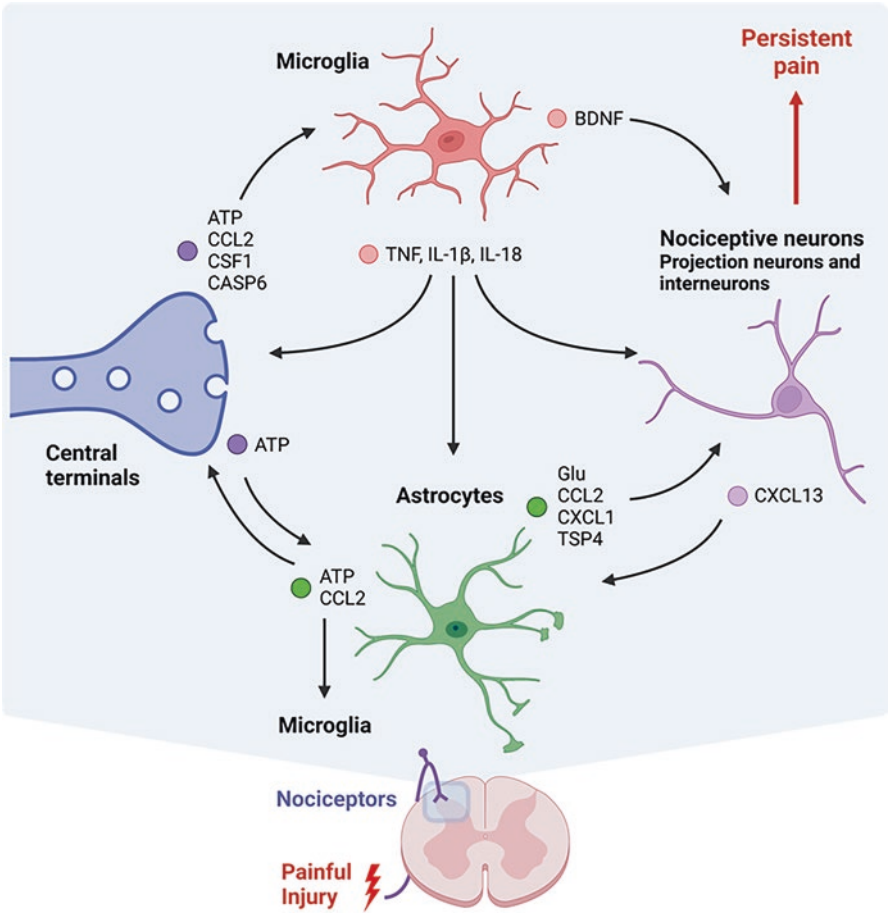

Fig. 5.2 Neuron–glial interactions in the spinal cord for the amplification of chronic pain Painful injuries such as nerve injury, arthritis, cancer, and treatment (chemotherapy) cause hyperactivity of nociceptors and secretion of glial modulators from their central terminals. As a result, microglia and astrocytes in the spinal cord dorsal horn are activated by the glial mediators. Following their activation, microglia and astrocytes secrete neuromodulators, such as cytokines (TNF, IL-1β, and IL-18) and chemokines (CCL2, CXCL1), to induce and maintain chronic pain by driving synaptic and neuronal plasticity. Pre- and postsynaptic neurons can both "listen" and "talk" to microglia and astrocytes. Notably, neurons can produce chemokine CXCL13, which can activate astrocytes via CCR5 receptor. *BDNF* brain-derived growth factor, *CASP6* caspase-6, *CSF-1* colony-stimulating factor 1, *Glu* glutamate, *TSP4* thrombospondin-4. (Modified from Ji et al., Science, Ji et al. 2016 with permission)

5.5 Astrocytes in Pain

5.5.1 Astrocytes in Physiological Conditions

Astrocytes are a particularly interesting group of glial cells, with a number of unique characteristics. Named for their star-like shape, astrocytes make up nearly 20–40% of all glial cells. Unlike other glial cells, astrocytes are physically joined to each other via proteins known as gap-junction protein complexes, named connexins (e.g., Cx-43). These complexes allow astrocytes to exchange materials with each other and mediate long-range signaling. Like microglia, astrocytes play an important physiological role in the CNS (Verkhratsky and Nedergaard 2018).

Traditionally, these star-shaped cells were believed to provide structure and nutrition to neurons; however, they are now also known to be actively involved in a number of neural processes. Uniquely, the cytosol of astrocytes is physically joined to each other via gap-junction protein complexes; such a connection expedites the exchange of substances between cells. Glial fibrillary acidic protein (GFAP) expression is another defining feature of astrocytes possessed by no other CNS glial cells. Astrocytes use GFAP in all primary branches and processes; when astrocytes enter injury-induced reactive states, GFAP expression will change as well. Notably, each astrocyte has its own domain or territory, which other astrocytes usually do not enter. When astrocytes enter reactive states; however, such territorial organization is disturbed. Further research must be done for us to understand the significance of this organization, however. Additionally, astrocytes are able to control blood flow during neuronal activation due to their considerable contact with cerebral blood vessels.

Likewise, astrocytes have substantial contact with inter-neuronal synapses; in rodent brains, just one cortical astrocyte can contract 4–6 neuronal soma and 300–600 neuronal dendrites, as well as wrap a shocking 140,000 synapses. Astrocytes differ dramatically in size and morphological complexity from species to species; for example, a human cortical astrocyte is double the size of a rodent cortical astrocyte in diameter. A human cortical astrocyte can also extend 10 times as many primary processes as a rodent cortical astrocyte can, allowing it to contact upward of two million synapses. More and more evidence points to associations between increased astrocyte morphological complexity and increased cognitive function (Oberheim et al. 2006). Astrocytes secrete several adhesin molecules, such as neuroligins. These adhesin molecules not only control astrocyte morphogenesis but also regulate synaptogenesis. Notably, synapses are covered by astrocytic processes as sculpture (Stogsdill et al. 2017).

5.5.2 *Astrocytes in Physiological Pain*

Under normal, nonpathological conditions, astrocytes supply metabolic support to neurons and help maintain healthy levels of glutamate, extracellular potassium ions (K^+), and water via expressing specific transporters for these molecules. Using their connections via gap-junction proteins, astrocytes can spread calcium waves, thereby spreading several long-distance signaling molecules. Additionally, astrocytes wrap around synapses to create a barrier that both supplies metabolic support and protects synapses from glutamate spillover. During synaptic development and transmission, the substantial contact astrocytes have with synapses that allow the cells to control local interstitial chemical and ionic environments. Furthermore, astrocytes are importantly involved in regulating the formation, maturation, maintenance, and elimination of synapses via a number of contact-mediated and diffusible signals. Increasingly, researchers have come to realize that astrocytes are actively involved in synaptic transmission regulation in the CNS; this fact suggests that processes of astrocytes may be parts of synapses as well, communicating with both presynaptic and postsynaptic terminals to regulate synaptic transmission (Araque et al. 1999). Moreover, astrocytes also make up the foundation of the glymphatic system, a fluid transport system that is responsible for clearing proteinaceous waste products (including excess beta-amyloid, tau protein, synuclein, and metabolic byproducts) throughout the brain (Ji et al. 2019; Nedergaard 2013).

Recent studies show that astrocytes in the spinal cord dorsal horn (SDH) may inhibit pain signaling under physiological conditions in normal animals (Ji et al. 2019). ATP derived from astrocytes produces adenosine in the extracellular space when hydrolyzed. Adenosine can inhibit nociception by activating adenosine A1 receptors located on sensory neurons including nociceptors. When gliotransmission was weakened via overexpression of a dominant-negative SNARE domain (dnSNARE mice) in glial cells, mechanical pain thresholds decreased. This result points to gliotransmission as a modulator of baseline mechanical nociception that normally inhibits pain (Foley et al. 2011). Additionally, spinal astrocytes can produce type-1 interferons (IFN-1) and anti-inflammatory cytokines, which can bind to IFN-I receptors on primary afferent presynaptic terminals to prevent spinal cord nociceptive synaptic transmission (Liu et al. 2016). Allodynia in naive animals could also be manifested by downregulating connexin (CX43), which primarily mediates communication between spinal astrocytes. Recently, astrocytes were shown to regulate pain gating in the spinal cord of noninjured animals following spinal cord stimulation of large Aβ-afferents, in further support of an antinociceptive role of astrocyte network in homeostatic status (Xu et al. 2021).

5.5.3 *Astrocytes in Pathological Pain*

Increasingly, evidence also points to an active role of astrocytes in driving CNS diseases. Astrocytes have been identified to be the key regulators of several diseases of the CNS, including neurodegenerative, neuropsychiatric, and neurodevelopmental diseases, as well as gliomas (Hasel et al. 2021; Stogsdill et al. 2017). Recent progress revealed that neurotoxic reactive astrocytes (A1) are induced by activated microglia under disease conditions (Liddelow et al. 2017). Astrocytes are thought to regulate such diseases via neuroinflammation, which can result in persistent chronic pain (Hasel et al. 2021; Ji et al. 2019).

Astrocytes also play a crucial role in the development and maintenance of chronic pain. For example, a correlation between spinal cord dorsal horn (SDH) astrocyte hypertrophy and pain hypersensitivity has been seen after a number of chronic pain-inducing injuries. Nerve trauma, spinal cord injury, chronic opioid exposure, intraplantar injection of CFA, bone cancer, chemotherapy, and HIV-induced neuropathy have all produced significant astrogliosis in rodents, as seen from the higher levels of GFAP expression. After nerve injury, astrogliosis lasts much longer than microgliosis, suggesting that astrocytes may play a more important role in maintaining neuropathic pain than microglia. Furthermore, many painful insults, such as inflammatory pain, chemotherapy-induced pain, and some cancer-induced pain, are more associated astrogliosis than microgliosis (Ji et al. 2013). Analysis of post-mortem spinal cord tissue has further shown that SDH astrocyte activation in HIV patients is tightly associated with chronic pain (Shi et al. 2012).

Several signaling molecules have been shown to induce and maintain inflammatory and neuropathic pain, including ERK, JNK (c-Jun N-terminal kinase), chemokines (CCL2, CXCL1, CXCL13), and Cx43 (Gao and Ji 2010; Fig. 5.2). After nerve injury, activation of Cx43 in astrocytes results in a release of chemokines (CXCL1 and CCL2), which can drive neuropathic pain via interaction with their neuronal receptors (CXCR2 and CCR2) (Chen et al. 2014). Nerve injury was shown to upregulate CXCL13 in spinal cord neurons, which then activates its CCR5 receptor in astrocytes, driving persistent activation of astrocytes that maintains neuropathic pain (Jiang et al. 2016). A population of astrocytes located in the superficial laminae of SDH is genetically defined by the transcription factor Hes5, and chemogenetic stimulation of Hes5+ SDH astrocytes is sufficient to produce mechanical pain hypersensitivity (Kohro et al. 2020). Recently, it was reported that by combining a transient nerve block to inhibit noxious afferent input from injured peripheral nerves, which is combined with simultaneous activation of astrocytes in the somatosensory cortex (S1), the authors were able to reverse neuropathic pain after nerve injury: activation of S1 astrocytes either low intensity transcranial direct current stimulation (tDCS) or via the chemogenetic DREADD system reversed allodynia. This study suggests a possible role of astrocytes in resolving chronic pain (Takeda et al. 2022).

5.6 Satellite Glial Cells, Schwann Cells, and Oligodendrocytes in Pain

5.6.1 Satellite Glial Cells (SGCs)

SGCs are prominent glial cells in the PNS and wrap around neuronal cell bodies and form a complete envelope, allowing for close neuron-SGC interactions in dorsal root ganglia (DRG) and trigeminal ganglia where the cell bodies of primary sensory neurons including nociceptive neurons are present (Hanani and Spray 2020, Fig. 5a). SGCs are derived from neural crest cells and are characterized by thin cellular sheaths that surround the individual neurons. Despite different locations in the PNS and CNS, SGCs share many features with astrocytes. For example, they express similar markers such as GFAP, GLAST, ALDH1L1, and Hevin/SPARCL1, and are interconnected by gap-junctions (Ji et al. 2013). Like astrocytes, SGCs express high levels of inwardly rectifying K+ channels 4.1, Kir4.1, coded by KCNJ10 (Vit et al. 2008), which enables SGCs to control the perineural potassium homeostasis and neuronal excitability. Several lines of evidence indicate that SGCs participate in the generation and maintenance of chronic pain. SGCs are rapidly activated after painful injuries and play an active role in the development of persistent pain (Hanani et al. 2002; Jasmin et al. 2010). The gap-junction coupling between SGCs surrounding individual neurons is augmented in pathological pain conditions such as nerve injury and inflammation (Chen et al. 2008; Hanani et al. 2002). Kir4.1 is downregulated under pathological pain conditions. Strikingly, knockdown of Kir4.1 expression in SGCs is sufficient to induce pain hypersensitivity in naïve animals (Vit et al. 2008). Furthermore, upon activation, SGCs release proinflammatory cytokines, such as TNF-α and IL-1β that can drive hyperexcitability of surrounding sensory neurons (Hanani and Spray 2020; Kawasaki et al. 2008a).

SGC activation (e.g., GFAP upregulation) has been shown after multiple painful injuries (Ji et al. 2013). SGC's reaction after nerve injury occurs very rapidly, becoming evident within 4 h, peaking at 1 week but declining after 3 weeks. This time course of SGC reaction suggests a possible role of SCGs in the induction and early maintenance of neuropathic pain (Zhang et al. 2009). Continuous infusion of fluorocitrate, a glial metabolism inhibitor, to the affected DRG via mini-osmotic pump alleviated mechanical allodynia in the early phase of nerve injury (Liu et al. 2012). Notably, a brief subcutaneous injection of morphine is sufficient to activate SGCs within 2 h, and this rapid SGC activation contributes to opioid-induced hyperalgesia (Berta et al. 2012).

5.6.2 Schwann Cells

Schwann cells are the most abundant cells in the PNS and include two broad categories: myelinating and nonmyelinating Schwann cells (Chen et al. 2003). It was generally thought that noxious stimuli directly activate nociceptive sensory nerve endings in the skin. A group of scientists in Karolinska Institute discovered a specialized cutaneous glial cell type with extensive processes forming a mesh-like network in the subepidermal border of the skin. They also demonstrate a direct excitatory functional connection to sensory neurons. These specialized cutaneous Schwann cells can initiate pain sensation (Abdo et al. 2019). Schwann cells physically support the long axons in the peripheral nerves (e.g., sciatic nerve) and release a variety of growth factors to nourish and myelinate the associated axons. After the sciatic nerve injury, which is commonly associated with neuropathic pain, activated Schwann cells undergo dramatic changes such as proliferation, migration, and release of numerous pronociceptive mediators, including proinflammatory cytokines and chemokines (e.g., TNF, IL-1β, IL-6, CCL2), growth factors (NGF and BDNF), and proteases (MMP-9 and MMP-2). Schwann cells also express ATP receptors (P2X2/3, P2X7), TLR2/4, and TRPA1 (Goncalves et al. 2017; Wei et al. 2019). Schwann cells also play a protective role against the pathogenesis of diabetic neuropathy through multicell interactions among Schwann cells, axons, and microvessels (Goncalves et al. 2017; Wei et al. 2019).

Under acute injury conditions, physical or metabolic damage to the peripheral nerves induces rapid and robust changes in the synthesis of neurotrophins in neurons and Schwann cells to guide and support regeneration. It has been shown that macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. Mechanistically, hypoxia upregulates vascular endothelial growth factor (VEGF) production after nerve injury, initiating migration of Schwann cells. Furthermore, VEGF stimulates the formation of endoneurial blood vessels into hypoxic areas, therefore, directing Schwann cells to bridge the gap between the proximal and distal nerve stumps (Cattin et al. 2015). However, diabetes can reduce the expression of the Schwann-cell-derived neurotrophic factors such as ciliary neurotrophic factor, leading to neuropathy (Goncalves et al. 2017).

5.6.3 Oligodendrocytes

Preterm birth can be caused by systemic inflammation (maternal/fetal infection) that leads to neuroinflammation and white matter injury. Various cytokines and chemokines are upregulated in oligodendrocytes in response to inflammation. Oligodendrocytes express several receptors for immune related molecules, allowing them to sense inflammation and to react. An in-vivo model mimicking inflammation-mediated white matter injury of preterm born infants consisting of intraperitoneal injection of IL-1β from P1 to P5 showed that in the IL-1β-treated animals,

upregulation was higher in O4+ immature oligodendrocytes as compared to PDGFRα+ oligodendrocyte precursor cells (OPCs), suggesting a different sensitivity to neuroinflammation. In oligodendrocytes primary cultures, cells treated with TLR3 agonist Poly(I:C) during differentiation showed a stronger upregulation of proinflammatory chemokines CCL2 and CXCL10 compared to cells treated during proliferation and led to decreased expression of myelin genes. Oligodendrocytes were also able to modulate microglia phenotype and function, depending on their maturation. Overall, inflammation in the response of oligodendrocytes can play an autonomous role in the blockade of their own differentiation. In addition, the immune activation of oligodendrocytes may play an important role in shaping the response of microglia during inflammation (Boccazzi et al. 2021).

5.7 Gliopathy and Neuroinflammation

5.7.1 Gliopathy

It has been proposed that chronic pain can manifest not only by "neuropathy" but also by "gliopathy," that is, dysfunction of glial cells (Ji et al. 2013). Under normal physiological conditions, astrocytes and SGCs provide trophic support to neurons and maintain the homeostasis of potassium, glutamate, and water in the CNS and PNS (Ji et al. 2019; Verkhratsky et al. 2012). Astrocytes and SGCs could also "insulate" the neural circuit of pain in the CNS and PNS, by (1) forming structural barrier and (2) keep the circuit silent via releasing inhibitory mediators, such as adenosine (Nedergaard and Verkhratsky 2012; Xu et al. 2021). Following nerve injury, chronic pain is not only associated with neuropathy but also manifest as "gliopathy." Astrocytes and SGCs lose their ability to maintain the homeostasis of K+ and glutamate, leading to hyperexcitability of nociceptors and spinal cord pain transmission neurons, due to increased extracellular levels of glutamate and K+. Astrocytes also express the water channel aquaporin 4 (AQP4) to maintain the water homeostasis and mediate the glymphatic function (Ji et al. 2019; Nedergaard 2013; Simard and Nedergaard 2004). Dysfunction of astrocytes will also result in edema in the CNS and PNS (Verkhratsky et al. 2012). As a result of gliopathy, glia can no longer insulate the pain circuit, and rather, they serve as an amplifier of pain, by producing proinflammatory and pronociceptive mediators. Recently, Schwannopathy was proposed as an integral factor in the pathogenesis of diabetic neuropathy; disruption of the interactions between Schwann cells, axons, and microvessels contributes to the disease (Goncalves et al. 2017). Furthermore, gliopathy also drives comorbidities of chronic pain, including depression, sleep deprivation, and anxiety, which will further exacerbate pain (Ji et al. 2019).

5.7.2 Neuroinflammation

Neuroinflammation, as mentioned above, refers to inflammation of the nervous tissue. It is a localized form of inflammation that can occur in both the peripheral nervous system and the central nervous system. Notable features of neuroinflammation include increased vascular permeability, leukocyte infiltration, glial cell activation, and increased production of inflammatory mediators such as chemokines and cytokines (Ji et al. 2014). Increased vascular permeability allows for immune cells, such as neutrophils, to travel more easily through blood vessels to the site of injury. During neuroinflammation, the blood–brain barrier (BBB) is likely to become more permeable. The blood–brain barrier refers to a layer of endothelial cells (cells that line blood vessels) that selectively allow substances diffused in the blood to access the brain (these substances are usually lipid-soluble, such as oxygen and carbon dioxide). However, when the blood–brain barrier is disrupted, substances that previously could not access the brain gain access. The combined effects of both glial cells and peripheral immune cells often significantly exacerbate neuroinflammation.

Neuroinflammation is involved in a variety of neurological and neuropsychiatric diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, autism, depression, and schizophrenia, as well as neuroinflammation associated with major surgeries (Yang et al. 2020). Neuroinflammation has been implicated in a variety of chronic pain conditions, including neuropathic pain after diabetic and HIV neuropathy (Ellis and Bennett 2013), arthritic pain, and postsurgical chronic pain, as well as complex regional pain syndrome (CRPS). Notably, neuroinflammation drives widespread pain such as fibromyalgia. Fibromyalgia is a condition affecting the musculoskeletal system that causes widespread pain throughout the body, which can disturb sleep, memory, and mood (Ji et al. 2018). Fibromyalgia may also involve small fiber neuropathy, which is associated with neuroinflammation in the PNS (Üçeyler et al. 2013). Currently, researchers are testing several novel therapies that may control neuroinflammation, including regenerative therapies and neuromodulation (Buchheit et al. 2020; Tao et al. 2020).

5.8 Central Sensitization and Neuroinflammation

The strength of a synapse, or connection between neurons, can vary widely. Sometimes synapses may be completely silent, transmitting no signals whatsoever between neurons; other times, synapses may be completely effective, transmitting even the slightest of signals. Synaptic strength changes depending on the concentration of neurotransmitters released from the presynaptic terminal (presynaptic modulation) and the sensitivity of the postsynaptic membrane to those neurotransmitters (postsynaptic modulation). Changes in synaptic strength and structure are known as synaptic plasticity, a mechanism crucial to our daily functioning. For example, long-term potentiation (LTP), a mechanism that depends on synaptic plasticity,

creates a long-lasting increase in synaptic strength when a synapse is frequently stimulated. LTP activity in the hippocampus is the reason why we are able to create lasting memories. Without these memories, we would be incapable of learning. Thus, without neural plasticity, we would be utterly unable to adapt to the various stimuli in our environment.

Synaptic plasticity also plays an enormous role in pain. Nociceptive sensitization refers to how an injury can lower an organism's pain threshold. Evolutionarily, such a mechanism makes sense; it prevents an organism from overexerting itself so that the organism can rest and heal. However, nociceptive sensitization can also result in debilitating pain, like chronic pain, that does no good whatsoever. Usually, nociceptive pain generated in such a way has a threshold and is short-lived. However, postinjury pain—pain arising from damage or inflammation in peripheral tissue or lesions to the nervous system—often leads to lasting pain hypersensitivity. Central sensitization importantly contributes to such postinjury pain hypersensitivity.

Activity-dependent central sensitization begins with a high-frequency burst of nociceptor input, resulting in a reduction in threshold, increase in responsiveness of dorsal horn neurons, and increase of receptive fields (Ji et al. 2003; Latremoliere and Woolf 2009). Central sensitization has clinical utility for the diagnosis and treatment of clinical pain (Woolf 2011; Treede et al. 2022). Activity-dependent central sensitization can occur just seconds after the nociceptive conditioning stimulus and can persist for hours after the stimulus has subsided. If even low levels of the stimulus remain, the central sensitization will continue (e.g., following peripheral nerve injury, persistent ectopic activity of the sensory fibers can cause central sensitization to continue). Most of the input dorsal horns receive is below threshold. As a result, under normal circumstances, no signal would be transmitted. However, just a 10–20 s burst of nociceptor-conditioning stimulus produces activity-dependent central sensitization, increasing synaptic effectiveness to the point that even subthreshold these signals can be transmitted. Notably, the increase in synaptic effectiveness applies to both nociceptive central terminal synapses (activated by conditioned stimulus) and synapses produced by Aβ fibers, low-threshold mechanosensitive fibers, on dorsal horn neurons. Usually, Aβ fibers are not activated by conditioned stimuli. However, due to heterosynaptic plasticity, a form of synaptic plasticity where the activity of one neuron can impact other nonactivated neurons. This result is alarming, as it allows innocuous stimuli to activate low-threshold fibers, which in turn activate usually high threshold nociceptive neurons. The increased excitability in neurons of the CNS results in a reduction of pain threshold and expansion of the receptive field. Long-term potentiation (LTP) in spinal cord neurons is a special form of central sensitization and serves as an important cellular mechanism for the development and maintenance of chronic pain (Ruscheweyh et al. 2011).

Pain researchers have tried to connect between memory and pain (Ji et al. 2003; Price and Inyang 2015). Evolutionarily, such a connection is crucial. Pain normally serves as a warning signal, alerting an organism to danger; to survive, then, an organism must remember what causes pain, and by extension, what to avoid. One

method of managing pain, then, is to block the connections between pain and memory.

Pain changes the way the nervous system functions, causing parts of it to become sensitive to pain for far longer than it ought to be—sometimes hours, sometimes months. Such a memory is what causes us to experience lasting pain or soreness. Extracellular signal-related kinases (ERK1 and ERK2) are proteins that transmit signals from cell surfaces to their nuclei, leading to the transcription of pain-inducing genes (e.g., NK-1, BDNF) via the transcription factor CREB. Interestingly, ERK is only activated by noxious stimulation in spinal cord neurons (Ji et al. 1999) and DRG primary sensory neurons (Dai et al. 2002). It is well established that ERK activation in spinal cord neurons drives central sensitization (Karim et al. 2001; Kawasaki et al. 2004). Another critical contributor to central sensitization is NMDA receptor (NMDAR), which is activated after neuronal depolarization (Woolf and Salter 2000). There are bidirectional interactions between ERK and NMDAR that can strengthen central sensitization. Central sensitization is maintained by neuroinflammation, as cytokines (TNF-α, IL-1β) and chemokines (CCL2 and CXCL1) are sufficient to evoke central sensitization (Ji et al. 2016) (Fig. 5.2). Strikingly, activation of glial cells may be sufficient to drive LTP in spinal cord nociceptive pathways (Kronschlager et al. 2016).

5.9 Concluding Remarks

Accumulating evidence suggests that non-neuronal cells such as immune cells, glial cells, and keratinocytes play active roles in the pathogenesis and resolution of pain. We review how non-neuronal cells interact with nociceptive neurons by secreting neuroactive signaling molecules that modulate pain. Neuroinflammation refers to a local form of inflammation in the PNS or CNS. Its defining characteristics include elevated levels of vascular permeability, leukocyte infiltration, and glial cell activation, which induces the production of inflammatory mediators, including chemokines and proinflammatory cytokines. Both chemokines and proinflammatory cytokines serve as potent neuromodulators that can produce central sensitization, leading to hyperalgesia and allodynia in chronic pain. Furthermore, non-neuronal cells also generate anti-inflammatory and proresolving mediators that can promote the resolution of acute pain.

References

Abdo H, Calvo-Enrique L, Lopez JM, Song J, Zhang MD, Usoskin D, El Manira A, Adameyko I, Hjerling-Leffler J, Ernfors P. Specialized cutaneous Schwann cells initiate pain sensation. Science. 2019;365(6454):695–9.

Aguilera-Lizarraga J, Florens MV, Viola MF, Jain P, Decraecker L, Appeltans I, Cuende-Estevez M, Fabre N, Van Beek K, Perna E, Balemans D, Stakenborg N, Theofanous S, Bosmans G, Mondelaers SU, Matteoli G, Ibiza Martínez S, Lopez-Lopez C, Jaramillo-Polanco J, Talavera K, Alpizar YA, Feyerabend TB, Rodewald HR, Farre R, Redegeld FA, Si J, Raes J, Breynaert C, Schrijvers R, Bosteels C, Lambrecht BN, Boyd SD, Hoh RA, Cabooter D, Nelis M, Augustijns P, Hendrix S, Strid J, Bisschops R, Reed DE, Vanner SJ, Denadai-Souza A, Wouters MM, Boeckxstaens GE. Local immune response to food antigens drives meal-induced abdominal pain. Nature. 2021;590(7844):151–6.

Andriessen AS, Donnelly CR, Ji RR. Reciprocal interactions between osteoclasts and nociceptive sensory neurons in bone cancer pain. Pain Rep. 2021;6(1):e867.

Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 1999;22(5):208–15.

Arriaga-Gomez E, Kline J, Emanuel E, Neamonitaki N, Yangdon T, Zacheis H, Pasha D, Lim J, Bush S, Boo B, Mengistu H, Kinnamon R, Shields-Cutler R, Wattenberg E, Chatterjea D. Repeated vaginal exposures to the common cosmetic and household preservative methylisothiazolinone induce persistent, mast cell-dependent genital pain in ND4 mice. Int J Mol Sci. 2019;20(21):5361.

Bali KK, Kuner R. Therapeutic potential for leukocyte elastase in chronic pain states harboring a neuropathic component. Pain. 2017;158(11):2243–58.

Bang S, Xie YK, Zhang ZJ, Wang Z, Xu ZZ, Ji RR. GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. J Clin Invest. 2018;128(8):3568–82.

Baumbauer KM, DeBerry JJ, Adelman PC, Miller RH, Hachisuka J, Lee KH, Ross SE, Koerber HR, Davis BM, Albers KM. Keratinocytes can modulate and directly initiate nociceptive responses. elife. 2015;4:e09674.

Berta T, Liu T, Liu YC, Xu ZZ, Ji RR. Acute morphine activates satellite glial cells and up-regulates il-1beta in dorsal root ganglia in mice via matrix metalloprotease-9. Mol Pain. 2012;8:18.

Boccazzi M, Van Steenwinckel J, Schang AL, Faivre V, Le Charpentier T, Bokobza C, Csaba Z, Verderio C, Fumagalli M, Mani S, Gressens P. The immune-inflammatory response of oligodendrocytes in a murine model of preterm white matter injury: the role of TLR3 activation. Cell Death Dis. 2021;12(2):166.

Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and regenerative pain medicine. J Clin Invest. 2020;130(5):2164–76.

Carreira EU, Carregaro V, Teixeira MM, Moriconi A, Aramini A, Verri WA Jr, Ferreira SH, Cunha FQ, Cunha TM. Neutrophils recruited by CXCR1/2 signalling mediate post-incisional pain. Eur J Pain. 2013;17(5):654–63.

Cattin AL, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJ, Garcia Calavia N, Guo Y, McLaughlin M, Rosenberg LH, Quereda V, Jamecna D, Napoli I, Parrinello S, Enver T, Ruhrberg C, Lloyd AC. Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. Cell. 2015;162(5):1127–39.

Chakrabarti S, Hore Z, Pattison LA, Lalnunhlimi S, Bhebhe CN, Callejo G, Bulmer DC, Taams LS, Denk F, Smith ESJ. Sensitization of knee-innervating sensory neurons by tumor necrosis factor-α-activated fibroblast-like synoviocytes: an in vitro, coculture model of inflammatory pain. Pain. 2020;161(9):2129–41.

Chen S, Rio C, Ji RR, Dikkes P, Coggeshall RE, Woolf CJ, Corfas G. Disruption of ErbB receptor signaling in adult non-myelinating Schwann cells causes progressive sensory loss. Nat Neurosci. 2003;6(11):1186–93.

Chen Y, Zhang X, Wang C, Li G, Gu Y, Huang LY. Activation of P2X7 receptors in glial satellite cells reduces pain through downregulation of P2X3 receptors in nociceptive neurons. Proc Natl Acad Sci U S A. 2008;105(43):16773–8.

Chen G, Park CK, Xie RG, Berta T, Nedergaard M, Ji RR. Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. Brain. 2014;137(Pt 8):2193–209.

Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-β secretion. J Clin Invest. 2015;125(8):3226–40.
Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100(6):1292–311.
Chen O, Donnelly CR, Ji RR. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020;62:17–25.
Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005;438(7070):1017–21.
Dai Y, Iwata K, Fukuoka T, Kondo E, Tokunaga A, Yamanaka H, Tachibana T, Liu Y, Noguchi K. Phosphorylation of extracellular signal-regulated kinase in primary afferent neurons by noxious stimuli and its involvement in peripheral sensitization. J Neurosci. 2002;22(17):7737–45.
Davies AJ, Kim HW, Gonzalez-Cano R, Choi J, Back SK, Roh SE, Johnson E, Gabriac M, Kim MS, Lee J, Lee JE, Kim YS, Bae YC, Kim SJ, Lee KM, Na HS, Riva P, Latremoliere A, Rinaldi S, Ugolini S, Costigan M, Oh SB. Natural killer cells degenerate intact sensory afferents following nerve injury. Cell. 2019;176(4):716–28.e18.
Ellis A, Bennett DL. Neuroinflammation and the generation of neuropathic pain. Br J Anaesth. 2013;111(1):26–37.
Foley JC, McIver SR, Haydon PG. Gliotransmission modulates baseline mechanical nociception. Mol Pain. 2011;7:93.
Gao YJ, Ji RR. Targeting astrocyte signaling for chronic pain. Neurotherapeutics. 2010;7(4):482–93.
Goebel A, Andersson D, Helyes Z, Clark JD, Dulake D, Svensson C. The autoimmune aetiology of unexplained chronic pain. Autoimmun Rev. 2022;21(3):103015.
Goncalves NP, Vaegter CB, Andersen H, Ostergaard L, Calcutt NA, Jensen TS. Schwann cell interactions with axons and microvessels in diabetic neuropathy. Nat Rev Neurol. 2017;13(3):135–47.
Grace PM, Wang X, Strand KA, Baratta MV, Zhang Y, Galer EL, Yin H, Maier SF, Watkins LR. DREADDed microglia in pain: implications for spinal inflammatory signaling in male rats. Exp Neurol. 2018;304:125–31.
Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, Guan AK, Evans-Reinsch Z, Braz J, Devor M, Abboud-Werner SL, Lanier LL, Lomvardas S, Basbaum AI. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci. 2016;19(1):94–101.
Guo W, Wang H, Zou S, Gu M, Watanabe M, Wei F, Dubner R, Huang GT, Ren K. Bone marrow stromal cells produce long-term pain relief in rat models of persistent pain. Stem Cells. 2011;29(8):1294–303.
Guo W, Imai S, Yang JL, Zou S, Watanabe M, Chu YX, Mohammad Z, Xu H, Moudgil KD, Wei F, Dubner R, Ren K. In vivo immune interactions of multipotent stromal cells underlie their long-lasting pain-relieving effect. Sci Rep. 2017a;7(1):10107.
Guo TZ, Shi X, Li WW, Wei T, Clark JD, Kingery WS. Passive transfer autoimmunity in a mouse model of complex regional pain syndrome. Pain. 2017b;158(12):2410–21.
Hanani M, Spray DC. Emerging importance of satellite glia in nervous system function and dysfunction. Nat Rev Neurosci. 2020;21(9):485–98.
Hanani M, Huang TY, Cherkas PS, Ledda M, Pannese E. Glial cell plasticity in sensory ganglia induced by nerve damage. Neuroscience. 2002;114(2):279–83.
Hanisch UK. Microglia as a source and target of cytokines. Glia. 2002;40(2):140–55.
Hasel P, Rose IVL, Sadick JS, Kim RD, Liddelow SA. Neuroinflammatory astrocyte subtypes in the mouse brain. Nat Neurosci. 2021;24(10):1475–87.
Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14(4):388–405.

Hosoi J, Murphy GF, Egan CL, Lerner EA, Grabbe S, Asahina A, Granstein RD. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. Nature. 1993;363(6425):159–63.

Hua Z, Liu L, Shen J, Cheng K, Liu A, Yang J, Wang L, Qu T, Yang H, Li Y, Wu H, Narouze J, Yin Y, Cheng J. Mesenchymal stem cells reversed morphine tolerance and opioid-induced hyperalgesia. Sci Rep. 2016;6:32096.

Huh Y, Ji RR, Chen G. Neuroinflammation, bone marrow stem cells, and chronic pain. Front Immunol. 2017;8:1014.

Inoue K, Tsuda M. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci. 2018;19(3):138–52.

Jasmin L, Vit JP, Bhargava A, Ohara PT. Can satellite glial cells be therapeutic targets for pain control? Neuron Glia Biol. 2010;6(1):63–71.

Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain. 2007;3:33.

Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. Nat Neurosci. 1999;2(12):1114–9.

Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003;26(12):696–705.

Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013;154(Suppl 1(0 1)):S10–28.

Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533–48.

Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science. 2016;354(6312):572–7.

Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology. 2018;129(2):343–66.

Ji RR, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20(11):667–85.

Ji J, He Q, Luo X, Bang S, Matsuoka Y, McGinnis A, Nackley AG, Ji RR. IL-23 enhances C-fiber-mediated and blue light-induced spontaneous pain in female mice. Front Immunol. 2021;12:787565.

Jiang BC, Cao DL, Zhang X, Zhang ZJ, He LN, Li CH, Zhang WW, Wu XB, Berta T, Ji RR, Gao YJ. CXCL13 drives spinal astrocyte activation and neuropathic pain via CXCR5. J Clin Invest. 2016;126(2):745–61.

Jin SX, Zhuang ZY, Woolf CJ, Ji RR. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. J Neurosci. 2003;23(10):4017–22.

Karim F, Wang CC, Gereau RW. Metabotropic glutamate receptor subtypes 1 and 5 are activators of extracellular signal-regulated kinase signaling required for inflammatory pain in mice. J Neurosci. 2001;21(11):3771–9.

Kawasaki Y, Kohno T, Zhuang ZY, Brenner GJ, Wang H, Van Der Meer C, Befort K, Woolf CJ, Ji RR. Ionotropic and metabotropic receptors, protein kinase A, protein kinase C, and Src contribute to C-fiber-induced ERK activation and cAMP response element-binding protein phosphorylation in dorsal horn neurons, leading to central sensitization. J Neurosci. 2004;24(38):8310–21.

Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Gao YJ, Roy K, Corfas G, Lo EH, Ji RR. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008a;14(3):331–6.

Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci. 2008b;28(20):5189–94.

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. Physiol Rev. 2011;91(2):461–553.

Kohno K, Shirasaka R, Yoshihara K, Mikuriya S, Tanaka K, Takanami K, Inoue K, Sakamoto H, Ohkawa Y, Masuda T, Tsuda M. A spinal microglia population involved in remitting and relapsing neuropathic pain. Science. 2022;376(6588):86–90.

Kohro Y, Matsuda T, Yoshihara K, Kohno K, Koga K, Katsuragi R, Oka T, Tashima R, Muneta S, Yamane T, Okada S, Momokino K, Furusho A, Hamase K, Oti T, Sakamoto H, Hayashida K, Kobayashi R, Horii T, Hatada I, Tozaki-Saitoh H, Mikoshiba K, Taylor V, Inoue K, Tsuda M. Spinal astrocytes in superficial laminae gate brainstem descending control of mechanosensory hypersensitivity. Nat Neurosci. 2020;23(11):1376–87.

Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996;19(8):312–8.

Kronschlager MT, Drdla-Schutting R, Gassner M, Honsek SD, Teuchmann HL, Sandkuhler J. Gliogenic LTP spreads widely in nociceptive pathways. Science. 2016;354(6316):1144–8.

Krukowski K, Eijkelkamp N, Laumet G, Hack CE, Li Y, Dougherty PM, Heijnen CJ, Kavelaars A. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic Pain. J Neurosci. 2016;36(43):11074–83.

Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain. 2009;10(9):895–926.

Li WW, Guo TZ, Shi X, Birklein F, Schlereth T, Kingery WS, Clark JD. Neuropeptide regulation of adaptive immunity in the tibia fracture model of complex regional pain syndrome. J Neuroinflammation. 2018;15(1):105.

Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung WS, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA. Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481–7.

Liu FY, Sun YN, Wang FT, Li Q, Su L, Zhao ZF, Meng XL, Zhao H, Wu X, Sun Q, Xing GG, Wan Y. Activation of satellite glial cells in lumbar dorsal root ganglia contributes to neuropathic pain after spinal nerve ligation. Brain Res. 2012;1427:65–77.

Liu XJ, Zhang Y, Liu T, Xu ZZ, Park CK, Berta T, Jiang D, Ji RR. Nociceptive neurons regulate innate and adaptive immunity and neuropathic pain through MyD88 adapter. Cell Res. 2014;24(11):1374–7.

Liu CC, Gao YJ, Luo H, Berta T, Xu ZZ, Ji RR, Tan PH. Interferon alpha inhibits spinal cord synaptic and nociceptive transmission via neuronal-glial interactions. Sci Rep. 2016;6:34356.

Liu YU, Ying Y, Li Y, Eyo UB, Chen T, Zheng J, Umpierre AD, Zhu J, Bosco DB, Dong H, Wu LJ. Neuronal network activity controls microglial process surveillance in awake mice via norepinephrine signaling. Nat Neurosci. 2019 Nov;22(11):1771–81.

Luo X, Huh Y, Bang S, He Q, Zhang L, Matsuda M, Ji RR. Macrophage toll-like receptor 9 contributes to chemotherapy-induced neuropathic pain in male mice. J Neurosci. 2019;39(35):6848–64.

Luo X, Chen O, Wang Z, Bang S, Ji J, Lee SH, Huh Y, Furutani K, He Q, Tao X, Ko MC, Bortsov A, Donnelly CR, Chen Y, Nackley A, Berta T, Ji RR. IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice. Neuron. 2021;109(17):2691–706.e5.

Mantyh PW. Cancer pain and its impact on diagnosis, survival and quality of life. Nat Rev Neurosci. 2006;7(10):797–809.

Moore C, Cevikbas F, Pasolli HA, Chen Y, Kong W, Kempkes C, Parekh P, Lee SH, Kontchou NA, Yeh I, Jokerst NM, Fuchs E, Steinhoff M, Liedtke WB. UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin-1 signaling. Proc Natl Acad Sci U S A. 2013;110(34):E3225–34.

Mousa SA, Zhang Q, Sitte N, Ji R, Stein C. Beta-endorphin-containing memory-cells and mu-opioid receptors undergo transport to peripheral inflamed tissue. J Neuroimmunol. 2001;115(1–2):71–8.

Nedergaard M. Neuroscience. Garbage truck of the brain. Science. 2013;340(6140):1529–30.

Nedergaard M, Verkhratsky A. Artifact versus reality – how astrocytes contribute to synaptic events. Glia. 2012;60(7):1013–23.

Niehaus JK, Taylor-Blake B, Loo L, Simon JM, Zylka MJ. Spinal macrophages resolve nociceptive hypersensitivity after peripheral injury. Neuron. 2021;109(8):1274–82.e6.
Oberheim NA, Wang X, Goldman S, Nedergaard M. Astrocytic complexity distinguishes the human brain. Trends Neurosci. 2006;29(10):547–53.
Parisien M, Lima LV, Dagostino C, El-Hachem N, Drury GL, Grant AV, Huising J, Verma V, Meloto CB, Silva JR, Dutra GGS, Markova T, Dang H, Tessier PA, Slade GD, Nackley AG, Ghasemlou N, Mogil JS, Allegri M, Diatchenko L. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci Transl Med. 2022;14(644):eabj9954.
Parusel S, Yi MH, Hunt CL, Wu LJ. Chemogenetic and optogenetic manipulations of microglia in chronic pain. Neurosci Bull. 2022. https://doi.org/10.1007/s12264-022-00937-3.
Price TJ, Inyang KE. Commonalities between pain and memory mechanisms and their meaning for understanding chronic pain. Prog Mol Biol Transl Sci. 2015;131:409–34.
Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther. 2003;306(2):624–30.
Regauer S. Mast cell activation syndrome in pain syndromes bladder pain syndrome/interstitial cystitis and vulvodynia. Transl Androl Urol. 2016;5(3):396–7.
Ruscheweyh R, Wilder-Smith O, Drdla R, Liu XG, Sandkühler J. Long-term potentiation in spinal nociceptive pathways as a novel target for pain therapy. Mol Pain. 2011;7:20.
Salter MW, Stevens B. Microglia emerge as central players in brain disease. Nat Med. 2017;23(9):1018–27.
Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB J. 2012;26(4):1755–65.
Shi Y, Gelman BB, Lisinicchia JG, Tang SJ. Chronic-pain-associated astrocytic reaction in the spinal cord dorsal horn of human immunodeficiency virus-infected patients. J Neurosci. 2012;32(32):10833–40.
Silva JR, Iftinca M, Fernandes Gomes FI, Segal JP, Smith OMA, Bannerman CA, Silva Mendes A, Defaye M, Robinson MEC, Gilron I, Cunha TM, Altier C, Ghasemlou N. Skin-resident dendritic cells mediate postoperative pain via CCR4 on sensory neurons. Proc Natl Acad Sci U S A. 2022;119(4):e2118238119.
Simard M, Nedergaard M. The neurobiology of glia in the context of water and ion homeostasis. Neuroscience. 2004;129(4):877–96.
Simeoli R, Montague K, Jones HR, Castaldi L, Chambers D, Kelleher JH, Vacca V, Pitcher T, Grist J, Al-Ahdal H, Wong LF, Perretti M, Lai J, Mouritzen P, Heppenstall P, Malcangio M. Exosomal cargo including microRNA regulates sensory neuron to macrophage communication after nerve trauma. Nat Commun. 2017;8(1):1778.
Singhmar P, Trinh RTP, Ma J, Huo X, Peng B, Heijnen CJ, Kavelaars A. The fibroblast-derived protein PI16 controls neuropathic pain. Proc Natl Acad Sci U S A. 2020;117(10):5463–71.
Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18(8):1081–3.
Stogsdill JA, Ramirez J, Liu D, Kim YH, Baldwin KT, Enustun E, Ejikeme T, Ji RR, Eroglu C. Astrocytic neuroligins control astrocyte morphogenesis and synaptogenesis. Nature. 2017;551(7679):192–7.
Takeda I, Yoshihara K, Cheung DL, Kobayashi T, Agetsuma M, Tsuda M, Eto K, Koizumi S, Wake H, Moorhouse AJ, Nabekura J. Controlled activation of cortical astrocytes modulates neuropathic pain-like behaviour. Nat Commun. 2022;13(1):4100.
Tan PH, Ji J, Yeh CC, Ji RR. Interferons in pain and infections: emerging roles in neuro-immune and neuro-glial interactions. Front Immunol. 2021;12:783725.
Tao X, Lee MS, Donnelly CR, Ji RR. Neuromodulation, specialized proresolving mediators, and resolution of Pain. Neurotherapeutics. 2020;17(3):886–99.

Treede RD, Hoheisel U, Wang D, Magerl W. Central sensitization: clinical utility of a physiological concept for the International Statistical Classification of Diseases and Related Health Problems and for nociplastic pain. Pain. 2022 Nov 1;163(Suppl 1):S99–S107.

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature. 2003;424(6950):778–83.

Tsuda M, Inoue K, Salter MW. Neuropathic pain and spinal microglia: a big problem from molecules in "small" glia. Trends Neurosci. 2005;28(2):101–7.

Üçeyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C. Small fibre pathology in patients with fibromyalgia syndrome. Brain. 2013;136(Pt 6):1857–67.

van der Vlist M, Raoof R, Willemen HLDM, Prado J, Versteeg S, Martin Gil C, Vos M, Lokhorst RE, Pasterkamp RJ, Kojima T, Karasuyama H, Khoury-Hanold W, Meyaard L, Eijkelkamp N. Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. Neuron. 2022;110(4):613–626.e9.

Verkhratsky A, Nedergaard M. Physiology of astroglia. Physiol Rev. 2018;98(1):239–389.

Verkhratsky A, Sofroniew MV, Messing A, deLanerolle NC, Rempe D, Rodriguez JJ, Nedergaard M. Neurological diseases as primary gliopathies: a reassessment of neurocentrism. ASN Neuro. 2012;4(3):e00082.

Verma V, Drury GL, Parisien M, Özdağ Acarli AN, Al-Aubodah TA, Nijnik A, Wen X, Tugarinov N, Verner M, Klares R 3rd, Linton A, Krock E, Morado Urbina CE, Winsvold B, Fritsche LG, Fors EA, Piccirillo C, Khoutorsky A, Svensson CI, Fitzcharles MA, Ingelmo PM, Bernard NF, Dupuy FP, Üçeyler N, Sommer C, King IL, Meloto CB, Diatchenko L, HUNT-All In Pain. Unbiased immune profiling reveals a natural killer cell-peripheral nerve axis in fibromyalgia. Pain. 2022;163(7):e821–36.

Vit JP, Ohara PT, Bhargava A, Kelley K, Jasmin L. Silencing the Kir4.1 potassium channel subunit in satellite glial cells of the rat trigeminal ganglion results in pain-like behavior in the absence of nerve injury. J Neurosci. 2008;28(16):4161–71.

Wang L, Jiang X, Zheng Q, Jeon SM, Chen T, Liu Y, Kulaga H, Reed R, Dong X, Caterina MJ, Qu L. Neuronal FcγRI mediates acute and chronic joint pain. J Clin Invest. 2019;129(9):3754–69.

Wang K, Gu Y, Liao Y, Bang S, Donnelly CR, Chen O, Tao X, Mirando AJ, Hilton MJ, Ji RR. PD-1 blockade inhibits osteoclast formation and murine bone cancer pain. J Clin Invest. 2020;130(7):3603–20.

Wei Z, Fei Y, Su W, Chen G. Emerging role of Schwann cells in neuropathic pain: receptors, glial mediators and myelination. Front Cell Neurosci. 2019;13:116.

Wei K, Nguyen HN, Brenner MB. Fibroblast pathology in inflammatory diseases. J Clin Invest. 2021;131(20):e149538.

Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152(3 Suppl):S2–15.

Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000;288(5472):1765–9.

Xu Q, Ford NC, He S, Huang Q, Anderson M, Chen Z, Yang F, Crawford LK, Caterina MJ, Guan Y, et al. Astrocytes contribute to pain gating in the spinal cord. Sci Adv. 2021;7(45):eabi6287.

Yang T, Velagapudi R, Terrando N. Neuroinflammation after surgery: from mechanisms to therapeutic targets. Nat Immunol. 2020;21(11):1319–26.

Yao Y, Echeverry S, Shi XQ, Yang M, Yang QZ, Wang GY, Chambon J, Wu YC, Fu KY, De Koninck Y, Zhang J. Dynamics of spinal microglia repopulation following an acute depletion. Sci Rep. 2016;6:22839.

Zhang H, Mei X, Zhang P, Ma C, White FA, Donnelly DF, LaMotte RH. Altered functional properties of satellite glial cells in compressed spinal ganglia. Glia. 2009;57(15):1588–99.

Zhao A, Jiao Y, Ye G, Kang W, Tan L, Li Y, Deng Y, Liu J, Alzheimer's Disease Neuroimaging Initiative (ADNI). Soluble TREM2 levels associate with conversion from mild cognitive impairment to Alzheimer's disease. J Clin Invest. 2022;132(24):e158708.

Chapter 6
Sex Differences in Pain with Emphasis on Neuroimmune Interactions

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Abstract To date, the mechanisms underlying how neuroimmune interactions contribute to sex dimorphism of chronic pain remain elusive. Although women suffer from chronic pain at greater rates than men, the current mechanistic understanding of chronic pain has been predominantly derived from the study of male animals. As such, a greater emphasis will be needed to investigate female-specific signaling mechanisms in chronic pain. These efforts will improve our understanding of sex dimorphism in chronic pain and improve the ability of pain medicine address specific patient backgrounds in the future. In this chapter, we will discuss pain-related sex differences in neural, immune, and glial mechanisms, with a focus on sex dimorphism in neuroimmune interactions.

Keywords Females · IL-17 · IL-23 · Macrophages · Males · Microglia · Sex dimorphism · Spinal cord · T cells · TRPV1

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6.1 Introduction

Since the introduction of the National Institutes of Health Revitalization Act in 1993 clinical studies have been required to include female participants. However, no such requirement currently exists for preclinical research, due in part to perceived complications of hormone fluctuations related to menstruation in female animals, and in part due to the belief that results seen in male animals could be generalized across sexes (Mogil 2021; Navratilova et al. 2021; Sorge and Totsch 2017). Such biases are particularly pronounced in neuroscience, where nearly five times as many male single-sex studies have been conducted versus female single-sex studies (Beery and Zucker 2011). This discrepancy in research design poses a significant problem in chronic pain studies, as clinical statistics indicate that women are more likely to suffer from chronic pain conditions, experience more severe levels of pain, and have weaker responses to medical treatment for pain when compared with men. Clinical studies by research teams from around the world, including from the United States (Dahlhamer et al. 2016), Canada (Shupler et al. 2019), the European Union (Langley 2011), and China (Wu et al. 2019) have recognized that sex dimorphism present in chronic pain. Women are at higher risk for chronic pain conditions such as neuropathic pain, chronic fatigue syndrome, interstitial cystitis, and fibromyalgia (Fillingim et al. 2009; Maixner and Humphrey 1993; Navratilova et al. 2021). In addition, women with chronic pain conditions often report pain that is more severe, frequent, and longer lasting than men with the same conditions, and may also suffer from more chronic pain-related disability than men (Unruh 1996). Moreover, some studies have also suggested that women may not experience as much opioid-induced pain relief as men do, though further research is necessary to confirm these results (Fillingim et al. 2009).

In any case, the evidence of sex dimorphism in chronic pain is abundant, yet the mechanisms underlying this phenomenon remain largely unclear, which has been a major impediment toward translating research findings and increasing failure rates in clinical trials (Mogil 2012). Recent studies suggest that chronic pain is not only controlled by neuronal sensitization in the peripheral sensitization (PNS) and central sensitization (CNS), but it is also driven by immune cells and glial cells (Chen et al. 2018a, b; Chiu et al. 2012; Grace et al. 2014; Inoue and Tsuda 2018; Ji et al. 2013, 2016, 2019; Salter and Stevens 2017). In this chapter, we will discuss pain-related sex differences in neurons, immune cells, and glial cells, with specific focus on the sex dimorphism in neuroimmune interactions.

6.2 Sexual Dimorphism in Neuronal Modulation of Pain

6.2.1 Sex Dimorphism in Capsaicin Response, TRPV1 Signaling, and CGRP Response

Capsaicin receptor TRPV1 is specifically expressed by C-fiber nociceptors. Several reports suggest that females may show greater responses to capsaicin stimulation and TRPV1 activation versus males (Frot et al. 2004; Gazerani et al. 2005; Hartmann et al. 2015). Interestingly, estrogen (estradiol or E2) may not regulate *Tprv1* expression in sensory neurons (Diogenes et al. 2006), as no sex differences were found in the overall TRPV1 expression in mouse or human DRGs (Luo et al. 2021). RNAscope in situ hybridization revealed a significant sex difference in the size distribution of TRPV1 (or *Trpv1*)-positive neurons in mouse DRGs. As expected, we observed that TRPV1 is mainly expressed in small (cell area: 100–400 μm^2) and medium-sized neurons (400–600 μm^2) in mice of both sexes. However, while percentages in other size categories remained comparable, female DRGs showed a significantly greater percentage of TRPV1-positive neurons than males in the 100–200 μm^2 size category. While further research is required to identify the role of these neurons in female-dominant pain, it is possible that TRPV1-positive neurons in the 100–200 μm^2 size category in females may be regulated by estrogen, thus contributing to chronic pain in females. Furthermore, we found that optogenetic blue light stimulation of ChR2-expressing nociceptors in the hind paw of TRPV1-ChR2 transgenic mice, in which ChR2 is selectively expressed in TRPV1-expressing neurons, produces greater levels of pain behavior (indicated by the mouse licking and flinching in response to light stimulation) in females than males (Ji et al. 2021).

Additionally, estrogen was shown to regulate TRPV1 signaling. After a brief 10 min incubation, estrogen prevented TRPV1 desensitization in dissociated sensory neurons (Payrits et al. 2017). Estrogen was found to facilitate TRPV1 agonist-induced mechanical hyperalgesia in ovariectomized (OVX) mice, as well as ocular pain in OVX rats (Payrits et al. 2017; Yamagata et al. 2016). Furthermore, women were shown to have higher facial pain intensity and unpleasantness than men in response to topical capsaicin administration (Frot et al. 2004). Capsaicin was shown to induce mechanical pain via neurogenic inflammation that can release the neuropeptide calcitonin gene-related peptide (CGRP) (Warwick et al. 2019). It was found that intraplantar administration of a very low dose of capsaicin (50 ng) elicited mechanical pain only in female mice (Luo et al. 2021). Additionally, injection of a low dose of CGRP (1 pg) produced mechanical pain only in female rats as well (Avona et al. 2019).

6.2.2 *Prolactin (PRL) and Prolactin Receptor (PRLR)*

Prolactin (PRL) is a hormone produced in the anterior pituitary gland, and under inflammatory conditions, PRL may also be synthesized by cells outside of the pituitary gland. Prolactin was shown to modulate TRPV1 activity in female sensory neurons in an estrogen-dependent manner (Diogenes et al. 2006). PRL was also shown to regulate TRPA1 and TRPM8 in sensory neurons in a sex-dependent manner in inflammatory pain (Patil et al. 2013). Notably, prolactin produces female-specific pain signals via prolactin receptors expressed on nociceptors (Chen et al. 2020a, b, Patil et al. 2019). Prolactin receptors have two isoforms: a short isoform (PRLR-S) and a long isoform (PRLR-L). Homodimer signaling through PRLR-S heightens neuronal excitability and pain sensitization, while signaling through PRLR-L *protects* against the nociceptive effects of PRLR-S by interfering with PRLR-S signaling. Normally, PRLR-L expression is higher in female trigeminal ganglion neurons compared to males, but under pathological conditions, inverted PRLR-S upregulation and PRLR-L downregulation may result in heightened pain responses in females. For example, studies have shown that priming with hyperalgesia-promoting medications results in PRLR-L downregulation in female animals only. These prolactin signaling processes may therefore play an important role in stress-related pain conditions that are prevalent in females (e.g., chronic migraine). Females have higher serum prolactin concentrations compared to males, and stressful events (e.g., injury and trauma) further increase serum prolactin concentrations in females only. Studies have shown that priming with stressors can result in allodynia and PRLR-L downregulation in females, and inhibition of prolactin release was able to block stress-induced pain (Navratilova et al. 2021).

In addition, sex differences have also been shown in the regulation of nociceptor translatomes. In Nav1.8-positive neurons (presumably nociceptors), it was found that 66 genes whose messenger RNAs were sex differentially actively translated. Among the notable genes in the nociceptor translatome, prostaglandin PGD2 synthesizing enzyme (PTGDS) is enriched in female mouse DRG (Tavares-Ferreira et al. 2022).

6.3 Sex Differences in Glial and Immune Modulation of Pain

A growing body of research considers neuroimmune interactions an important biological cause for sex dimorphism in chronic pain (Chen et al. 2018b; Mogil 2012, 2020; Rosen et al. 2017; Sorge and Strath 2018). This section will focus on the current knowledge and research advancements concerning the roles of immune cells, including microglia, macrophages, T cells, B cells, and astrocytes in the sex dimorphism of chronic pain (Table 6.1).

Table 6.1 Immune and glial cell signaling molecules with sex dimorphic functions in pathological pain

Signaling pathway	Experimental approaches	Sex preference	Pain models	References
Microglia				
p38	Pharmacology	Male	CCI, HP	Taves et al. (2016), Luo et al. (2018), Paige et al. (2018)
TLR4	KO, pharmacology	Male	SNI, CFA, CRPS	Sorge et al. (2011), Huck et al. (2021)
HMGB1	Pharmacology	Male		Agalave et al. (2021b)
Caspase-6	KO, pharmacology	Male	Formalin	Chen et al. (2018a, b)
Mu opioid receptor	KO	Male	Morphine analgesia	Reiss et al. (2022)
PI3K/Akt	Pharmacology	Male	Incision	Xu et al. (2019)
$P2X_4R$	KO, pharmacology	Male	SNI, CCI	Sorge et al. (2015), Paige et al. (2018)
BDNF	KO	Male	SNI	Sorge et al. (2015)
Macrophage				
TLR9	KO, pharmacology	Male	CIPN	Luo et al. (2019)
CSF1	KO	Male	SNI	Yu et al. (2020)
HMGB1	Pharmacology	Male	CAIA	Rudjito et al. (2021)
IL-23/ IL-17A	KO, pharmacology, electrophysiology, Ca^{2+} imaging, Optogenetics	Female	CIPN, CCI, DN, Formalin	Luo et al. (2021), Ji et al. (2021)
T cells				
PPARα	Pharmacology	Male	SNI	Sorge et al. (2015)
PPARγ	Pharmacology	Female	SNI	Sorge et al. (2015)
Astrocyte				
eIF4E	KO	Male	CIPN	Agalave et al. (2021a)

Abbreviations: *KO* knockout, *CCI* chronic constrictive injury, *HP* hyperalgesic priming, *SNI* spared nerve injury, *CFA* complete Freund's adjuvant, *CRPS* complex regional pain syndrome, *CIPN* chemotherapy-induced peripheral neuropathy, *CAIA* collagen antibody-induced arthritis, *DN* diabetic neuropathy

6.3.1 Sex Dimorphism in Microglial Signaling

In multiple chronic pain models, microglial reactions, such as morphological changes, microgliosis, and microglial proliferation, exhibit no sex differences in the spinal cord. For example, spinal levels of $Iba1^+$ and/or $CX3CR1^+$ microglia increase in both male and female animals in neuropathic pain models of spared nerve injury (SNI) (Sorge et al. 2015) and chronic constrictive injury (CCI) (Taves et al. 2016). However, several lines of evidence have identified male-specific microglial function in multiple chronic pain conditions, using transgenic and pharmacological methods, including selective microglial activation, selective depletion of microglia, general

inhibition of microglial function with microglial inhibitors, and specific inhibition of microglial signaling pathways (Sorge et al. 2015).

Selective activation of spinal microglia by intrathecal clozapine-*N*-oxide (CNO), using a chemogenetic approach called designer receptor exclusively activated by a designer drug (DREADD), elicits mechanical allodynia in male but not female rats and mice (Grace et al. 2018; Saika et al. 2020). Moreover, CCI-induced allodynia was attenuated by intrathecal CNO in male rats intrathecally transfected with Gi (inhibitory) DREADDs (Grace et al. 2018).

Despite the estrous cycles in females, male and female rodents develop comparable baseline pain sensitivities under normal conditions and also exhibit comparable pain hypersensitivity under pathological conditions, showing no sex differences. However, intrathecal injections of microglial inhibitors (e.g., minocycline) produce male-specific antiallodynic effects in pathological pain models of spared nerve injury (SNI, Sorge et al. 2015), formalin-induced acute inflammatory pain (Chen et al. 2018a), CCI (Chen et al. 2018a; Mapplebeck et al. 2018; Taves et al. 2016), complex regional pain syndrome (CRPS) (Guo et al. 2019), and collagen antibody-induced arthritis (CAIA) (Fernandez-Zafra et al. 2019).

For instance, 7 days after inducing the SNI, minocycline, a nonselective inhibitor of microglia, was intrathecally injected into both male and female mice and their pain thresholds were tested over the next 2 h. It was found that while the minocycline dose-dependently reversed mechanical hypersensitivity in males, the inhibitor was actually ineffective in reversing mechanical hypersensitivity in females regardless of the dose. A similar experiment that tested inflammatory pain instead of neuropathic pain, yielded similar results (Sorge et al. 2015). However, because minocycline has other functions besides suppressing glial function at high doses, the researchers of this study carried out additional experiments to confirm that microglia are necessary to induce mechanical allodynia in males (Sorge et al. 2015). In one experiment, microglia in mice of both sexes were briefly depleted via an intrathecal injection of sporin toxin conjugated with macrophage antigen complex-1 (MAC1). Four hours after injection, microglial levels were similarly deleted in both males and females. This resulted in a noticeable reversal of mechanical allodynia in males, but once again, the treatment had no effect on female mechanical pain levels. The researchers soon found that $P2X_4$ receptor ($P2X_4R$), an ATP receptor induced in microglia after nerve injury, is also required for SNI-induced allodynia in male animals (Sorge et al. 2015).

In a mouse model of fibromyalgia, intramuscular injections of acidic saline into the gastrocnemius produce mechanical allodynia in both male and female mice. Notably, intracerebroventricular minocycline only reduces pain in male mice in the late-stage fibromyalgia, suggesting that brain microglia may contribute to fibromyalgia in a sex- and stage-dependent manner (Ueda et al. 2020). In both male and female adult mice, neonatal priming incisions can cause long-term alteration into adult life of somatosensory function and enhance the second injection-induced pain response, which is reversed by minocycline treatment in early life, but only in male animals (Moriarty et al. 2019).

Numerous studies indicate that several microglial signaling pathways including toll-like receptor 4 (TLR4), p38 MAP kinase, caspase-6, mu opioid receptor, phosphatidylinositol 3-kinase (PI3K)/Akt, $P2X_4$ receptor (P2X4R), and brain-derived neurotrophic factor (BDNF) contribute to chronic pain in a male-specific manner (Chen et al. 2018a, b; Mapplebeck et al. 2018; Sorge et al. 2015). Sorge et al. found that toll-like receptor 4 (TLR4), the activation of which results in inflammatory cytokine production, was involved in the production of mechanical allodynia in only males. Knowing that TLR4 is located on microglia, they suspected that microglia might be the reason for this sex difference. The group further suspected that perhaps microglia were not even necessary for the processing of pain in female mice (Sorge et al. 2011).

TLR4 recognizes lipopolysaccharide (LPS), one of the most potent activators of microglia (Xu et al. 2013). Intrathecal injection of LPS produces robust mechanical allodynia in male but not female mice, whereas systemic injection of LPS induces pain hypersensitivity in both sexes. Such male-selective pronociceptive effects of LPS are abolished by testosterone deficiency or *Tlr4* knockout. Concordantly, intrathecal injection of the TLR4 antagonist LPS-RS results in male-specific analgesia in mouse models of inflammatory pain (complete Freund's adjuvant, CFA) and SNI (Sorge et al. 2011). Using transgenic mice with inducible and selective depletion of the *Tlr4* gene in myeloid-lineage cells ($Cx3cr1^{Cre\text{-}ERT2\text{-}eYFP}/Tlr4^{fl/fl}$), the role of microglial TLR4 signaling was examined in the tibial fracture model of postoperative pain. *Tlr4* conditional knockout (cKO), induced before the tibial fracture surgery, alleviated mechanical allodynia and spontaneous pain only in male mice and not in female mice. However, *Tlr4* cKO, induced *after* surgery, partially reduced mechanical allodynia in both sexes. These findings suggest that microglial TLR4 may play an essential role in the transition from acute pain to chronic pain (Huck et al. 2021).

p38 is a member of the mitogen-activated protein kinases (MAPK) family and primarily expressed by microglia in the spinal dorsal horn, which contributes to pathological pain processing (Ji and Suter 2007). In the mouse CCI model, nerve injury induces more p38 activation (indicated by p38 phosphorylation) in the spinal cord in males, as opposed to CX_3CR1^+ microglia in females. Of note, intrathecal injection of the p38 inhibitor skepinone reduced CCI-induced mechanical allodynia only in male mice, whereas systemic or perineural injection of skepinone produced analgesia in both sexes, suggesting that the peripheral and central p38 pathways may have separate mechanisms (Taves et al. 2016). Intrathecal injection of p38α antisense oligonucleotides (ASO) causes a nearly 50% reduction in spinal p38α mRNA levels, which attenuates CCI-induced mechanical allodynia in male but not female animals (Luo et al. 2018). In the mouse hyperalgesic priming model, the p38 inhibitor skepinone prevented the development of persistent mechanical allodynia produced by interleukin-6R and prostaglandin E_2 (PGE2), but only in male animals (Paige et al. 2018).

Microglia-derived BDNF is crucial for intercellular communication between microglia and neurons in pain signaling (Coull et al. 2005). Selective depletion of *Bdnf* in CX3CR1$^+$ microglia ($Cx3cr1^{Cre\text{-}ER}/Bdnf^{fl/fl}$) does not affect microgliosis in

the spinal dorsal horn in male and female mice. However, *Bdnf* cKO attenuates SNI-induced mechanical allodynia only in male animals (Sorge et al. 2015).

High mobility group box 1 protein (HMGB1) is a pathogenic mediator of various diseases and injured states, and it is an endogenous agonist of TLR4. Intrathecal injection of disulfide HMBG1 increases spinal levels of Iba1^{+} microglia and promotes mechanical allodynia in both male and female mice with no sex differences. Interestingly, selective depletion of *Tlr4* in microglia prevents disulfide HMBG1-induced mechanical allodynia only in male *LysM*^{Cre}*/TLr4*$^{fl/fl}$ mice (*Tlr4* cKO), suggesting that the mechanisms underlying disulfide HMBG1-mediated pain may be sex dimorphic (Agalave et al. 2021b).

Neuron-derived Caspase-6 can regulate microglial function in chronic pain (Berta et al. 2014). Intrathecal injection of caspase-6 produces mechanical allodynia in male but not female mice. Accordingly, the caspase-6 inhibitor ZVEID or *Caspase6*$^{-/-}$ knockout exhibits male-dominant analgesia against formalin-induced pain hypersensitivity (Berta et al. 2016).

P2X receptors are ligand-gated ion channels, which are activated by extracellular ATP. Microglial $P2X_4R$ drives pain hypersensitivity in pathological conditions (Beggs et al. 2012). In the mouse SNI model, surgery upregulates *P2rx4* gene expression in the spinal dorsal horn of male but not female mice. Intrathecal injection of the P2X4R inhibitor TNP-ATP produces analgesia only in male SNI-injured mice (Sorge et al. 2015). In the rat CCI model, surgery promotes $P2X_4R$ expression and function in spinal dorsal horn microglia of males but not females. Additionally, intrathecal injection of TNP-ATP alleviates mechanical allodynia only in male but not female CCI-injured rats. Moreover, adoptive transfer of ATP-stimulated primary cultured microglia from male but not female rats produced mechanical allodynia in male naïve rats (Mapplebeck et al. 2018). In the mouse hyperalgesic priming model, the P2X4R inhibitor TNP-ATP prevented persistent mechanical allodynia induced by the IL-6R/PGE2 pathway in male but not female mice (Paige et al. 2018).

The mu opioid receptor (MOR) is encoded by OPRM1 gene and mediates opioid-induced analgesia and side-effects. Using *Cx3cr1*^{CRE}*/egfp-Oprm1-mCherry* reporter mice, MOR was found to be expressed in about 40% of spinal microglia across male and female animals (Maduna et al. 2019). Intrathecal injection of minocycline improved morphine analgesia in male but not female rats (Posillico et al. 2015). Selective depletion of *Oprm1* in microglia (*Oprm1* cKO) alleviated morphine analgesic tolerance in the hot plate test in male but not female *Cx3cr1*^{CRE}*/Oprm1*$^{fl/fl}$ mice (Reiss et al. 2022).

Additionally, a recent transcriptional profiling study revealed 10 major subtypes of microglial cells (Masuda et al. 2019). It is unclear though, which microglia subtypes have sex-dimorphic roles in chronic pain. As microglial activation and microgliosis occur in spinal dorsal horn of both sexes in chronic pain (Sorge et al. 2015), we cannot exclude the possibility that some aspects microglial signaling may be female specific in pain processing, as has been demonstrated in males. Moreover, the male-selective role of microglia in pain may be site dependent.

Microglia may play a role in females under conditions not mentioned above. Female microglia have been shown to enhance neuropathic pain in mice and rats

after spinal cord injury and in rats with bone cancer (Chen et al. 2012; Hains and Waxman 2006; Yang et al. 2015). It is well known that females require more morphine than males for comparable levels of analgesia. A recent study reported that female mice exhibit greater microglial activation in the periaqueductal gray (PAG) than male mice under naïve and LPS-priming conditions. Intra-PAG inhibition of microglial activation by minocycline can reverse such sex dimorphism in morphine analgesia (Doyle et al. 2017).

6.3.2 Sex Dimorphism in Macrophage Signaling

Macrophages overall do not exhibit sex-dimorphic changes in response to chronic pain. In the chemotherapy-induced peripheral neuropathy (CIPN) model, the chemotherapy agent paclitaxel increases DRG levels of F4/80^{+} macrophages in both male and female mice (Luo et al. 2019). Using the macrophage Fas-induced apoptosis (MAFIA) transgenic mouse line, the Basbaum Lab showed that systematic administration of the apoptosis inducer AP20187 causes a significant loss of CX3CR1^{+} macrophages in lumbar DRGs but not in spinal cord microglia. Of note, macrophage depletion attenuates SNI-induced mechanical allodynia in both male and female mice (Yu et al. 2020).

Emerging studies indicate that male and female macrophages may utilize signaling distinct from microglia to modulate chronic pain. In macrophages of male animals, signaling through Toll-like receptor (TLR9), colony stimulating factor-1 (CSF1) and HMGB1 (Luo et al. 2019; Yu et al. 2020) play major roles in chronic pain, and in female animals the IL-23/IL-17A axis has a particularly important function (Luo et al. 2021).

Neuronal colony-stimulating factor 1 (CSF1) can regulate macrophage function through its receptor CSF1R (Chitu et al. 2016). Depletion of *Csf1* in DRG sensory neurons ($Adv^{CRE}/Csf1^{fl/fl}$) prevents the development of neuropathic pain in mice (Guan et al. 2016). Neuronal depletion of *Csf1* reduces SNI-induced CX3CR1^{+} macrophage expansion in DRGs of male but not female $Adv^{CRE}/Csf1^{fl/fl}$ mice (Yu et al. 2020).

In the collagen antibody-induced arthritis (CAIA) model, intraarticular injection of collagen antibodies causes mechanical allodynia and increases *Hmgb1* gene expression in both male and female mice. Intraarticular injection of disulfide HMGB1 produces mechanical allodynia and promotes joint expression of proinflammatory cytokines (*Tnf, Il1b, Il6, and Ccl2 mRNA*) in male but not female mice. In primary macrophage cultures, disulfide HMGB1 causes greater release of TNF, IL-6 and CXCL1 in male macrophages versus female macrophages. When minocycline is coinjected into the joint, there is a reversal of disulfide HMGB1-induced pain hypersensitivity in male but not female mice, suggesting an involvement of myeloid-derived cells (e.g., macrophages). Moreover, selective depletion of *Tlr4* in LysM+ myeloid-derived cells abolishes disulfide HMGB1-induced joint pain hypersensitivity in male but not female $LysM^{Cre}/Tlr4^{fl/fl}$ mice,

suggesting HMGB1-mediated pain signaling may require macrophage expression by macrophages in male animals (Rudjito et al. 2021).

Male-specific TLR9 signaling has also been implicated in the development of neuropathic pain (Luo et al. 2019). In immune cells, TLR9 is localized to endolysosomes and senses endocytosed single-stranded DNA-containing CpG motifs derived from bacterial DNA (Krieg 2002). Intraplantar injection of the TLR9 agonist ODN1826 induces mechanical allodynia in both male and female mice. However, in a mouse model of chemotherapy-induced peripheral neuropathy (CIPN), TLR9 inhibition by ODN2088 or *Tlr9* mutation reduced mechanical allodynia in male but not female mice (Fig. 6.1). Of note, in both female and male mice, adoptive transfer of paclitaxel-activated macrophages induced potent and persistent mechanical allodynia, which is reversed by *Tlr9* mutation only in males. In primary macrophage cultures, paclitaxel treatment promotes TNF and CXCL1 production, which is blocked by *Tlr9* mutation only in male cells. These results suggest a predominance of TLR9 signaling in male macrophages. Additionally, T cell deficiency enables antinociceptive effects of ODN2088 in female nude mice, suggesting an involvement of T cells in regulating TLR9 pathways in females (Fig. 6.1) (Luo et al. 2019).

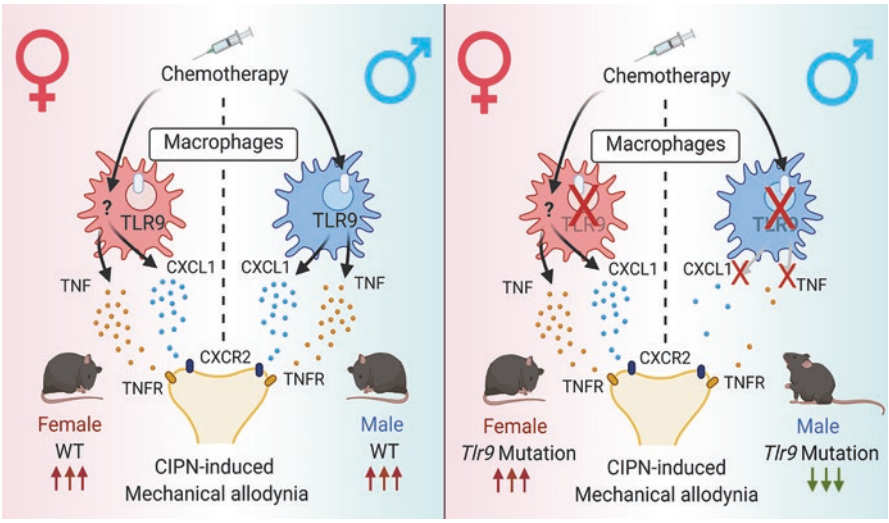

Fig. 6.1 Schematic of macrophage TLR9 signaling in WT and *Tlr9* mutant mice in CIPN. (**a**) Macrophage infiltration and activation is induced by PTX in DRGs of mice in both males and females. Release of TNF and CXCL1 from macrophages in DRGs, as well as subsequent binding to TNFR1/R2 and CXCR2 on sensory neurons, is likewise promoted by PTX. These actions drive mechanical allodynia in CIPN mice by causing hyperexcitability in nociceptive DRG neurons. (**b**) Mechanical allodynia elicited by PTX is attenuated by blocking TLR9, downregulating release of PTX-induced TNF and CXCL1 release from macrophages in male, but not female mice. In addition, T- and B-cell-deficient female mice may use TLR9 signaling in CIPN instead. (Reproduced from Luo et al. (J Neurosci 2019) with permission)

6.3.3 Sex Dimorphism in T Cells, B Cells, and Astrocytes

T cells may primarily affect pain processing in females (Sorge et al. 2015). Several studies demonstrate a female-dominant T cell reaction in chronic pain at different sites. In the mouse SNI model, females exhibit higher levels of $CD4^+$ and $CD8^+$ T cells in the blood compared to males (Sorge et al. 2015). In the mouse CCI model, females exhibit a higher infiltration of $CD3^+$ T cells in the sciatic nerve than males (Vacca et al. 2021). In the mouse partial sciatic nerve injury (PSNL) model, females exhibit a higher ratio of certain populations of T cells in DRGs than males (Lopes et al. 2017). Furthermore, several studies indicate T cell involvement in the sex dimorphism of pain. Peroxisome proliferator activated receptor (PPAR) is a transcription regulator; its subtypes, PPARα and PPARγ, are selectively expressed in male and female T cells respectively. The PPARα inhibitor GW6471 produces analgesia against SNI-induced mechanical allodynia in male mice, whereas the PPARγ inhibitor GW9662 does so in females.

These sexually dimorphic roles of PPAR subtypes require sex hormones. Moreover, T cell-deficient mice (nude and *Rag1*$^{-/-}$) display normal mechanical allodynia in the SNI model. Notably, T cell-deficiency can restore the analgesic effects of intrathecal minocycline in females with SNI or CFA, and adoptive transfer of wildtype splenocytes render female *Rag1*$^{-/-}$ mice insensitive to minocycline in the CFA model (Sorge et al. 2015). In another study, female mice exhibit less sensitivity to morphine analgesia, with females showing a right-shift of the morphine dose–response curve compared to males. T cell deficient (nude) mice of both sexes show reduced morphine analgesia with no sex differences. Notably, adoptive transfer of male WT $CD4^+$ T cells restores greater morphine analgesia in nude mice than female WT $CD4^+$ T cells, suggesting a sex dimorphic role of T cells in regulating morphine analgesia (Rosen et al. 2019).

Recent studies have pointed toward a sex dimorphic role of B cells in chronic pain. In the mouse PSNL model, males exhibit a higher ratio of $CD19^+$ B cells in DRG tissues than females (Lopes et al. 2017). In the tibial fracture pain model, WT mice developed mechanical allodynia in both sexes, which was attenuated by B cell deficiency (muMT) only in male animals. Of note, such effects of B cell deficiency can be blunted by treating the animals with serum collected from male but not female mice with tibial fracture (Guo et al. 2019).

Several studies show that astrocytes exhibit no sex dimorphism in their response to and functioning in chronic pain. Spinal levels of $GFAP^+$ and/or Connexin-43^+ astrocytes increase in mice of both sexes in chronic pain models of CCI (Chen et al. 2018a), CAIA (Fernandez-Zafra et al. 2019), and CIPN (Agalave et al. 2021a). Moreover, in mouse models of acute inflammatory pain, intrathecal injection of the astroglial toxin L-AA reduced formalin-induced spontaneous pain in both male and female animals. Spinal inhibition of astrocyte-selective pathways, such as JNK and Connexin-43, also produces analgesia against formalin-induced pain in both male and female animals (Chen et al. 2018a).

However, studies also indicate that astrocytes may have sex dimorphic roles in chronic pain. Several glial toxins have been used to study glial cells by inhibiting their function. While minocycline mainly targets microglia, fluorocitrate preferentially inhibits astrocytes, and propentofylline may inhibit both microglia and astrocytes (Ji et al. 2019; Sweitzer et al. 2001). Sorge et al. showed all these inhibitors reduced neuropathic pain predominantly in males (Sorge et al. 2015). During puerperium, female rats exhibit a lack of astrocyte activation following spinal nerve ligation compared to their normal female counterparts (Gutierrez et al. 2013). In the CAIA model, spinal astrocytic inhibition by pentoxifylline alleviates mechanical allodynia only in male mice (Fernandez-Zafra et al. 2019). Eukaryotic translation initiation factor 4E (eIF4E) is a cytosolic regulator of mRNA translation. Interestingly, *eif4e* mutation reduces CIPN-induced $GFAP^+$ astrocyte reaction only in males (Agalave et al. 2021a). Thus, astrocytes may only show sex dimorphism under specific pain conditions.

6.4 IL-23/IL-17 Axis-Mediated Neuroimmune Interactions Exhibit Multilevel Sex Differences in Females

Interleukin 23 (IL-23) is a proinflammatory cytokine and belongs to the interleukin 12 (IL-12) family. IL-23 is released by antigen-presenting cells such as dendritic cells and macrophages (Gaffen et al. 2014). It was found that intraplantar injection of IL-23 elicits mechanical allodynia in female mice but not in male mice (Luo et al. 2021). Moreover, loss of IL-23 function, whether through $Il23^{-/-}$ or $Il23r^{-/-}$ knockout, or by treatment with the IL-23R antagonist P2305, can reduce CIPN-induced mechanical allodynia in female but not male mice. Additionally, intraplantar P2305 produces female-specific analgesia in pathological pain models of CCI, diabetic neuropathy, and formalin-induced acute inflammatory pain. These results suggest a female-specific role of the IL-23/IL-23R axis in pain processing (Luo et al. 2021).

Depletion of macrophages, but not T cells, reverses IL-23-induced pain in females, demonstrating a macrophage-dependent mechanism. In both male and female recipient mice, adoptive transfer of paclitaxel-activated macrophages produces comparable levels of mechanical allodynia. However, this allodynia effect is reversed by *Il23* or *Il23r* deficiency only in females, suggesting a female-specific role of the macrophage IL-23/IL-23R axis in pain. It was also found that IL-23-induced pain requires $TRPV1^+$ C-fiber nociceptors. Interestingly, Ca^{2+} imaging and electrophysiological evidence indicates that IL-23 does not act on these nociceptors directly. Instead, another proinflammatory cytokine, IL-17A, is required for IL-23-induced pain and it can directly activate nociceptors. In primary macrophage cultures, IL-23 treatment causes higher production of IL-17A in female cells compared to male cells. Intraplantar administration of IL-17A produces female-dominant mechanical allodynia in mice, which also requires TRPV1. Consequently, IL-17A

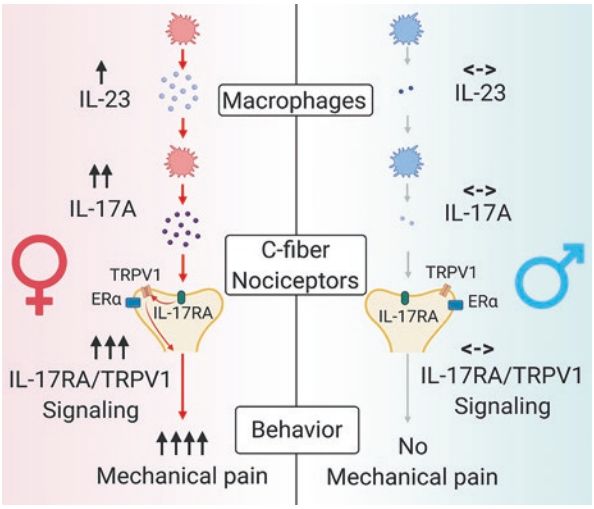

Fig. 6.2 Estrogen/estrogen receptor (ER) signaling in neurons promotes female-dominant pain mediated by IL-23/IL-17, which may explain the female-specific modulation of mechanical pain by the IL-23/IL-17A/TRPV1 axis. Notably, both macrophage and nociceptive signaling have sex-dimorphic characteristics. (Reproduced from Luo et al. (Neuron 2021) with permission)

evokes Ca^{2+} influx and potentiates action potential firing in a female-dominant manner. These results indicate that IL-17A functions as a downstream effector of the IL-23/IL-23R axis in pain modulation. The TRPV1 agonist capsaicin also produces female-dominant mechanical allodynia in mice (Luo et al. 2021).

Notably, the link between IL-17A receptor (IL-17RA) and TRPV1 in DRG sensory neurons is essential to female-specific pain mediated by the IL-23/IL-17A axis, which is regulated by estrogen receptor α (ERα). In situ hybridization studies indicate that male and female mice exhibit comparable ratios of $Il17ra^{+}/Trpv1^{+}/Er\alpha^{+}$ neuron subsets in DRGs. However, selective depletion of *Erα* in $TRPV1^{+}$ neurons ($Trpv1^{CRE}/Er\alpha^{fl/fl}$) diminishes IL-23, IL-17A, and capsaicin-evoked mechanical allodynia in females, suggesting that estrogen signaling is an essential component of IL-23/IL-17A-mediated pain in females (Fig. 6.2). We also observed that in human DRG tissues, ERα but not ERβ shows sex dimorphism, where females have significantly higher expression levels of Erα expression (Luo et al. 2021).

Using an optogenetic approach in which ChR2 is expressed in TPRV1+ nociceptors, we found blue light stimulation induced greater pain in females. We also found that intraplantar IL-23 injection can potentiate blue light-induced pain, but this only occurs in female mice and not in male mice (Ji et al. 2021).

6.5 Summary and Future Directions

The incidence of chronic pain in women is higher than men. Women also suffer disproportionately from inflammatory diseases associated with pain such as fibromyalgia, osteoarthritis, chronic migraine, and autoimmune diseases (rheumatoid arthritis and systemic lupus erythematosus). Because scientists have mainly used male rodents due to built-up inertia within the research field and the fact that male rodents do not have cyclical hormonal fluctuations like female rodents do, sex-related differences and sex dimorphism in pain were poorly understood until very recently. As the previous chapters have shown, immune and glial cells are crucial to the modulation of pain. This chapter has highlighted several glial and immune cell types and how they contribute to sex dimorphism in chronic pain (Table 6.1). Notably, the mechanisms of most sex-dimorphic immune pain molecules have predominantly been characterized in male animals. As such, further studies will be necessary to investigate novel female-specific pain signaling pathways in these immune cells across various chronic pain conditions. These efforts will improve our understanding of sex dimorphism in chronic pain to improve the treatment of pain for patients in the future.

References

Agalave NM, Mody PH, Szabo-Pardi TA, Jeong HS, Burton MD. Neuroimmune consequences of eIF4E phosphorylation on chemotherapy-induced peripheral neuropathy. Front Immunol. 2021a;12:642420.

Agalave NM, Rudjito R, Farinotti AB, Khoonsari PE, Sandor K, Nomura Y, Szabo-Pardi TA, Urbina CM, Palada V, Price TJ, Erlandsson Harris H, Burton MD, Kultima K, Svensson CI. Sex-dependent role of microglia in disulfide high mobility group box 1 protein-mediated mechanical hypersensitivity. Pain. 2021b;162(2):446–58.

Avona A, Burgos-Vega C, Burton MD, Akopian AN, Price TJ, Dussor G. Dural calcitonin gene-related peptide produces female-specific responses in rodent migraine models. J Neurosci. 2019;39(22):4323–31.

Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev. 2011;35(3):565–72.

Berta T, Park CK, Xu ZZ, Xie RG, Liu T, Lü N, Liu YC, Ji RR. Extracellular caspase-6 drives murine inflammatory pain via microglial TNF-α secretion. J Clin Invest. 2014;124(3):1173–86.

Berta T, Qadri YJ, Chen G, Ji RR. Microglial signaling in chronic pain with a special focus on caspase 6, p38 MAP kinase, and sex dependence. J Dent Res. 2016;95(10):1124–31.

Chen MJ, Kress B, Han X, Moll K, Peng W, Ji RR, Nedergaard M. Astrocytic CX43 hemichannels and gap junctions play a crucial role in development of chronic neuropathic pain following spinal cord injury. Glia. 2012;60(11):1660–70.

Chen G, Luo X, Qadri MY, Berta T, Ji RR. Sex-dependent glial signaling in pathological pain: distinct roles of spinal microglia and astrocytes. Neurosci Bull. 2018a;34(1):98–108.

Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018b;100(6):1292–311.

Chen O, Donnelly CR, Ji RR. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020a;62:17–25.

Chen Y, Moutal A, Navratilova E, Kopruszinski C, Yue X, Ikegami M, Chow M, Kanazawa I, Bellampalli SS, Xie J, Patwardhan A, Rice K, Fields H, Akopian A, Neugebauer V, Dodick D, Khanna R, Porreca F. The prolactin receptor long isoform regulates nociceptor sensitization and opioid-induced hyperalgesia selectively in females. Sci Transl Med. 2020b;12(529):eaay7550.

Chitu V, Gokhan Ş, Nandi S, Mehler MF, Stanley ER. Emerging roles for CSF-1 receptor and its ligands in the nervous system. Trends Neurosci. 2016;39(6):378–93.

Chiu IM, von Hehn CA, Woolf CJ. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. Nat Neurosci. 2012;15(8):1063–7.

Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature. 2005;438(7070):1017–21.

Dahlhamer J, Lucas J, Zelaya C, Nahin R, Mackey S, DeBar L, Kerns R, Von Korff M, Porter L, Helmick C. Prevalence of chronic pain and high-impact chronic pain among adults – United States, 2016. Morb Mortal Wkly Rep. 2016;67(36):1001–6.

Diogenes A, Patwardhan AM, Jeske NA, Ruparel NB, Goffin V, Akopian AN, Hargreaves KM. Prolactin modulates TRPV1 in female rat trigeminal sensory neurons. J Neurosci. 2006;26(31):8126–36.

Doyle HH, Eidson LN, Sinkiewicz DM, Murphy AZ. Sex differences in microglia activity within the periaqueductal gray of the rat: a potential mechanism driving the dimorphic effects of morphine. J Neurosci. 2017;37(12):3202–14.

Fernandez-Zafra T, Gao T, Jurczak A, Sandor K, Hore Z, Agalave NM, Su J, Estelius J, Lampa J, Hokfelt T, Wiesenfeld-Hallin Z, Xu X, Denk F, Svensson CI. Exploring the transcriptome of resident spinal microglia after collagen antibody-induced arthritis. Pain. 2019;160(1):224–36.

Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL 3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. J Pain. 2009;10(5):447–85.

Frot M, Feine JS, Bushnell CM. Sex differences in pain perception and anxiety. A psychophysical study with topical capsaicin. Pain 2004;08(3):230–36.

Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol. 2014;14(9):585–600.

Gazerani P, Andersen OK, Arendt-Nielsen L. A human experimental capsaicin model for trigeminal sensitization. Gender-specific differences. Pain 2005;118(1–2):155–63.

Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol. 2014;14(4):217–31.

Grace PM, Wang X, Strand KA, Baratta MV, Zhang Y, Galer EL, Yin H, Maier SF, Watkins LR. DREADDed microglia in pain: implications for spinal inflammatory signaling in male rats. Exp Neurol. 2018;304:125–31.

Guan Z, Kuhn JA, Wang X, Colquitt B, Solorzano C, Vaman S, Guan AK, Evans-Reinsch Z, Braz J, Devor M, Abboud-Werner SL, Lanier LL, Lomvardas S, Basbaum AI. Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. Nat Neurosci. 2016;19(1):94–101.

Guo TZ, Shi X, Li WW, Wei T, Clark JD, Kingery WS. Sex differences in the temporal development of pronociceptive immune responses in the tibia fracture mouse model. Pain. 2019;160(9):2013–27.

Gutierrez S, Hayashida K, Eisenach JC. The puerperium alters spinal cord plasticity following peripheral nerve injury. Neuroscience. 2013;228:301–8.

Hains BC, Waxman SG. Activated microglia contribute to the maintenance of chronic pain after spinal cord injury. J Neurosci. 2006;26(16):4308–17.

Hartmann EM, Handwerker HO, Forster C. Gender differences in itch and pain-related sensations provoked by histamine, cowhage and capsaicin. Acta Derm Venereol. 2015;95(1):25–30. doi: https://doi.org/10.2340/00015555-1894. PMID: 24819823.

Huck NA, Siliezar-Doyle J, Haight ES, Ishida R, Forman TE, Wu S, Shen H, Takemura Y, Clark JD, Tawfik VL. Temporal contribution of myeloid-lineage TLR4 to the transition to chronic pain: a focus on sex differences. J Neurosci. 2021;41(19):4349–65.

Inoue K, Tsuda M. Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci. 2018;19(3):138–52.
Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. Mol Pain. 2007;3:33.
Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013;154(Suppl 1(01)):S10–28.
Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science. 2016;354(6312):572–7.
Ji RR, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20(11):667–85.
Ji J, He Q, Luo X, Bang S, Matsuoka Y, McGinnis A, Nackley AG, Ji RR. IL-23 Enhances C-Fiber-Mediated and Blue Light-Induced Spontaneous Pain in Female Mice. Front Immunol. 2021;12:787565.
Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol. 2002;20:709–60.
Langley PC. The prevalence, correlates and treatment of pain in the European Union. Curr Med Res Opin. 2011;27(2):463–80.
Lopes DM, Malek N, Edye M, Jager SB, McMurray S, McMahon SB, Denk F. Sex differences in peripheral not central immune responses to pain-inducing injury. Sci Rep. 2017;7(1):16460.
Luo X, Fitzsimmons B, Mohan A, Zhang L, Terrando N, Kordasiewicz H, Ji RR. Intrathecal administration of antisense oligonucleotide against p38α but not p38β MAP kinase isoform reduces neuropathic and postoperative pain and TLR4-induced pain in male mice. Brain Behav Immun. 2018;72:34–44.
Luo X, Huh Y, Bang S, He Q, Zhang L, Matsuda M, Ji RR. Macrophage toll-like receptor 9 contributes to chemotherapy-induced neuropathic pain in male mice. J Neurosci. 2019;39(35):6848–64.
Luo X, Chen O, Wang Z, Bang S, Ji J, Lee SH, Huh Y, Furutani K, He Q, Tao X, Ko MC, Bortsov A, Donnelly CR, Chen Y, Nackley A, Berta T, Ji RR. IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice. Neuron. 2021;109(17):2691–2706.e5.
Maduna T, Audouard E, Dembélé D, Mouzaoui N, Reiss D, Massotte D, Gaveriaux-Ruff C. Microglia express mu opioid receptor: insights from transcriptomics and fluorescent reporter mice. Front Psych. 2019;9:726.
Maixner W, Humphrey C. Gender differences in pain and cardiovascular responses to forearm ischemia. Clin J Pain. 1993;9(1):16–25.
Mapplebeck JCS, Dalgarno R, Tu Y, Moriarty O, Beggs S, Kwok CHT, Halievski K, Assi S, Mogil JS, Trang T, Salter MW. Microglial P2X4R-evoked pain hypersensitivity is sexually dimorphic in rats. Pain. 2018;159(9):1752–63.
Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Sagar SC, Nessler S, Kunz P, van Loo G, Coenen VA, Reinacher PC, Michel A, Sure U, Gold R, Grün D, Priller J, Stadelmann C, Prinz M. Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature. 2019;566(7744):388–92.
Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci. 2012;13(12):859–66. doi: https://doi.org/10.1038/nrn3360. PMID: 23165262.
Mogil JS. Qualitative sex differences in pain processing: emerging evidence of a biased literature. Nat Rev Neurosci. 2020;21(7):353–65.
Mogil JS. Sex differences in pain and pain inhibition: multiple explanations of a controversial phenomenon. Nat Rev Neurosci. 2021;13(12):859–66.
Moriarty O, Tu Y, Sengar AS, Salter MW, Beggs S, Walker SM. Priming of adult incision response by early-life injury: neonatal microglial inhibition has persistent but sexually dimorphic effects in adult rats. J Neurosci. 2019;39(16):3081–93.
Navratilova E, Fillingim RB, Porreca F. Sexual dimorphism in functional pain syndromes. Sci Transl Med. 2021;13(619):eabj7180.
Paige C, Maruthy GB, Mejia G, Dussor G, Price T. Spinal inhibition of P2XR or p38 signaling disrupts Hyperalgesic priming in male, but not female, mice. Neuroscience. 2018;385:133–42.

Patil MJ, Ruparel SB, Henry MA, Akopian AN. Prolactin regulates TRPV1, TRPA1, and TRPM8 in sensory neurons in a sex-dependent manner: contribution of prolactin receptor to inflammatory pain. Am J Physiol Endocrinol Metab. 2013;305(9):E1154–64.

Patil M, Belugin S, Mecklenburg J, Wangzhou A, Paige C, Barba-Escobedo PA, Boyd JT, Goffin V, Grattan D, Boehm U, Dussor G, Price TJ, Akopian AN. Prolactin regulates pain responses via a female-selective nociceptor-specific mechanism, vol. iScience 20; 2019. p. 449–65.

Payrits M, Sághy É, Cseko K, Pohóczky K, Bölcskei K, Ernszt D, Barabás K, Szolcsányi J, Ábrahám IM, Helyes Z, Szoke É. Estradiol sensitizes the transient receptor potential Vanilloid 1 receptor in pain responses. Endocrinology. 2017;158(10):3249–58.

Posillico CK, Terasaki LS, Bilbo SD, Schwarz JM. Examination of sex and minocycline treatment on acute morphine-induced analgesia and inflammatory gene expression along the pain pathway in Sprague-Dawley rats. Biol Sex Differ. 2015;6:33.

Reiss D, Maduna T, Maurin H, Audouard E, Gaveriaux-Ruff C. Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice. J Neurosci Res. 2022;100(1):203–19.

Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. J Neurosci Res. 2017;95(1–2):500–8.

Rosen SF, Ham B, Haichin M, Walters IC, Tohyama S, Sotocinal SG, Mogil JS. Increased pain sensitivity and decreased opioid analgesia in T-cell-deficient mice and implications for sex differences. Pain. 2019;160(2):358–66.

Rudjito R, Agalave NM, Farinotti AB, Lundbäck P, Szabo-Pardi TA, Price TJ, Harris HE, Burton MD, Svensson CI. Sex- and cell-dependent contribution of peripheral high mobility group box 1 and TLR4 in arthritis-induced pain. Pain. 2021;162(2):459–70.

Salter MW, Stevens B. Microglia emerge as central players in brain disease. Nat Med. 2017;23(9):1018–27.

Saika F, Matsuzaki S, Kobayashi D, Ideguchi Y, Nakamura TY, Kishioka S, Kiguchi N. Chemogenetic regulation of CX3CR1-expressing microglia using Gi-DREADD exerts sex-dependent anti-allodynic effects in mouse models of neuropathic pain. Front Pharmacol. 2020;11:925.

Shupler MS, Kramer JK, Cragg JJ, Jutzeler CR, Whitehurst DGT. Pan-Canadian estimates of chronic pain prevalence from 2000 to 2014: a repeated cross-sectional survey analysis. J Pain. 2019;20(5):557–65.

Sorge RE, Totsch SK. Sex Differences in Pain. J Neurosci Res. 2017;95(6):1271–81.

Sorge RE, Strath LJ. Sex differences in pain responses. Curr Opin Physio. 2018;6:75–81.

Sorge RE, LaCroix-Fralish ML, Tuttle AH, Sotocinal SG, Austin JS, Ritchie J, Chanda ML, Graham AC, Topham L, Beggs S, Salter MW, Mogil JS. Spinal cord toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neurosci. 2011;31(43):15450–4.

Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18(8):1081–3.

Sweitzer SM, Schubert P, DeLeo JA. Propentofylline, a glial modulating agent, exhibits antiallodynic properties in a rat model of neuropathic pain. J Pharmacol Exp Ther. 2001;297(3):1210–7.

Tavares-Ferreira D, Ray PR, Sankaranarayanan I, Mejia GL, Wangzhou A, Shiers S, Uttarkar R, Megat S, Barragan-Iglesias P, Dussor G, Akopian AN, Price TJ. Sex differences in nociceptor translatomes contribute to divergent prostaglandin signaling in male and female mice. Biol Psychiat. 2022;91(1):129–40.

Taves S, Berta T, Liu DL, Gan S, Chen G, Kim YH, Van de Ven T, Laufer S, Ji RR. Spinal inhibition of p38 MAP kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. Brain Behav Immun. 2016;55:70–81.

Ueda H, Dozono N, Tanaka K, Kaneko S, Neyama H, Uchida H. Allodynia by splenocytes from mice with acid-induced fibromyalgia-like generalized pain and its sexual dimorphic regulation by brain microglia. Front Neurosci. 2020;14:600166.

Unruh AM. Gender variations in clinical pain experience. Pain. 1996;65(2–3):123–67.

Vacca V, Marinelli S, De Angelis F, Angelini DF, Piras E, Battistini L, Pavone F, Coccurello R. Sexually dimorphic immune and Neuroimmune changes following peripheral nerve injury in mice: novel insights for gender medicine. Int J Mol Sci. 2021;22(9):4397.

Warwick CA, Shutov LP, Shepherd AJ, Mohapatra DP, Usachev YM. Mechanisms underlying mechanical sensitization induced by complement C5a: the roles of macrophages, TRPV1, and CGRP receptors. Pain. 2019;160(3):702–11.

Wu A, Dong W, Liu S, Cheung JPY, Kwan KYH, Zeng X, Zhang K, Sun Z, Wang X, Cheung KMC, Zhou M, Zhao J. The prevalence and years lived with disability caused by low back pain in China, 1990 to 2016: findings from the global burden of disease study 2016. Pain. 2019;160(1):237–45.

Xu ZZ, Berta T, Ji RR. Resolvin E1 inhibits neuropathic pain and spinal cord microglial activation following peripheral nerve injury. J Neuroimmune Pharmacol. 2013;8(1):37–41.

Xu B, Mo C, Lv C, Liu S, Li J, Chen J, Wei Y, An H, Ma L, Guan X. Post-surgical inhibition of phosphatidylinositol 3-kinase attenuates the plantar incision-induced postoperative pain behavior via spinal Akt activation in male mice. BMC Neurosci. 2019;20(1):36.

Yamagata K, Sugimura M, Yoshida M, Sekine S, Kawano A, Oyamaguchi A, Maegawa H, Niwa H. Estrogens exacerbate nociceptive pain via up-regulation of TRPV1 and ANO1 in trigeminal primary neurons of female rats. Endocrinology. 2016;157(11):4309–17.

Yang Y, Li H, Li TT, Luo H, Gu XY, Lü N, Ji RR, Zhang YQ. Delayed activation of spinal microglia contributes to the maintenance of bone cancer pain in female Wistar rats via P2X7 receptor and IL-18. J Neurosci. 2015;35(20):7950–63.

Yu X, Liu H, Hamel KA, Morvan MG, Yu S, Leff J, Guan Z, Braz JM, Basbaum AI. Dorsal root ganglion macrophages contribute to both the initiation and persistence of neuropathic pain. Nat Commun. 2020;11(1):264.

Chapter 7
Neuroimmune Interactions in Acute and Chronic Itch

Ouyang Chen, Sreya Rahman, Madelynne Olexa, Jasmine Ji, Yul Huh, and Ru-Rong Ji

Abstract Itch is a sensory experience of the skin that is familiar to all humans. Recent studies have established that the immune system and central and peripheral nervous systems engage in extensive interactions termed "crosstalk," which gives rise to both acute and chronic itch pathologies. Peripheral sensory neurons detect itch-triggering stimuli from the environment to transduce pruritic signals. Itch signals travel from nerve fibers in the skin and peripheral nervous system to the spinal cord and brain, eliciting scratch behavior as a response to relieve the irritation caused by itch. The mechanisms underlying itch have also been shown to overlap with molecular circuits involved in pain, suggesting a relationship between the two sensations. We discuss various types of pruritogens, released from immune cells, keratinocytes, neurons, glial cells, and cancer cells, as well as the pruritogen receptors expressed by primary sensory neurons (pruriceptors) and spinal cord neurons. Understanding how neuroimmune interactions modulate acute and chronic itch will be necessary to develop more effective treatments for these pathologies.

Keywords Pruritus · Itch · Neuroimmune interaction · Toll-like receptors (TLRs) · Gastrin-releasing peptide (GRP) · GRP receptor (GRPR) · Glial cells · Skin · miRNA · Lymphoma · TRPA1 · TRPV1

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7.1 Introduction to Itch

Itch (or pruritus) is an unpleasant sensation on the skin that provokes scratching behavior. It is normally a benign, though unpleasant, physiologic response. However, when itch becomes severe, chronic, or occurs with certain medical conditions, it has a significant effect on a patient's well-being. Clinically, itch is considered either acute (lasting less than 6 weeks) or chronic (continuing beyond 6 weeks). Chronic itch is experienced by 15% of the population and negatively affects sleep, mood, and personal relationships, significantly reducing overall quality of life as a result.

Although our understanding of the mechanisms and pathways that carry pruritic stimuli and process itch sensation is still limited, the most well-supported distinction between types of itch is between histaminergic and non-histaminergic itch (Ikoma et al. 2006; Yosipovitch and Papoiu 2008). The different types of itch correspond to differences in disease and treatment responses. Acute itch is coded through a histamine-induced neuronal pathway, whereas chronic itch signaling is non-histaminergic. Chronic itch is the result of interactions between nerves that sense non-histaminergic stimuli, the immune system, and keratinocytes in the skin (Yosipovitch and Bernhard 2013). On the molecular level, pruriceptors, the itch-sensing primary sensory neurons, are responsible for responding to itch-inducing stimuli called pruritogens and modulating itch signaling throughout the nervous system (Ringkamp et al. 2011; Akiyama and Carstens 2013; LaMotte et al. 2014). The sensation of itch results from nerve fiber activation in the dermo-epidermal junction of the skin (Han and Dong 2014). Neuropathic itch can result from damage to the nervous system, including nerve fiber compression or degeneration and glial cell damage (Misery et al. 2014).

The nervous system and the immune system work in tandem to protect and warn individuals of threats by sensing the presence of pathogens as well as injured and dying cells. As a result of their coevolution and overlapping roles, neurons and immune cells respond to both environmental inputs as well as signals from each other. This bilateral interaction tunes the responses of the two systems in different circumstances. Nociceptive neurons express various receptors for soluble mediators produced by the immune system, including ions, amines, lipids, cytokines, and chemokines (see Chap. 2). Conversely, immune cells such as macrophages and mast cells can be directly activated by neuropeptides and other neuromodulators released from the peripheral terminals of activated nociceptors. Therefore, the extensive crosstalk between the nervous and immune systems is being increasingly recognized as an essential aspect of itch-related homeostasis and disease. In the following sessions, we discuss cells, mediators, receptors, and effectors of itch (Table 7.1), with specific focus on neuroimmune and neuroglial interactions.

Table 7.1 Cells, mediators, receptors, and effectors of itch

Resources	Pruritogens	Receptors	Effectors
Neurons	Substance P	NK1R	NK1R
	GRP	GRPR	GRPR
Keratinocytes	NGF	TrkA	TRPV1
	Endothelin-1	ETA	TRPV1
	TSLP1	TSLPR	TRPA1
	BAM8-22 peptide	MrgprC11	TRPV1/TRPA1
	IL-33	IL-33R	TRPV1/TRPA1
	miR-146a	TRPV1	TRPV1
Mast cells	Histamine	H1R/H4R	TRPV1/Nav1.7
	Tryptase	PAR1/2	TRPV1
	Serotonin	5HTR	TRPA1
T cells	IL-31	IL-31R	TRPV1/TRPA1
T cell lymphoma	miR-711	TRPA1	TRPA1
Drugs	Chloroquine	MagprA3	TRPA1/Nav1.7
	β-alanine	MrgprD	TRPA1
	Imiquimod	TLR7	TRPA1
	Morphine	MOR	MOR
Oxidative stress	ROS (H_2O_2)	TRPA1	TRPA1

Itch mediators are secreted from primary sensory neurons, keratinocytes, and immune cells. In addition, commonly used drugs are also known to elicit itch. Furthermore, oxidative stress such as reactive oxygen species (ROS) can also elicit itch via activation of TRPA1 (Liu and Ji 2012)

7.2 Epidermis and Keratinocytes in Itch

Itch is a unique sensory modality, as it is restricted to the skin, mucous membranes, and cornea. No other tissues or organs are capable of experiencing itch (Yosipovitch and Papoiu 2008). It has been demonstrated that removal of the epidermis abolishes the perception of pruritus, but not pain (Schmelz et al. 1997). The sensation of itch originates from the itch-specific nerve fibers located in the epidermis and dermal-epidermal junction, termed pruriceptors. Itch-specific nerve fibers (predominantly C fibers) are characterized by mechano-insensitivity, low conduction velocities, large areas of innervation, and high transcutaneous electrical thresholds (Schmelz et al. 2003). These itch fibers have close relation to epidermal cells such as keratinocytes.

Keratinocytes are a cell type in the skin that form the protective barrier between the body and the external environment (e.g., regulation of water loss, antimicrobial peptide secretion). Keratinocytes contribute to itch by releasing pruritogens, including the alarmin thymic stromal lymphopoietin (TSLP), to directly activate pruriceptive neurons (Schwendinger-Schreck et al. 2015; Wilson et al. 2013; Ziegler et al. 2013). Keratinocytes also express the same receptors expressed on neurons to

mediate pruritus. For example, mast cell proteases not only trigger pruritus via activation of PAR2+ sensory neurons (Schwendinger-Schreck et al. 2015), but they also stimulate the release of TSLP from keratinocytes (Wilson et al. 2013). In addition, TSLP secreted from keratinocytes may indirectly promote skin inflammation and pruritus pathways by activating immune cells. TSLP binds to TSLP receptor (TSLPR) on Th2 cells and type 2 innate lymphoid cells leading to the production of pruritogenic cytokines (Indra 2013; Ochiai et al. 2018; Soumelis et al. 2002). IL-4 and IL-13 produced by immune cells can also stimulate keratinocytes to secrete TSLP (Bogiatzi et al. 2007). Additionally, keratinocytes release nerve growth factor (NGF) in response to neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), in order to modulate innervation and leakiness of the skin barrier (neurogenic inflammation). Thus, keratinocyte–neuron interactions create powerful bi-directional feedback loops that can worsen chronic skin conditions.

7.3 Immune Cells and Mediators in Itch

Activation of the immune system can affect neurophysiological, neurochemical, and neuroendocrine activities. Cytokines, chemokines, peptides, micro-RNAs, and other factors produced and released by immune cells may directly act on peripheral neurons, especially pruriceptors (Fig. 7.1 and Table 7.1). We will summarize the interaction among different immune cells and nociceptors below.

Mast cells are the “frontier soldiers” of the immune system. They are involved in the innate and adaptive immune system (Galli et al. 2005; Kubo 2017). Mast cells are found in externally exposed surfaces such as the epithelial lining of the skin, airways of the lung, mucosa of the gastrointestinal tract, and meningeal membrane of the central nervous system (CNS). This feature allows them to induce a rapid immune response to environmental stimuli, such as allergens and pathogens. Increasing evidence has demonstrated that mast cells contribute to various diseases via their interactions with the vasculature and CNS (Aich et al. 2015; Galli et al. 2005; Xanthos et al. 2011). Mast cell degranulation is critical for the innate immune response and allergic reactions. The release of histamine, bradykinin, and other mediators upon degranulation may contribute to itch sensitization in pathological conditions. It has been found that the degranulation of mast cells partly requires interaction between mast cells and peripheral nerve terminals. The process is mediated by the calcium-dependent cell adhesion molecule N-cadherin, which is expressed in both mast cells and nociceptor neurons (Cyphert et al. 2009; Suzuki et al. 2004).

Histaminergic itch is induced through the H1 and H4 receptors on sensory nerves. Combined antagonism of H1 and H4 receptors may be a useful strategy in controlling itch and inflammation in diseases such as atopic dermatitis (AD) and psoriasis (Ohsawa and Hirasawa 2014). Non-histaminergic itch may be associated with several distinct neural pathways. One of them is Proteinase-activated receptor-2 (PAR2), as PAR2+ fibers and the PAR2 agonist tryptase are increased in lesioned

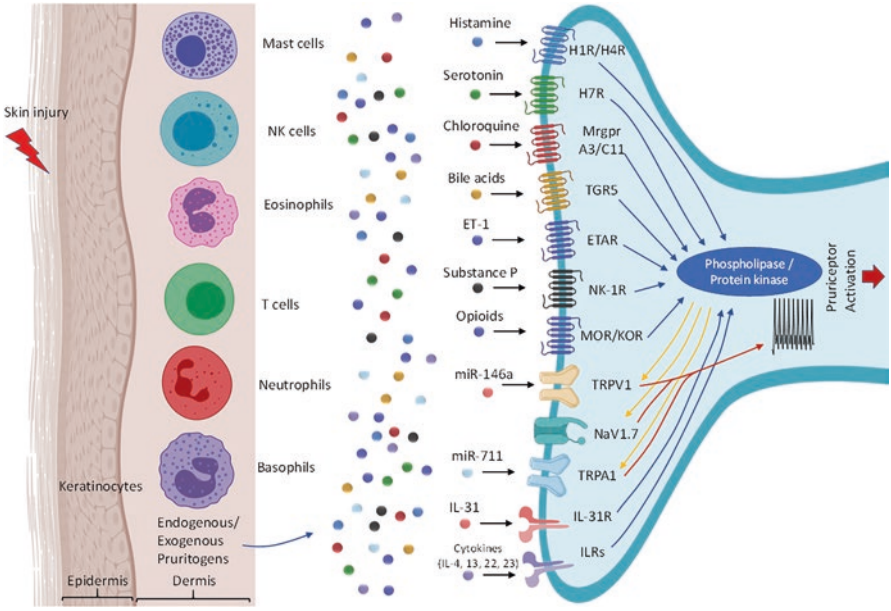

Fig. 7.1 Neuroimmune interactions in the skin for the induction of itch. Following skin injury, keratinocytes and immune cells such as mast cells, NK cells, eosinophils, T cells, neutrophils, and basophils, produce various itch mediators, including histamine released by mast cells and cytokines from immune cells. These pruritogens then bind to specific receptors expressed on pruriceptors (itch-sensing neurons), which can result in the activation of key enzymes (e.g., phospholipase) and protein kinases that can further activate critical ion channels (e.g., transient receptor potential ion channels TRPA1, TRPV1, and TRPV4 and sodium channels Nav1.7). The subsequent propagation of action potentials through the pruriceptive neurons to the central nervous system causes itch sensation. Furthermore, miRNAs, such as miR-711 released from cutaneous T cell lymphoma (CTCL) and miR-146a released from keratinocytes can also induce pruritus via direct activation of TRPA1 and TRPV1, respectively. Additionally, drugs such as opioids (morphine), anti-malaria drug chloroquine, and anti-cancer drug imiquimod also elicit itch via activation of pruriceptors

AD skin. Upon activation of PAR2+ fibers, SP and CGRP are released, leading to the potentiation of neurogenic inflammation (Kempkes et al. 2014; Steinhoff et al. 2003a).

Macrophages are derived from circulating monocytes and recruited within hours of an injury. Regional macrophages take on a phagocytic role almost immediately after injury to remove worn out cells and other cellular debris in both innate and adaptive immune responses. After their activation and recruitment, macrophages release numerous soluble mediators, which sensitize nociceptors/pruriceptors toward pruritus, for instance, during pruritus induced by hydroxyethyl starch (Bork 2005). Mice with pruritic inflammatory skin disease lack heterogeneous nuclear ribonucleoprotein D (hnRNPD), which is a regulator of inflammatory cytokine mRNA stability (Sadri and Schneider 2009).

Skin dendritic cells are antigen-presenting cells (APCs) which initiate the adaptive immune response against invading pathogens by presenting antigens to T cells (Tay et al. 2014). It has been shown that overexpression of the cysteine protease cathepsin S induces elevated PAR2 expression in dendritic cells and the expression of Type 1 helper T cell-(Th1) related cytokines, leading to spontaneous scratching behavior (Kim et al. 2012).

Activation of type 2 helper T-cells (Th2 cells) is important for moderate to severe AD. IL-4 and IL-13 are cytokines required for the development, initiation, and maintenance of the Th2 subset of the T cells. Increased expression of IL-4 and IL-13 are associated with eosinophilic infiltration and increased production of NGF and the NGF receptor TrkA (Leung et al. 2004; Simon et al. 2004). Overexpression of IL-4 and IL-13 in the epidermis of transgenic mice causes AD and pruritus (Chan et al. 2001; Zheng et al. 2009). The expression levels of IL-4 and IL-13 are elevated in human skin samples of AD patients, with IL-13 elevated in the serum (Jeong et al. 2003). Serum levels of IL-13 in turn are correlated with disease severity (Metwally et al. 2004). Recently, researchers found that TRPA1 is linked with IL-13-related pruritus in AD (Oh et al. 2013). Monoclonal antibodies targeting IL-4 and IL-13 can significantly reduce the severity of pruritus in AD patients (Griffiths et al. 2021). IL-33 is a promoter of Th2-mediated inflammation in the pathogenesis of AD (Kroeger et al. 2009; Rankin et al. 2010; Savinko et al. 2012), and the expression of IL-33 is elevated in the skin cells of AD patients (Savinko et al. 2012).

IL-31 is one of the most prominent itch mediators produced by Th2 cells and its receptor (IL-31R) is expressed on keratinocytes, epithelial cells, and primary sensory neurons including nociceptors/pruriceptors (Dillon et al. 2004; Heise et al. 2009; Kremer et al. 2014; Sonkoly et al. 2006). IL-31RA is also localized in small diameter neurons of human DRG (Cevikbas et al. 2014; Sonkoly et al. 2006). IL-31R activation leads to activation of the JAK family of tyrosine kinases, which leads to the activation of transcription factors (STAT-1/5 and ERK-1/2) and an MAP kinase signaling cascade (Cornelissen et al. 2012; Kasraie et al. 2013). IL-31 expression is elevated in many pruritic disorders including AD, allergic contact dermatitis, and cutaneous T-cell lymphoma (CTCL) (Ohmatsu et al. 2012; Raap et al. 2008; Singer et al. 2013; Sonkoly et al. 2006).

Nerve growth factor (NGF) is a neurotrophin which is critical for peripheral nervous system development, growth, differentiation, and regeneration (Yamamoto et al. 2007). It has been demonstrated that serum levels of NGF correlate with the degree of scratching behavior in mice (Yamamoto et al. 2007). Eosinophils serve as a main source of NGF. Thus, NGF is thought to participate in neural hyperplasia (Kanda and Watanabe 2003). In mouse models, NGF stimulates increased levels of substance P and calcitonin gene-related peptide (CGRP), which are factors involved in neurogenic inflammation and the hypersensitization of pruriceptors (Verge et al. 1995). In addition, NGF primes sensory nerves by lowering the threshold for itch sensation. NGF can also induce TRKA activation, which increases the phosphorylation of phosphoinositide 3-kinases (PI3K) and stimulates TRPV1 expression and calcium influx (Nockher and Renz 2006; Zhuang et al. 2004).

7.4 Itch Receptors and Signaling in Itch-Sensing Sensory Neurons (Pruriceptors)

7.4.1 G Protein-Coupled Receptor (GPCR)

To detect external changes, nerve fibers of the pruriceptors (a subset of nociceptors) express specialized receptors. Different receptors are present in different types of nerve fibers to respond to specific ligands (Han and Dong 2014). One common characteristic of these receptors is that they are members of the G protein-coupled receptor (GPCR) family.

Itching and vasodilation can be induced by various types of itch mediators (Fig. 7.1). For example, histamine is released from immune cells due to tissue inflammation and exposure to environmental allergens (Ikoma et al. 2006; Shim et al. 2007; Simons and Simons 2011). There are four histamine receptors, all of which are GPCRs (Simons and Simons 2011). H1R and H4R are expressed in the DRG and have been identified as potential mediators of pruriception (Simons and Simons 2011). H1R is an important receptor for histamine-induced itch reactions, and H1R-specific inhibitors can completely suppress histamine-induced itch in human skin (Simons and Simons 2011). In addition, H4R inhibitors can block itch in a contact dermatitis mouse model, indicating that H4R is another mediator of itch (Rossbach et al. 2009). A novel H4R antagonist (JNJ 39758979) reduced histamine-induced pruritus in a randomized control trial (Kollmeier et al. 2014). Furthermore, H1R and H4R dual blockade was more effective at reducing itch and inflammation than blockade of either alone (Cowden et al. 2010).

Mas-related G protein-coupled receptors (Mrgprs) are a subgroup of GPCRs (Dong et al. 2001). Mice express more than 50 different Mrgprs, while humans express 10 members of the receptor family (Dong et al. 2001; Zylka et al. 2003). Many Mrgprs are expressed by nociceptors in the DRG and the trigeminal ganglia, which project axons into the skin to sense noxious mechanical stimuli and temperature (Dong et al. 2001; McNeil and Dong 2012). Mrgprs are involved in chloroquine (CQ)-induced itch (Liu et al. 2009). In Mrgpr knockout mice, while neither nociception nor histaminergic itch was reduced, but CQ-induced calcium influx and action potentials were abolished (Liu et al. 2009). Further research has revealed that mouse MrgprA3 and human MrgprX1 are involved in CQ-induced itch (Sikand et al. 2011). In a mouse model in which investigators genetically expressed TRPV1 in MrgprA3+ neurons, capsaicin administration resulted in activation of MrgprA3+ neurons and induced itching but not pain (Han et al. 2013). Thus, MrgprA3+ neurons mediate an itch-specific circuit. These critical findings have demonstrated that sensation does not depend on stimulus type but rather on the specific activated neuronal pathway (Han et al. 2013).

Protease-activated receptors (PAR) are another family of itch-related GPCRs found on different cell types, including keratinocytes and pruriceptive neurons in the DRG (Han and Dong 2014). Activation of PAR2 and PAR4 leads to the induction of non-histaminergic itch (Reddy et al. 2008; Vergnolle et al. 2003). PAR2 can

be activated by various endogenous and exogenous proteases, such as cathepsin S, tryptase, dust mites, and Staphylococcus aureus (Reddy et al. 2008, 2010; Soh et al. 2010; Steinhoff et al. 2006). Tryptase, an endogenous agonist of PAR2, is significantly increased in lesioned skin of AD patients (Steinhoff et al. 2003a). More interestingly, histamine levels are not increased in lesioned skin from AD patients compared to healthy controls, further demonstrating that histaminergic nerves are not involved in AD-related itch (Buddenkotte et al. 2005).

7.4.2 Transient Receptor Potential Ion Channels

The transient receptor potential (TRP) family consists of several major ion channels that transmit positively charged ions across the cell membrane. They are important for sensory perception, including itch, and other sensory modalities (Minke and Cook 2002; Moran et al. 2011). Several TRP family members have been implicated in itch including TRPV1, TRPA1, and TRPM8 (Moore et al. 2018).

TRPV1 is expressed broadly in nociceptors, keratinocytes, and mast cells (Han et al. 2013; Shim et al. 2007; Stander et al. 2004). TRPV1 signaling mediates both histaminergic and non-histaminergic itch; especially the former. TRPV1 is activated by various stimuli including capsaicin, high temperature (over 42 °C), acid (pH < 5.9), ATP, and changes in chemical and inflammatory conditions. Histamine-induced TRPV1 activation results in itch (Imamachi et al. 2009; Shim et al. 2007) and requires the activation of phospholipase A2 and 12-lipoxygenase. TRPV1 expression in histamine-sensitive C nerve fibers is correlated with an associated burning sensation of histamine-induced itch (Stander et al. 2004). TRPV1 signaling also causes the release of neuropeptides such as substance P and calcitonin gene-related peptide, leading to neurogenic inflammation (Aubdool and Brain 2011).

TRPA1 is expressed by nociceptors, sensory C fibers, keratinocytes, and fibroblasts (Atoyan et al. 2009; Kwan et al. 2009). TRPA1 responds to pain and itch (Toth et al. 2014). TRPA1 is a mediator of non-histaminergic itch and is involved in Mrgpr-mediated itch (Wilson et al. 2011), as well as pruritus induced by oxidative stress (Liu et al. 2012), miR-711 (Han et al. 2018), and serotonin (Table 7.1). Lesioned skin samples from AD patients have increased TRPA1 expression compared to healthy controls (Oh et al. 2013).

It was found that lymphoma-secreted miR-711 is sufficient to elicit potent pruritus via direct activation of TRPA1 (Han et al. 2018, Fig. 7.2). Unlike conventional activation of TRPA1 from the intracellular side of the channel, miR-711 binds the channel on the extracellular loop and rapidly activates TRPA1, without causing neurogenic inflammation (Han et al. 2018).

TRPM8 is a temperature-sensitive calcium channel expressed by primary sensory neurons, mast cells, and keratinocytes (Denda and Tsutsumi 2011; McCoy et al. 2011). Menthol, eucalyptol, and icillin are the notable ligands of TRPM8 (Valdes-Rodriguez et al. 2013). Activation of TRPM8 causes intracellular calcium influx and a cooling sensation (Patel et al. 2007). TRPM8 is expressed on

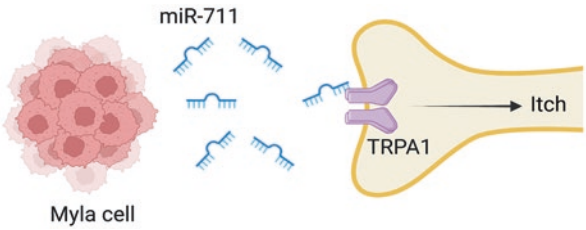

Fig. 7.2 miRNA-mediated cancer itch. Cutaneous T cell lymphoma (CTCL) is a chronic itch condition and CTCL patients suffer from serve pruritus. Inoculation of human Myla cells from CTCL is sufficient to induce lymphoma and chronic itch in immune-deficient mice (Chen et al. 2022). Both mouse and human CTCL produced miR-711, which was shown to induce robust pruritus following intradermal injection. miR-711 directly activates transient receptor potential ankyrin 1 (TRPA1) on sensory neurons. TRPA1 was believed to be activated mainly through intracellular interactions with its ligands; however, recent studies show that it can also be activated extracellularly via RNA-protein interactions (Han et al. 2018). Notably, miR-711 only activates a subset of neurons that also respond to histamine and chloroquine, indicating a population of pruriceptors

myelinated cutaneous nerves and implicated in the transmission of itch signals, accounting for the heterogeneity of symptoms reported by chronic pruritus patients (Ringkamp et al. 2011).

7.4.3 Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are pattern-recognition receptors that initiate innate immune responses by recognizing pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) (Akira et al. 2006). Liu et al. showed that TLR7 is expressed in small DRG neurons to mediate itch sensation (Liu et al. 2010). Immunohistochemistry revealed that TLR7 colocalizes with gastrin-releasing peptide (GRP), MrgprA3, and TRPV1 (Liu et al. 2010). Imidazoquinoline derivatives (imiquimod and resiquimod) and guanosine analogs (loxoribine) are ligands for TLR7 (Hemmi et al. 2002). Intradermal injection of imiquimod, resiquimod, and loxoribine induced itch-indicative scratching behavior in wild-type mice, and scratching is reduced in *Tlr7*$^{-/-}$ knockout mice (Liu et al. 2010). Strikingly, bath application of TLR7 ligands on dissociated DRG neurons elicits inward currents and action potentials, and this effect is abolished in *Tlr7*$^{-/-}$ knockout mice (Liu et al. 2010). *Tlr7*$^{-/-}$ knockout mice show a significant reduction in scratching behaviors in response to non-histaminergic pruritogens, including chloroquine, endothelin-1, and SLIGRL-NH2, which is a PAR2 agonist (Liu et al. 2010). However, TLR7 null mice exhibit normal scratching behaviors elicited by histamine, HTMT, or compound 48/80 (Liu et al. 2010). In addition to TLR7, the activation of TLR3 also generates itch, and its expression in DRGs is colocalized with GRP and TRPV1 (Liu et al. 2012). The TLR3 agonist poly (I:C) leads to

excitation of DRG neurons which results in scratching behavior (Liu et al. 2012). Similarly, global deletion of TLR3 abolishes scratching behaviors induced by the TLR3 agonists chloroquine and histamine. Thus, TLR3 is required for histaminergic and non-histaminergic itch, and both TLR7 and TLR3 are critical itch receptors that regulate the excitability of pruriceptive neurons (Liu and Ji 2014). Thus, TLRs may serve as novel targets for therapies against pruritis. Please see Chap. 8 for more details on TLR signaling in pain and itch.

7.5 Neuroimmune Interactions in Skin Diseases and Chronic Itch

The skin is the largest organ of the human body, with vital roles in maintaining homeostasis, providing a protective barrier, and defending against foreign invaders and pathogens. Nerve fibers innervating the skin are located in the vicinity of keratinocytes, fibroblasts, endothelial cells, Schwann cells, and resident immune cell populations. The cutaneous sensory nerve fibers (CSNFs), which innervate the dermis and epidermis, represent the majority of nerve fibers in the skin. CSNFs originate from the dorsal root ganglia (DRG) and the trigeminal ganglia. The afferent fibers of DRG neurons extend outwards to the body's skin, and stimuli at these projections send signals back to the sensory neuron cell bodies within the DRGs. These signals are then delivered to the dorsal horn of the spinal cord and upwards to the brainstem and thalamus (Fig. 7.3a). Trigeminal ganglia neurons innervate the skin of the head and face.

The autonomic nervous system also innervates the skin but makes up a small overall percentage of the nerve fibers. These nerves are only found in the dermal layer and innervate hair follicles, blood vessels, lymphatic vessels, apocrine and eccrine glands, and erector pili muscles. The resident dermal immune population ensures both protection against pathogens and maintenance of tolerance against innocuous antigens. The skin is highly innervated, and neuroimmune interactions are important for communicating with and responding to the external environment.

Abnormal neuroimmune interactions have consistently been shown to cause a number of inflammatory skin conditions. Neuroimmune interactions play a key role

Fig. 7.3 (continued) expressing neuropeptide Y (NPY), which inhibits mechanical itch. The properties of the excitatory neurons involved in mechanical itch are still not fully understood. Primary sensory neurons of DRG are activated in response to chemical pruritogens and release glutamate, natriuretic polypeptide B (NppB), and gastrin-releasing peptide (GRP). NppB activates natriuretic peptide receptor A (Npra) to release GRP. GRP then activates GRP receptor (GRPR) to transduce the signal to projection neurons (PN) in the dorsal horn. In chronic itch, astrocytes can interact with GRPR-expressing neurons with secreting neuromodulators. Scratching sends signals to inhibitory neurons that express basic helix-loop-helix B5 (Bhlhb5). These interneurons release inhibitory neurotransmitters like dynorphin, glycine, and gamma-aminobutyric acid (GABA) that negatively act upon GRPR-positive neurons. This results in attenuation of the itch sensation

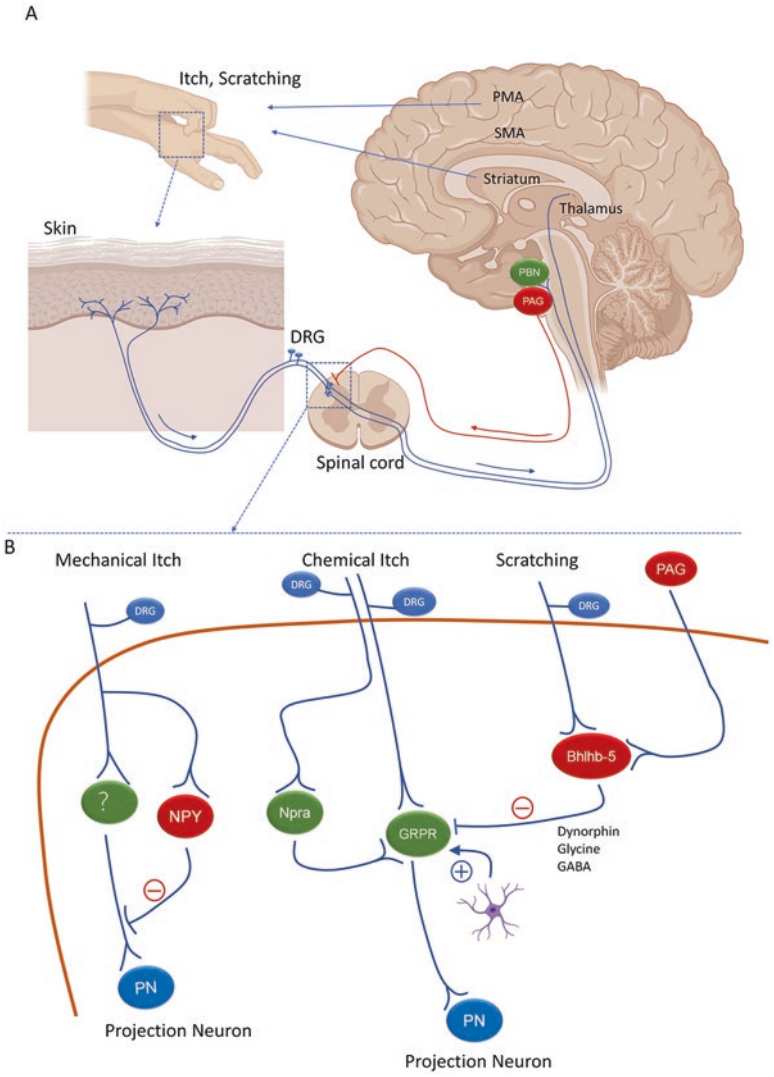

Fig. 7.3 Itch pathways for chemical and mechanical itch (**a**) Ascending and descending pathways of itch. Itch signals are peripherally detected in the skin by itch-sensing primary sensory neurons (pruriceptors), with their cell bodies resided in dorsal root ganglia (DRG). Then, the itch signals are transduced to the spinal cord, ascend through the spinothalamic tract to the thalamus, and finally to the sensory cortex, eliciting itch sensation (pruritus). Pruritic signals also travel through the spinoparabrachial pathway to reach the parabrachial nucleus (PBN). Once itch signals have reached the brain, they are further projected to the premotor area (PMA), supplementary motor area (SMA), and striatum. These areas play a role in sending signals to initiate scratching behavior as a motor reflex. Furthermore, the descending control from the periaqueductal gray (PAG) can also modulate the spinal cord itch circuit. (**b**) Spinal cord neurocircuits for mechanical itch and chemical itch and mechanism of itch relief by scratching. Mechanical itch stimuli from TLR5-expressing primary sensory neurons project onto spinal cord neurons

in the pathogenesis of allergic contact dermatitis (ACD), atopic dermatitis (AD), and psoriasis.

ACD is a T-cell-mediated hypersensitivity reaction caused by allergens and haptens, which causes itchy, inflamed skin. Cutaneous sensory nerves control the immune response via interactions with antigen-presenting cells. Recently, TRPV1 and TRPA1 were found to have different roles in contact hypersensitization in a mouse ACD model caused by treatment with squaric acid dibutylester (SADBE). While SADBE directly activated both TRPV1 and TRPA1 channels on neurons to produce itch, only TRPV1 ion channels played a role in inflammation (LaMotte et al. 2014). This was demonstrated when the removal of TRPV1+ neurons or genetic deficiency in TRPV1 led to increased inflammation. On the other hand, TRPA1 mediates both itch and inflammation (edema, leukocyte infiltration, keratinocyte hyperplasia) in ACD mouse models using urushiol (poison ivy component) and oxazolone, and interestingly, TRPV1 channels were not involved (Liu et al. 2013).

AD is a chronic inflammatory skin condition, with symptoms including constant itchiness, thick scaly skin, damaged epidermal barrier function, and a T helper 2 (TH2) cell-skewed allergic response. One major feature of AD lesions is hyperinnervation and penetration of sensory neurons into the epidermis, causing increased itch and release of neuropeptides. Lesions and blood samples from AD patients have been found to contain high concentrations of substance P and NGF, which lead to keratinocyte hyperproliferation. In an innervated skin model, where human AD skin samples were cultured alongside porcine DRGs, sensory nerves caused keratinocyte proliferation that was dependent on CGRP. Isolated AD skin samples used in this model also demonstrated increased innervation and neurite outgrowth, CGRP release, and epidermal thickening compared with healthy control skin samples (Erickson et al. 2021).

Psoriasis is an inflammatory disorder of the skin defined by dysregulation of the IL-17/IL-23 axis, acanthosis (dark discoloration in body folds), hyperkeratosis (abnormal thickening of the skin), and itch. Neuroimmune interactions control the induction of IL-23 signaling and formation of inflammatory lesions. The role of the sensory nervous system in psoriasiform skin inflammation was first demonstrated by cutaneous denervation of a psoriasis mouse model. When the skin was surgically axotomized (axons severed along an axis) in KC-Tie2 psoriasiform mice, acanthosis improved greatly, while CD4+ T cells and CD11c + DCs decreased. These effects were shown to depend on CGRP and substance P, pointing toward the nervous system's major role in psoriasis. In a follow-up study, botulinum neurotoxin A (BoNT-A), a neurotoxin that cleaves SNAP25, was injected intradermally into KC-Tie2 mice. BoNT-A prevented the release of CGRP and substance P, and greatly improved skin inflammation and epidermal hyperplasia (Riol-Blanco et al. 2014). Small-scale human clinical trials have confirmed the effectiveness of BoNT-A in improving plaque psoriasis (Todberg et al. 2018). Using a mouse of psoriasis model driven by TLR7/8 agonist imiquimod, TRPV1+ nerves were found to control IL-23

release via dermal dendritic cells. These dendritic cells control IL-17 expression via γδ T cells in the skin, thus promoting psoriatic inflammation (van der Fits et al. 2009).

Cutaneous T-cell lymphoma (CTCL) is a cancer that causes T-cells to mount an immunological attack against the skin, resulting in dermatological sequelae and the development of tumors. Approximately 88% of CTCL patients are affected by severe pruritus that requires separate treatment from the lymphoma itself. Emollients, topical steroids, and oral histamines fail to relieve the chronic itch, suggesting that CTCL pruritus is not caused by histamines (Cevikbas and Lerner 2020). Researchers, therefore, have sought histamine-independent mediators as possible treatment targets to alleviate chronic pruritus. It was found that CTCL is associated with increased levels of the IL-31 in plasma, which correlate with itch severity (Nattkemper et al. 2016). IL-31 is produced by Th2 cells, particularly those in the malignant population associated with CTCL (Singer et al. 2013). Researchers speculate that IL-31 exerts indirect effects on sensory nerves through T cells and keratinocytes to transduce itch sensations (Nattkemper et al. 2016). Studies have also suggested that IL-31 initiates basophil migration and the release of IL-4 and IL-13. Recently, histamine receptor 4 (H4R) has been observed on CD4+ T-cells. This receptor increases mRNA expression of IL-31. Additionally, a recent study showed no correlation between itch severity and IL-31 levels in the early stages of CTCL, which is dominated by Th1 cells rather than Th2 cells (Malek et al. 2015). Studies involving different treatments at different stages of CTCL indicate additional mechanisms involved in pruritus. Successful treatment with SP receptor antagonism has been reported, and treatment regimens with gabapentin and mirtazapine during advanced stages of CTCL were also proposed (Erickson et al. 2021). To study the mechanisms of CTCL, an animal model of CTCL was developed at Duke University using immune-deficient mice. Inoculation of Myla cells from a CTCL patient resulted in robust tumor growth and chronic pruritus that can last more than 2 months. Lymphoma is highly innervated by nerve fibers and intra-tumoral injection of nerve blockers (C-fibers or A-fibers) or IL-31 neutralizing antibody significantly attenuated pruritus (Chen et al. 2022). Moreover, lymphoma secreted miR-711, which can elicit itch via direct activation of TRPA1 (Fig. 7.2).

7.6 Neurocircuits of Itch

There is considerable overlap between the molecular mediators of pain and itch, and they even coexist in some chronic pathologies (Liu and Ji 2013). However, while there certainly does seem to be some intersections between the two, pain and itch remain separate processes. It is possible to knock out itch-related behaviors in mouse models while retaining symptoms of pain, demonstrating distinct pathways for each sensation (Fig. 7.3a).

7.6.1 Pain and Itch

Two theories of how signals of pain and itch are transmitted throughout the nervous system are the labeled-line coding model and the population-coding model. Labeled-line coding posits that primary afferent sensory neurons specific to either pain or itch will send information to central nervous system (CNS) neurons, which produce the respective sensation (Ma 2010). This theory holds that different sensory modalities are modulated by mutually exclusive neuronal populations (Sun et al. 2017). The labeled-line theory has been challenged by the finding that painful can suppress itch through a specific neurocircuit in the spinal cord involving inhibitory neurons (Fig. 7.3b).

The population-coding model proposes that a subpopulation of neurons receives pain and itch signals through distinct combinations of activated fibers (Akiyama and Carstens 2013; Ma 2010). Upon receiving either type of stimuli, the neurons will transmit a signal for the specific sensation. Population-coding reflects a complex and intricately integrated system of sensory transmission (LaMotte et al. 2014).

Intercommunication between different parts of the nervous system suggests how related etiological processes and mediators cause pain and itch. Chronic pain and itch negatively impact quality of life, and this is compounded by the fact that these pathologies often accompany one another (Liu and Ji 2013). Further studies on the molecular mechanisms underlying these sensations will contribute to the development of more effective treatments.

7.6.2 Neuropeptides as Itch Transmitters

Substance P (SP) is a neuropeptide which is highly involved in afferent neuronal signaling (De Felipe et al. 1998). SP is released by activated sensory neurons and binds to neurokinin receptors (NK1) expressed on mast cells, keratinocytes, and cutaneous nerves, resulting in the release of other itch mediators (Kremer et al. 2014). Intradermal injection of SP results in mast cell activation and histamine release (Jorizzo et al. 1983; van der Kleij et al. 2003). SP also induces itch that is not dependent on histamine, but rather on mast cells. Furthermore, SP-activated mast cells release inflammatory mediators such as leukotriene B4, prostaglandin D2, and TNF-α (Luger 2002; Steinhoff et al. 2003b). In addition to the mast cells, SP also triggers the release of pruritogenic compounds from keratinocytes, endothelial cells, and immune cells (Biro et al. 2007; Kulka et al. 2008). Recently, it was found that SP is an endogenous agonist of Mrgprb2 in mast cells and mediates immune cells' migration via Mrgprb2. SP was shown to activate human mast cells via MRGPRX2 (human homolog of Mrgprb2) for the release of inflammatory cytokines and chemokines (Green et al. 2019).

Gastrin-releasing peptide (GRP) is a neuropeptide involved in the sensation of itch. Intradermal or intrathecal injection of GRP elicited itch in mice (Sun and Chen

2007; Kulka et al. 2008). GRP receptor (GRPR) is expressed on spinal cord neurons, and activation of GRPR transmits the itch signal to higher order neurons (Mishra and Hoon 2013; Sun and Chen 2007; Sun et al. 2009). Ablation of GRPR-expressing neurons in the spinal cord results in substantial deficits in scratching behavior in response to all chemical itch stimuli (Sun et al. 2009). In monkey chronic idiopathic pruritus models, the expression of GRP and GRPRs in the spinal cord and skin are significantly increased (Nattkemper et al. 2013).

7.6.3 Spinal Cord Circuits of Chemical and Mechanical Itch

When pruritic neuronal signals are transduced to the spinal cord from primary sensory neurons, there is a circuit in the spinal cord modulating signaling and affecting conscious itch sensation (Fig. 7.3b). Mechanical itch, such as alloknesis (when normal mechanical stimuli are perceived as itchy stimuli) and hyperknesis (excessive itch perception to a pruritic stimuli) are conveyed by an itch-related circuit. It has been demonstrated that neuropeptide Y (NPY) positive neurons have an inhibitory effect on mechanical itch (Bourane et al. 2015). For chemical itch, primary sensory neurons respond to pruritic stimuli by releasing glutamate and natriuretic polypeptide B (NppB). Nppb activates natriuretic peptide receptor A (NPA) to release GRP. In addition, primary sensory neurons may release GRP themselves. Thus, GRP and GRPRs are critical molecules involved in chemical itch (Fig. 7.3b).

In chronic itch models, the chemical itch circuit can be modulated by astrocytes in the spinal cord (Ji et al. 2019). Lipocalin-2 released by astrocytes potentiates GRP signaling (Shiratori-Hayashi et al. 2015). Toll-like receptor 4 (TLR4) is also involved in chronic itch through astrogliosis in the spinal cord (Liu et al. 2016, Fig. 7.4).

To relieve the response from the system, there is a circuit to mitigate pruritus. Pain, cooling, and mechanical stimuli (scratching) can attenuate itch sensation and activate a specific population of inhibitory neurons, which is characterized by their expression of basic helix-loop-helix family member B5 (Bhlhb5) (Ross et al. 2010). These inhibitory interneurons release neurotransmitters such as dynorphin, glycine, and gamma-aminobutyric acid (GABA) to inhibit the activity of GPRP+ neurons (Fig. 7.3b).

After processing in the spinal cord, itch signals are transduced through the spinothalamic tract to the thalamus and through the spinoparabrachial pathway to the parabrachial nucleus (Mu et al. 2017), as shown in Fig. 7.3a. Pruritic signals are then projected to various brain regions such as the primary and secondary somatosensory cortex. These regions contribute to the localization and intensity of itch. Other projected regions are linked with different behaviors or sensitizations related to itch. The midcingulate cortex is linked to perception and motivation; the anterior cingulate cortex (ACC) and insula are associated with unpleasant sensations; the premotor area (PMA), supplementary motor areas, striatum, and cerebellum are

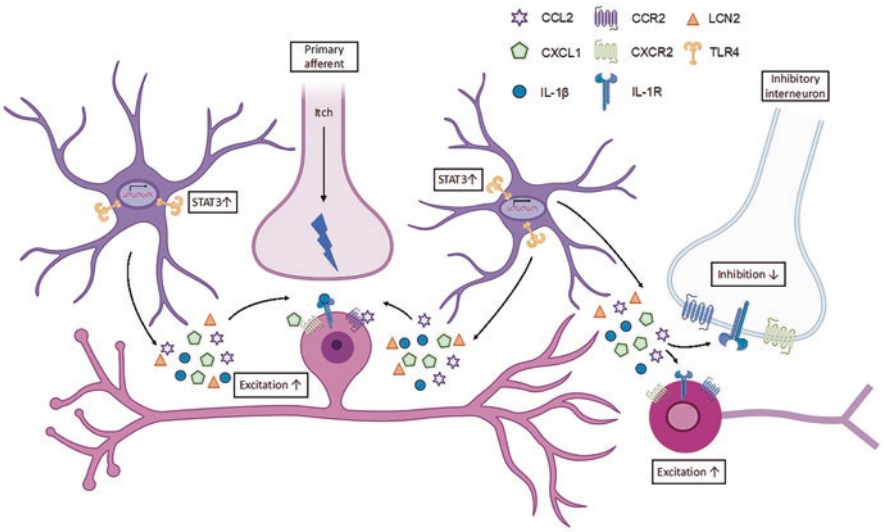

Fig. 7.4 Astrocyte activation drives chronic itch pathology via neuroglial interactions. Chronic itch conditions such as dry skin injury or cutaneous T cell lymphoma result in astroglial reaction in the spinal cord dorsal horn. Increased activity of TLR4 and signal transducer and activator of transcription 3 (STAT3) has been implicated in reactive astrogliosis (Liu et al. 2016; Shiratori-Hayashi et al. 2015). The activation of astrocytes in the spinal cord induces the release of neuroinflammatory signals that lead to itch, such as lipocalin-2 (LCN2). LCN2 potentiates itch symptoms by acting on neurons expressing gastrin-releasing peptide receptor (GRPR). Reactive astrocytes also upregulate the synthesis of inflammatory molecules like C-X-C chemokine ligand 1 (CXCL1), interleukin 1 beta (IL-1β), and C-C motif chemokine 2 (CCL2). CXCL1 binds to C-X-C chemokine receptor 1 (CXCR2), IL-1β binds to interleukin 1 receptor (IL-1R), and CCL2 binds to C-C motif chemokine receptor 2 (CCR2). It is possible that these molecules bind to their receptors and then act on GRP+ interneurons and GRPR+ neurons as well as pruriceptors in the periphery to drive chronic itch symptoms. These astrocyte-derived mediators could increase the excitation of postsynaptic neurons and disinhibition of inhibitory interneurons to drive chronic itch sensation. (Modified from Ji et al. 2019) (Nature Review Neuroscience, Author's own copyright)

involved in controlling and initiating scratching behavior, and the prefrontal area is implicated in decision-making (Fig. 7.3a).

7.6.4 Opioid-Induced Itch

Opioid-induced pruritus most commonly occurs following neuraxial administration of opioids, with 30–60% of patients reporting itch symptoms. Pregnant women who receive epidural or spinal morphine are often treated with opioids and are particularly prone to side effects related to itch (Melo et al. 2018; Wang et al. 2021). While itch symptoms are often associated with peripheral mechanisms, opioid-induced itch is primarily mediated by the central mechanisms (Liu et al. 2011). It is

noteworthy that opioid-induced itch is thought to arise through a mechanism largely independent of the analgesic effects of opioids (Wang et al. 2021). In the past, it was believed that opioid analgesia itself unmasked itch. While it was initially proposed that neurons expressing both mu opioid receptor (MOR) and GRPR were responsible for the induction of morphine-induced itch (Liu et al. 2011), recent studies found that there are distinct neuronal populations that express either MOR or GRPR, both of which are involved in the itch circuitry. Strikingly, it was found that pruritus induced by intrathecal injection of the mu opioid receptors agonists (morphine or DAMGO) was totally abolished in mice with a specific deletion of *Oprm1* (gene encoding MOR) in Vgat+ inhibitory neurons (Wang et al. 2021). Furthermore, ablating GRPR-expressing neurons blocked morphine-induced itch, and the administration of a GRPR antagonist successfully eliminates morphine-induced itch. Taken together, these findings suggest morphine inhibits GABAergic neurons with a downstream effect of disinhibiting excitatory GRPR neurons (Wang et al. 2021). Furthermore, it was found that kappa-opioid receptor agonists can alleviate morphine-induced itch without affecting the analgesic properties of morphine in mice (Nguyen et al. 2021). Additionally, opioid receptors in TRPV1-expressing sensory neurons may mediate peripheral opioid-induced itch (Melo et al. 2018). Different types of skin cells, such as keratinocytes, mast cells, fibroblasts, and macrophages, express MORs (Reich and Szepietowski 2012). Further studies are still required to fully characterize the mechanism of opioid-induced itch in both the peripheral and central nervous systems, but notable progress has been made in recent years.

7.7 Glial Cells in Itch

Recent studies have shown that reactive astrocytes in the spinal cord are involved in the pathogenesis of chronic itch. Intrathecal administration of astroglial inhibitors reduces chronic itch, as mouse models of dry skin and cutaneous T-cell lymphoma (CTCL) exhibit astrogliosis in itch-affected areas of the spinal cord (Fig. 7.4).

Astrocytes induce chronic itch by multiple mechanisms. Astrocyte activation is dependent on toll-like receptor 4 (TLR4) and transcription factor signal transducer and activator of transcription 3 (STAT3). Blocking TLR4 signaling and the expression of astrocytic STAT3 inhibits chronic itch, or suppression of STAT3-dependent astrogliosis decreased scratching behavior in chronic itch conditions (Shiratori-Hayashi et al. 2015; Liu et al. 2016; Chen et al. 2022). The activation of spinal astrocytes leads to the release of several immune factors, such as lipocalin-2 (LCN2). Intrathecally administering LCN2 together with GRP increased GRP-induced scratching in mice by acting on GRPR-expressing neurons. However, administering LCN2 alone did not increase itch and neuronal excitability (Shiratori-Hayashi and Tsuda 2021). Mouse models of chronic itch exhibit elevated LCN2 levels in the spinal dorsal horn, and suppressing astrocytic LCN2 activation can block pruritus (Furutani et al. 2022; Shiratori-Hayashi and Tsuda 2021). Astrocytic

LCN2 was also shown to increase the GRP-evoked excitability of GRPR+ postsynaptic neurons in a mouse model of chronic itch (Koga et al. 2020). These findings suggest that chronic itch induced by GRP is heightened by LCN2 release. Persistent STAT3 activation in astrocytes was evoked by IL-6 through inositol trisphosphate receptor type 1 (IP3R1) and transient receptor potential canonical (TRPC) channels (Shiratori-Hayashi and Tsuda 2021). Another cytokine involved in astrocyte-mediated chronic itch is IL-33, which maintains chronic itch through the JAK-STAT3 pathway. ST2, the receptor for IL-33, is highly expressed in astrocytes, and ST2-knockout mice exhibited reduced astrocyte activation and decreased scratching behavior (Du et al. 2019).

The chemokine receptor CXCR3 is also upregulated in the central nervous system of chronic itch models and is involved in astrocyte-mediated chronic itch. CXCR3-knockout mice exhibit normal acute itch symptoms but reduced scratching under chronic itch-inducing conditions, indicating that CXCR3 plays an important role in chronic itch. Administration of a CXCR3 antagonist reduced astrogliosis and chronic itch as well (Du et al. 2022; Jing et al. 2018). While more work is still required to fully understand the mechanisms underlying reactive spinal astrocyte-related chronic itch, it is a promising avenue of study for future therapeutic techniques to treat pruritus.

Activation of mitogen-activated protein (MAP) kinases in chronic itch conditions will lead to the release of pro-inflammatory molecules including C-X-C chemokine ligand 1 (CXCL1), interleukin 1 beta (IL-1β), and C-C motif chemokine 2 (CCL2), which bind to their receptors on GRP-expressing interneurons and postsynaptic GRPR-expressing neurons (Ji et al. 2019; Du et al. 2022). This may result in the disinhibition of inhibitory interneurons and excitation of postsynaptic neurons (Fig. 7.4).

7.8 Conclusion

Recent studies show the important effects of crosstalk between the nervous and immune systems in itch sensitization. Peripheral pruriceptors act as immune sensors and coordinate with immune cells to form a comprehensive, well-organized defense system. Immune-related receptors, such as Toll-like receptors, are expressed in peripheral nociceptors and can be activated by certain ligands and environmental stimuli. Immune cells can also be activated by neuropeptides and modulate peripheral nociceptors. The neuroimmune interaction of peripheral pruriceptors and immune cells play an important role in itch regulation for both physiological and pathological conditions and for future clinical treatments. Research has suggested that the central nervous system is also deeply involved in itch symptoms as itch signals from peripheral neurons are transduced through the spinal cord and brain. Itch circuits in the CNS, such as those for chemical or mechanical itch, are intertwined with the action of glial cells, molecular mediators of chronic pain, opioid receptors, and other circuits. While the nervous and immune systems are distinct

from each other, it is clear that studying their interactions will be critical to further develop clinical therapeutic techniques and improve patient outcomes. As our understanding develops of how these systems work together to generate different pathologies, more effective treatment strategies will be established.

References

Aich A, Afrin LB, Gupta K. Mast cell-mediated mechanisms of nociception. Int J Mol Sci. 2015;16(12):29069–92.

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell. 2006;124(4):783–801.

Akiyama T, Carstens E. Neural processing of itch. Neuroscience. 2013;250:697–714.

Atoyan R, Shander D, Botchkareva NV. Non-neuronal expression of transient receptor potential type A1 (TRPA1) in human skin. J Invest Dermatol. 2009;129(9):2312–5.

Aubdool AA, Brain SD. Neurovascular aspects of skin neurogenic inflammation. J Investig Dermatol Symp Proc. 2011;15(1):33–9.

Biro T, Toth BI, Marincsak R, Dobrosi N, Geczy T, Paus R. TRP channels as novel players in the pathogenesis and therapy of itch. Biochim Biophys Acta. 2007;1772(8):1004–21.

Bogiatzi SI, Fernandez I, Bichet JC, Marloie-Provost MA, Volpe E, Sastre X, Soumelis V. Cutting edge: proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. J Immunol. 2007;178(6):3373–7.

Bork K. Pruritus precipitated by hydroxyethyl starch: a review. Br J Dermatol. 2005;152(1):3–12.

Bourane S, Duan B, Koch SC, Dalet A, Britz O, Garcia-Campmany L, Kim E, Cheng L, Ghosh A, Ma Q, Goulding M. Gate control of mechanical itch by a subpopulation of spinal cord interneurons. Science. 2015;350(6260):550–4.

Buddenkotte J, Stroh C, Engels IH, Moormann C, Shpacovitch VM, Seeliger S, Vergnolle N, Vestweber D, Luger TA, Schulze-Osthoff K, et al. Agonists of proteinase-activated receptor-2 stimulate upregulation of intercellular cell adhesion molecule-1 in primary human keratinocytes via activation of NF-kappa B. J Invest Dermatol. 2005;124(1):38–45.

Cevikbas F, Lerner EA. Physiology and pathophysiology of itch. Physiol Rev. 2020;100(3):945–82.

Cevikbas F, Wang X, Akiyama T, Kempkes C, Savinko T, Antal A, Kukova G, Buhl T, Ikoma A, Buddenkotte J, et al. A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: involvement of TRPV1 and TRPA1. J Allergy Clin Immunol. 2014;133(2):448–60.

Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol. 2001;117(4):977–83.

Chen O, He Q, Han Q, Furutani K, Gu Y, Olexa M, Ji RR. Mechanisms and treatments of neuropathic itch in a mouse model of lymphoma. J Clin Invest. 2022:e160807. https://doi.org/10.1172/JCI160807.

Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Luscher-Firzlaff J, Luscher B, Baron JM. IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. J Allergy Clin Immunol. 2012;129(2):426-33, 433.e421–28

Cowden JM, Zhang M, Dunford PJ, Thurmond RL. The histamine H4 receptor mediates inflammation and pruritus in Th2-dependent dermal inflammation. J Invest Dermatol. 2010;130(4):1023–33.

Cyphert JM, Kovarova M, Allen IC, Hartney JM, Murphy DL, Wess J, Koller BH. Cooperation between mast cells and neurons is essential for antigen-mediated bronchoconstriction. J Immunol. 2009;182(12):7430–9.

De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJ, Laird JM, Belmonte C, Cervero F, Hunt SP. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. Nature. 1998;392(6674):394–7.

Denda M, Tsutsumi M. Roles of transient receptor potential proteins (TRPS) in epidermal keratinocytes. Adv Exp Med Biol. 2011;704:847–60.

Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR, Haugen HS, Maurer M, Harder B, Johnston J, et al. Interleukin 31, a cytokine produced by activated t cells, induces dermatitis in mice. Nat Immunol. 2004;5(7):752–60.

Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell. 2001;106(5):619–32.

Du L, Hu X, Yang W, Yasheng H, Liu S, Zhang W, Zhou Y, Cui W, Zhu J, Qiao Z, Maoying Q, Chu Y, Zhou H, Wang Y, Mi W. Spinal IL-33/ST2 signaling mediates chronic itch in mice through the astrocytic JAK2-STAT3 cascade. Glia. 2019;67(9):1680–93.

Du LX, Zhu JY, Mi WL. Cytokines and chemokines modulation of itch. Neuroscience. 2022;495:74–85.

Erickson S, Heul AV, Kim BS. New and emerging treatments for inflammatory itch. Ann Allergy Asthma Immunol. 2021;126(1):13–20.

Furutani K, Chen O, McGinnis A, Wang Y, Serhan CN, Hansen TV, Ji RR. Novel pro-resolving lipid mediator mimetic 3-oxa-PD1n-3 DPA reduces acute and chronic itch by modulating excitatory and inhibitory synaptic transmission and astroglial secretion of lipocalin-2 in mice. Pain. 2022; https://doi.org/10.1097/j.pain.0000000000002824.

Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nat Immunol. 2005;6(2):135–42.

Green DP, Limjunyawong N, Gour N, Pundir P, Dong X. A mast-cell-specific receptor mediates neurogenic inflammation and pain. Neuron. 2019;101(3):412–20.

Griffiths C, de Bruin-Weller M, Deleuran M, Fargnoli MC, Staumont-Salle D, Hong CH, Sanchez-Carazo J, Foley P, Seo SJ, Msihid J, et al. Dupilumab in adults with moderate-to-severe atopic dermatitis and prior use of systemic non-steroidal immunosuppressants: analysis of four phase 3 trials. Dermatol Ther (Heidelb). 2021;11(4):1357–72.

Han L, Dong X. Itch mechanisms and circuits. Annu Rev Biophys. 2014;43:331–55.

Han L, Ma C, Liu Q, Weng HJ, Cui Y, Tang Z, Kim Y, Nie H, Qu L, Patel KN, et al. A subpopulation of nociceptors specifically linked to itch. Nat Neurosci. 2013;16(2):174–82.

Han Q, Liu D, Convertino M, Wang Z, Jiang C, Kim YH, Luo X, Zhang X, Nackley A, Dokholyan NV, et al. Mirna-711 binds and activates TRPA1 extracellularly to evoke acute and chronic pruritus. Neuron. 2018;99(3):449–63. e446

Heise R, Neis MM, Marquardt Y, Joussen S, Heinrich PC, Merk HF, Hermanns HM, Baron JM. IL-31 receptor alpha expression in epidermal keratinocytes is modulated by cell differentiation and interferon gamma. J Invest Dermatol. 2009;129(1):240–3.

Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002;3(2):196–200.

Ikoma A, Steinhoff M, Stander S, Yosipovitch G, Schmelz M. The neurobiology of itch. Nat Rev Neurosci. 2006;7(7):535–47.

Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, Han SK. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci U S A. 2009;106:11330–5.

Indra AK. Epidermal TSLP: a trigger factor for pathogenesis of atopic dermatitis. Expert Rev Proteomics. 2013;10(4):309–11.

Jeong CW, Ahn KS, Rho NK, Park YD, Lee DY, Lee JH, Lee ES, Yang JM. Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. Clin Exp Allergy. 2003;33(12):1717–24.

Ji RR, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20:667–85.

Jing PB, Cao DL, Li SS, Zhu M, Bai XQ, Wu XB, Gao YJ. Chemokine receptor CXCR3 in the spinal cord contributes to chronic itch in mice. Neurosci Bull. 2018;34(1):54–63.
Jorizzo JL, Coutts AA, Eady RA, Greaves MW. Vascular responses of human skin to injection of substance p and mechanism of action. Eur J Pharmacol. 1983;87(1):67–76.
Kanda N, Watanabe S. Histamine enhances the production of nerve growth factor in human keratinocytes. J Invest Dermatol. 2003;121(3):570–7.
Kasraie S, Niebuhr M, Werfel T. Interleukin (IL)-31 activates signal transducer and activator of transcription (STAT)-1, STAT-5 and extracellular signal-regulated kinase 1/2 and down-regulates IL-12p40 production in activated human macrophages. Allergy. 2013;68(6):739–47.
Kempkes C, Buddenkotte J, Cevikbas F, Buhl T, Steinhoff M. Role of par-2 in neuroimmune communication and itch. In: Carstens E, Akiyama T, editors. Itch: mechanisms and treatment. Boca Raton: CRC Press; 2014.
Kim N, Bae KB, Kim MO, Yu DH, Kim HJ, Yuh HS, Ji YR, Park SJ, Kim S, Son KH, et al. Overexpression of cathepsin s induces chronic atopic dermatitis in mice. J Invest Dermatol. 2012;132(4):1169–76.
Koga K, Yamagata R, Kohno K, Yamane T, Shiratori-Hayashi M, Kohro Y, Tozaki-Saitoh H, Tsuda M. Sensitization of spinal itch transmission neurons in a mouse model of chronic itch requires an astrocytic factor. J Allergy Clin Immunol. 2020;145(1):183–191.e10. https://doi.org/10.1016/j.jaci.2019.09.034.
Kollmeier A, Francke K, Chen B, Dunford PJ, Greenspan AJ, Xia Y, Xu XL, Zhou B, Thurmond RL. The histamine H(4) receptor antagonist, JNJ 39758979, is effective in reducing histamine-induced pruritus in a randomized clinical study in healthy subjects. J Pharmacol Exp Ther. 2014;350(1):181–7.
Kremer AE, Feramisco J, Reeh PW, Beuers U, Oude Elferink RP. Receptors, cells and circuits involved in pruritus of systemic disorders. Biochim Biophys Acta. 2014;1842(7):869–92.
Kroeger KM, Sullivan BM, Locksley RM. IL-18 and iL-33 elicit Th2 cytokines from basophils via a MyD88- and p38alpha-dependent pathway. J Leukoc Biol. 2009;86(4):769–78.
Kubo M. Innate and adaptive type 2 immunity in lung allergic inflammation. Immunol Rev. 2017;278(1):162–72.
Kulka M, Sheen CH, Tancowny BP, Grammer LC, Schleimer RP. Neuropeptides activate human mast cell degranulation and chemokine production. Immunology. 2008;123(3):398–410.
Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL. TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J Neurosci. 2009;29(15):4808–19.
LaMotte RH, Dong X, Ringkamp M. Sensory neurons and circuits mediating itch. Nat Rev Neurosci. 2014;15(1):19–31.
Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. J Clin Invest. 2004;113(5):651–7.
Liu T, Ji RR. Oxidative stress induces itch via activation of transient receptor potential subtype ankyrin 1 in mice. Neurosci Bull. 2012;28:145–54.
Liu T, Ji RR. New insights into the mechanisms of itch: are pain and itch controlled by distinct mechanisms? Pflugers Arch. 2013;465(12):1671–85.
Liu T, Ji RR. Toll-like receptors and itch. In: Carstens E, Akiyama T, editors. Itch: mechanisms and treatment. Boca Raton: CRC Press; 2014.
Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, et al. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell. 2009;139(7):1353–65.
Liu T, Xu ZZ, Park CK, Berta T, Ji RR. Toll-like receptor 7 mediates pruritus. Nat Neurosci. 2010;13(12):1460–2.
Liu XY, Liu ZC, Sun YG, Ross M, Kim S, Tsai FF, Li QF, Jeffry J, Kim JY, Loh HH, Chen ZF. Unidirectional cross-activation of GRPR by MOR1D uncouples itch and analgesia induced by opioids. Cell. 2011;147(2):447–58.
Liu T, Berta T, Xu ZZ, Park CK, Zhang L, Lu N, Liu Q, Liu Y, Gao YJ, Liu YC, et al. TLR3 deficiency impairs spinal cord synaptic transmission, central sensitization, and pruritus in mice. J Clin Invest. 2012;122(6):2195–207.

Liu B, Escalera J, Balakrishna S, Fan L, Caceres AI, Robinson E, Sui A, McKay MC, McAlexander MA, Herrick CA, Jordt SE. TRPA1 controls inflammation and pruritogen responses in allergic contact dermatitis. FASEB J. 2013;27(9):3549–63.

Liu T, Han Q, Chen G, Huang Y, Zhao LX, Berta T, Gao YJ, Ji RR. Toll-like receptor 4 contributes to chronic itch, alloknesis, and spinal astrocyte activation in male mice. Pain. 2016;157:806–17.

Luger TA. Neuromediators – a crucial component of the skin immune system. J Dermatol Sci. 2002;30(2):87–93.

Ma Q. Labeled lines meet and talk: population coding of somatic sensations. J Clin Invest. 2010;120:3773–8.

Malek M, Gleń J, Rębała K, Kowalczyk A, Sobjanek M, Nowicki R, Ruckemann-Dziurdzińska K, Sokołowska-Wojdyło M. IL-31 does not correlate to pruritus related to early stage cutaneous T-cell lymphomas but is involved in pathogenesis of the disease. Acta Derm Venereol. 2015;95(3):283–8. https://doi.org/10.2340/00015555-1958.

McCoy DD, Knowlton WM, McKemy DD. Scraping through the ice: uncovering the role of TRPM8 in cold transduction. Am J Physiol Regul Integr Comp Physiol. 2011;300(6):R1278–87.

McNeil B, Dong X. Peripheral mechanisms of itch. Neurosci Bull. 2012;28(2):100–10.

Melo H, Basso L, Iftinca M, MacNaughton WK, Hollenberg MD, McKay DM, Altier C. Itch induced by peripheral mu opioid receptors is dependent on TRPV1-expressing neurons and alleviated by channel activation. Sci Rep. 2018 Oct 19;8(1):15551. https://doi.org/10.1038/s41598-018-33620-7.

Metwally SS, Mosaad YM, Abdel-Samee ER, El-Gayyar MA, Abdel-Aziz AM, El-Chennawi FA. IL-13 gene expression in patients with atopic dermatitis: relation to IgE level and to disease severity. Egypt J Immunol. 2004;11(2):171–7.

Minke B, Cook B. TRP channel proteins and signal transduction. Physiol Rev. 2002;82(2):429–72.

Misery L, Brenaut E, Le Garrec R, Abasq C, Genestet S, Marcorelles P, Zagnoli F. Neuropathic pruritus. Nat Rev Neurol. 2014;10(7):408–16.

Mishra SK, Hoon MA. The cells and circuitry for itch responses in mice. Science. 2013;340(6135):968–71.

Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. Regulation of pain and itch by TRP channels. Neurosci Bull. 2018;34:120–42.

Moran MM, McAlexander MA, Biro T, Szallasi A. Transient receptor potential channels as therapeutic targets. Nat Rev Drug Discov. 2011;10(8):601–20.

Mu D, Deng J, Liu KF, Wu ZY, Shi YF, Guo WM, Mao QQ, Liu XJ, Li H, Sun YG. A central neural circuit for itch sensation. Science. 2017;357(6352):695–9.

Nattkemper LA, Zhao ZQ, Nichols AJ, Papoiu ADP, Shively CA, Chen ZF, Yosipovitch G. Overexpression of the gastrin-releasing peptide in cutaneous nerve fibers and its receptor in the spinal cord in primates with chronic itch. J Invest Dermatol. 2013;133(10):2489–92.

Nattkemper LA, Martinez-Escala ME, Gelman AB, Singer EM, Rook AH, Guitart J, Yosipovitch G. Cutaneous T-cell lymphoma and pruritus: the expression of IL-31 and its receptors in the skin. Acta Derm Venereol. 2016;96(7):894–8.

Nguyen E, Lim G, Ding H, Hachisuka J, Ko MC, Ross SE. Morphine acts on spinal dynorphin neurons to cause itch through disinhibition. Sci Transl Med. 2021;13(579):eabc3774. https://doi.org/10.1126/scitranslmed.abc3774.

Nockher WA, Renz H. Neurotrophins in allergic diseases: from neuronal growth factors to intercellular signaling molecules. J Allergy Clin Immunol. 2006;117(3):583–9.

Ochiai S, Jagot F, Kyle RL, Hyde E, White RF, Prout M, Schmidt AJ, Yamane H, Lamiable O, Le Gros G, et al. Thymic stromal lymphopoietin drives the development of IL-13(+) Th2 cells. Proc Natl Acad Sci U S A. 2018;115(5):1033–8.

Oh MH, Oh SY, Lu J, Lou H, Myers AC, Zhu Z, Zheng T. Trpa1-dependent pruritus in IL-13-induced chronic atopic dermatitis. J Immunol. 2013;191(11):5371–82.

Ohmatsu H, Sugaya M, Suga H, Morimura S, Miyagaki T, Kai H, Kagami S, Fujita H, Asano Y, Tada Y, et al. Serum IL-31 levels are increased in patients with cutaneous T-cell lymphoma. Acta Derm Venereol. 2012;92(3):282–3.

Ohsawa Y, Hirasawa N. The role of histamine H1 and H4 receptors in atopic dermatitis: from basic research to clinical study. Allergol Int. 2014;63(4):533–42.
Patel T, Ishiuji Y, Yosipovitch G. Menthol: a refreshing look at this ancient compound. J Am Acad Dermatol. 2007;57(5):873–8.
Raap U, Wichmann K, Bruder M, Stander S, Wedi B, Kapp A, Werfel T. Correlation of IL-31 serum levels with severity of atopic dermatitis. J Allergy Clin Immunol. 2008;122(2):421–3.
Rankin AL, Mumm JB, Murphy E, Turner S, Yu N, McClanahan TK, Bourne PA, Pierce RH, Kastelein R, Pflanz S. IL-33 induces IL-13-dependent cutaneous fibrosis. J Immunol. 2010;184(3):1526–35.
Reddy VB, Iuga AO, Shimada SG, LaMotte RH, Lerner EA. Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. J Neurosci. 2008;28(17):4331–5.
Reddy VB, Shimada SG, Sikand P, Lamotte RH, Lerner EA. Cathepsin S elicits itch and signals via protease-activated receptors. J Invest Dermatol. 2010;130(5):1468–70.
Reich A, Szepietowski JC. Non-analgesic effects of opioids: peripheral opioid receptors as promising targets for future anti-pruritic therapies. Curr Pharm Des. 2012;18:6021–4. https://doi.org/10.2174/138161212803582405.
Ringkamp M, Schepers RJ, Shimada SG, Johanek LM, Hartke TV, Borzan J, Shim B, LaMotte RH, Meyer RA. A role for nociceptive, myelinated nerve fibers in itch sensation. J Neurosci. 2011;31(42):14841–9.
Riol-Blanco L, Ordovas-Montanes J, Perro M, Naval E, Thiriot A, Alvarez D, Paust S, Wood JN, von Andrian UH. Nociceptive sensory neurons drive interleukin-23-mediated psoriasiform skin inflammation. Nature. 2014;510(7503):157–61.
Ross SE, Mardinly AR, McCord AE, Zurawski J, Cohen S, Jung C, Hu L, Mok SI, Shah A, Savner EM, Tolias C, Corfas R, Chen S, Inquimbert P, Xu Y, McInnes RR, Rice FL, Corfas G, Ma Q, Woolf CJ, Greenberg ME. Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Neuron. 2010;65(6):886–98.
Rossbach K, Wendorff S, Sander K, Stark H, Gutzmer R, Werfel T, Kietzmann M, Baumer W. Histamine H4 receptor antagonism reduces hapten-induced scratching behaviour but not inflammation. Exp Dermatol. 2009;18(1):57–63.
Sadri N, Schneider RJ. Auf1/Hnrnpd-deficient mice develop pruritic inflammatory skin disease. J Invest Dermatol. 2009;129(3):657–70.
Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimaki S, Karisola P, Reunala T, Wolff H, Lauerma A, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. J Invest Dermatol. 2012;132(5):1392–400.
Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE. Specific C-receptors for itch in human skin. J Neurosci. 1997;17(20):8003–8.
Schmelz M, Schmidt R, Weidner C, Hilliges M, Torebjork HE, Handwerker HO. Chemical response pattern of different classes of c-nociceptors to pruritogens and algogens. J Neurophysiol. 2003;89(5):2441–8.
Schwendinger-Schreck J, Wilson SR, Bautista DM. Interactions between keratinocytes and somatosensory neurons in itch. Handb Exp Pharmacol. 2015;226:177–90.
Shim WS, Tak MH, Lee MH, Kim M, Kim M, Koo JY, Lee CH, Kim M, Oh U. TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and L2-lipoxygenase. J Neurosci. 2007;27(9):2331–7.
Shiratori-Hayashi M, Tsuda M. Spinal glial cells in itch modulation. Pharmacol Res Perspect. 2021;9(6):e00754. https://doi.org/10.1002/prp2.754.
Shiratori-Hayashi M, Koga K, Tozaki-Saitoh H, Kohro Y, Toyonaga H, Yamaguchi C, Hasegawa A, Nakahara T, Hachisuka J, Akira S, Okano H, Furue M, Inoue K, Tsuda M. STAT3-dependent reactive astrogliosis in the spinal dorsal horn underlies chronic itch. Nat Med. 2015;21(8):927–31.
Sikand P, Dong X, LaMotte RH. Bam8-22 peptide produces itch and nociceptive sensations in humans independent of histamine release. J Neurosci. 2011;31(20):7563–7.

Simon D, Braathen LR, Simon HU. Eosinophils and atopic dermatitis. Allergy. 2004;59(6):561–70.
Simons FE, Simons KJ. Histamine and H1-antihistamines: celebrating a century of progress. J Allergy Clin Immunol. 2011;128(6):1139–50. e1134
Singer EM, Shin DB, Nattkemper LA, Benoit BM, Klein RS, Didigu CA, Loren AW, Dentchev T, Wysocka M, Yosipovitch G, et al. IL-31 is produced by the malignant T-cell population in cutaneous T-cell lymphoma and correlates with CTCL pruritus. J Invest Dermatol. 2013;133(12):2783–5.
Soh UJ, Dores MR, Chen B, Trejo J. Signal transduction by protease-activated receptors. Br J Pharmacol. 2010;160(2):191–203.
Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, Alenius H, Dieu-Nosjean MC, Meller S, Rieker J, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol. 2006;117(2):411–7.
Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol. 2002;3(7):673–80.
Stander S, Moormann C, Schumacher M, Buddenkotte J, Artuc M, Shpacovitch V, Brzoska T, Lippert U, Henz BM, Luger TA, et al. Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. Exp Dermatol. 2004;13(3):129–39.
Steinhoff M, Neisius U, Ikoma A, Fartasch M, Heyer G, Skov PS, Luger TA, Schmelz M. Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci. 2003a;23(15):6176–80.
Steinhoff M, Stander S, Seeliger S, Ansel JC, Schmelz M, Luger T. Modern aspects of cutaneous neurogenic inflammation. Arch Dermatol. 2003b;139(11):1479–88.
Steinhoff M, Bienenstock J, Schmelz M, Maurer M, Wei E, Biro T. Neurophysiological, neuroimmunological, and neuroendocrine basis of pruritus. J Invest Dermatol. 2006;126(8):1705–18.
Sun YG, Chen ZF. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. Nature. 2007;448(7154):700–3.
Sun YG, Zhao ZQ, Meng XL, Yin J, Liu XY, Chen ZF. Cellular basis of itch sensation. Science. 2009;325(5947):1531–4.
Sun S, Xu Q, Guo C, Guan Y, Liu Q, Dong X. Leaky gate model: intensity-dependent coding of pain and itch in the spinal cord. Neuron. 2017;93(4):840–53.e5.
Suzuki A, Suzuki R, Furuno T, Teshima R, Nakanishi M. N-cadherin plays a role in the synapse-like structures between mast cells and neurites. Biol Pharm Bull. 2004;27(12):1891–4.
Tay SS, Roediger B, Tong PL, Tikoo S, Weninger W. The skin-resident immune network. Curr Dermatol Rep. 2014;3:13–22.
Todberg T, Zachariae C, Bregnhøj A, Hedelund L, Bonefeld KK, Nielsen K, Iversen L, Skov L. The effect of botulinum neurotoxin A in patients with plaque psoriasis – an exploratory trial. J Eur Acad Dermatol Venereol. 2018;32(2):e81–2. https://doi.org/10.1111/jdv.14536.
Toth BI, Olah A, Szollosi AG, Biro T. TRP channels in the skin. Br J Pharmacol. 2014;171(10):2568–81.
Valdes-Rodriguez R, Kaushik SB, Yosipovitch G. Transient receptor potential channels and dermatological disorders. Curr Top Med Chem. 2013;13(3):335–43.
van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, Lubberts E. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. J Immunol. 2009;182:5836–45.
van der Kleij HP, Ma D, Redegeld FA, Kraneveld AD, Nijkamp FP, Bienenstock J. Functional expression of neurokinin 1 receptors on mast cells induced by IL-4 and stem cell factor. J Immunol. 2003;171(4):2074–9.
Verge VM, Richardson PM, Wiesenfeld-Hallin Z, Hokfelt T. Differential influence of nerve growth factor on neuropeptide expression in vivo: a novel role in peptide suppression in adult sensory neurons. J Neurosci. 1995;15(3 Pt 1):2081–96.
Vergnolle N, Ferazzini M, D'Andrea MR, Buddenkotte J, Steinhoff M. Proteinase-activated receptors: novel signals for peripheral nerves. Trends Neurosci. 2003;26(9):496–500.

Wang Z, Jiang C, Yao H, Chen O, Rahman S, Gu Y, Zhao J, Huh Y, Ji RR. Central opioid receptors mediate morphine-induced itch and chronic itch via disinhibition. Brain. 2021;144(2):665–81.

Wilson SR, Gerhold KA, Bifolck-Fisher A, Liu Q, Patel KN, Dong X, Bautista DM. Trpa1 is required for histamine-independent, mas-related g protein-coupled receptor-mediated itch. Nat Neurosci. 2011;14(5):595–602.

Wilson SR, The L, Batia LM, Beattie K, Katibah GE, McClain SP, Pellegrino M, Estandian DM, Bautista DM. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. Cell. 2013;155(2):285–95.

Xanthos DN, Gaderer S, Drdla R, Nuro E, Abramova A, Ellmeier W, Sandkuhler J. Central nervous system mast cells in peripheral inflammatory nociception. Mol Pain. 2011;7:42.

Yamamoto M, Haruna T, Yasui K, Takahashi H, Iduhara M, Takaki S, Deguchi M, Arimura A. A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in Nc/Nga mice. Allergol Int. 2007;56(2):139–48.

Yosipovitch G, Bernhard JD. Clinical practice. Chronic pruritus. N Engl J Med. 2013;368(17):1625–34.

Yosipovitch G, Papoiu AD. What causes itch in atopic dermatitis? Curr Allergy Asthma Rep. 2008;8(4):306–11.

Zheng T, Oh MH, Oh SY, Schroeder JT, Glick AB, Zhu Z. Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. J Invest Dermatol. 2009;129(3):742–51.

Zhuang ZY, Xu H, Clapham DE, Ji RR. Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. J Neurosci. 2004;24(38):8300–9.

Ziegler SF, Roan F, Bell BD, Stoklasek TA, Kitajima M, Han H. The biology of thymic stromal lymphopoietin (TSLP). Adv Pharmacol. 2013;66:129–55.

Zylka MJ, Dong X, Southwell AL, Anderson DJ. Atypical expansion in mice of the sensory neuron-specific Mrg G protein-coupled receptor family. Proc Natl Acad Sci U S A. 2003;100(17):10043–8.

Chapter 8
Toll-Like Receptors in Pain and Itch

Amanda S. Andriessen, Elmira Hezarkhani, and Christopher R. Donnelly

Abstract Pattern recognition receptors (PRR) are genetically encoded proteins which recognize a host of highly conserved "danger signals" produced by microbial organisms (pathogen-associated molecular patterns, or PAMPs) or released by damaged host cells (damage-associated molecular patterns, or DAMPs). PAMP or DAMP-mediated activation of PRR-bearing immune cells is a critical step in initiating an immune response. The Toll-like receptors (TLRs) are a small family of proteins with deep evolutionary origins; they are present in both invertebrate and vertebrate species and all TLR members share a common Toll-Interleukin-1 Receptor (TIR) domain. There is considerable variation in the number of TLRs present in different species with Drosophila (9), mice (12), and humans (10) each having a slightly different number which pales in comparison to the number present in purple sea urchins (222). In this chapter, we discuss the basic biology of the TLRs, including their activation, subcellular localization, and downstream signaling. We highlight the critical role that TLRs play in initiating innate and adaptive immune responses and emphasize a discussion of how TLR-mediated proinflammatory signaling is coupled to pain or itch through neuro–immune interactions. We also highlight emerging evidence that suggests TLR signaling in sensory neurons can rapidly modulate neuronal excitability and discuss the physiological consequences of non-canonical TLR signaling in neurons. Overall, this chapter reviews the plethora of evidence which now exists to support the many ways in which TLR signaling can regulate sensory function via neuro–immune interactions.

Keywords Toll-like receptor (TLR) · TLR4 · TLR5 · Toll · Pattern recognition receptor (PRR) · Pathogen-associated molecular pattern (PAMP) · Damage-associated molecular pattern (DAMP) · Immunity · Nociception

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Abbreviations

AP1	Activator Protein-1
BDNF	Brain-Derived Neurotrophic Factor
CIPN	Chemotherapy-Induced Peripheral Neuropathy
CLR	C-type Lectin Receptor
CNS	Central Nervous System
DAMP	Damage-Associated Molecular Pattern
DRG	Dorsal Root Ganglion
ET-1	Endothelin-1
GPCR	G Protein Coupled Receptor
hBD2	Human Beta-Defensin-2
HMGB1	High Mobility Group Box Protein 1
HSP	Heat Shock Protein
IFM	Inflammatory Mediator
IFN-I	Type-I Interferon
IKK	Inhibitor of Nuclear Factor-κB (IκB) Kinase
IL	Interleukin
IL-1R	Interleukin-1 Receptor-Associated Kinase (Biragyn et al. 2002)
ISG	Interferon Stimulated Gene
LPS	Lipopolysaccharide
LTP	Long-Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MyD88	Myeloid Differentiation Primary Response 88
NF200	Neurofilament 200
NF-κB	Nuclear Factor-κB
NGF	Nerve Growth Factor
NLR	Nucleotide-Binding and Oligomerization Domain (NOD)-Like Receptor
PAMP	Pathogen-Associated Molecular Pattern
PGE_2	Prostaglandin E2
pION	Partial Infraorbital Nerve Ligation
PNS	Peripheral Nervous System
Poly(I:C)	Polyinosinic-Polycytidylic Acid
PRR	Pattern Recognition Receptor
PTX	Paclitaxel
SDH	Spinal Dorsal Horn
sEPSC	Spontaneous Excitatory Postsynaptic Current
SGC	Satellite Glial Cell
sIPSC	Spontaneous Inhibitory Postsynaptic Current
SMOC	Supramolecular Organizing Complex
TBK1	Tank-Binding Kinase-1
TG	Trigeminal Ganglion
TIR	Toll-Interleukin-1 Receptor [Homology Domain]

TIRAP	TIR Domain Containing Adaptor Protein
TLR	Toll-Like receptor
TNF	Tumor Necrosis Factor
TRAF	TNF Receptor Associated Factor (Caterina et al. 2000)
TRIF	TIR-Domain-Containing Adapter-Indued Interferon-Beta
TrkA	Tropomyosin Receptor Kinase A
TRPA1	Transient Receptor Potential Cation Channel Subfamily A Member 1
TRPM8	Transient Receptor Potential Cation Channel Subfamily M Member 8
TRPV1	Transient Receptor Potential Cation Channel Subfamily V Member 1

8.1 A Historical Perspective on Toll-Like Receptors

The overarching purpose of the immune system is to recognize, fight against, and ultimately dispel molecules in our body that we do not recognize and thus could be potentially harmful (Parkin and Cohen 2001). In our conceptual framework of the immune system, we view it as a system divided into two main components: innate immunity and adaptive immunity. Innate immunity encompasses a system designed to provide the first line of defense against invading microorganisms and potential pathogens, conferring rapid, but relatively non-specific protection. In contrast, adaptive immunity, mediated primarily by B and T lymphocytes, takes days or weeks to fully develop, but has the advantage of being specifically directed at a putative danger signal (e.g., a pathogenic microorganism) and also confers immunological memory, wherein the putative danger signal can be "remembered" by adaptive immune cells to enable their rapid activation in subsequent encounters (Parkin and Cohen 2001).

With a few noteworthy examples, our conceptual understanding of how the immune system functions have largely emerged from work performed over the last century. In 1884, Elie Metchnikoff discovered the presence of cells in a species of water flea which engulfed fungal spores, ultimately naming these cells "phagocytes." Through further experiments, his work led to antimicrobial defense, phagocytosis, and rapid microbial detection as key elements of the innate immune system (Modlin 2012), but the molecular mechanisms remained unresolved. An understanding of how the adaptive immune system functions was later to emerge. In 1955, Niels Jerne suggested that an expansive array of soluble antibodies is present in the blood prior to infection, which Frank Burnet further refined in 1957 when he put forth the clonal selection theory. The clonal selection theory suggested that B cells bear a single type of receptor, and its activation, induced by detection of a particular antigen, triggers clonal expansion to generate an expanded lineage of B cells bearing receptors identical to that of the initial parent cell (Burnet 1976). Thus, this theory explained how B cells produce identical antigen-specific clones for humoral antibody-mediated immunity and came to be a foundational concept in our understanding of adaptive immunity (Hodgkin et al. 2007). Thirty years following the work of Burnet, Charles Janeway proposed that innate immune recognition is

coupled with antigen-specific immunity through the use of genetically encoded pattern recognition receptors (PRRs), which recognize exogenous pathogen-associated molecular patterns, or PAMPs (Janeway 2013; Janeway Jr. 1989). Shortly after Janeway's discoveries, Polly Matzinger recognized that inflammation and immunity can also be initiated in the absence of exogenous stimuli, eventually developing the danger model of the immune response which proposed that damaged host cells are likewise capable of releasing signals that can induce immune responses, which eventually came to be known as damage-associated molecular patterns, or DAMPs (Seong and Matzinger 2004).

The understanding of the innate immune system significantly expanded in when Sims et al. cloned the gene for IL-1R1, but the sequence did not point to how Il-1R1 signals as there was a lack of any recognizable pattern in the cytosolic domain (Sims et al. 1988). Three years later, Gay and Keith discovered homology in the amino acid sequence of the IL-1R1 cytosolic domain and that of Toll, a protein in Drosophila melanogaster (Gay and Keith 1991). Toll was already noteworthy for its critical role in Drosophila embryogenesis (Anderson et al. 1985a, b), but the work of Gay and Keith suggested a potentially new function for Toll in inflammation and immunity. Similar to Toll, the drosophila protein, Dorsal, was also known to play an important role in embryonic development in flies, and it was found that the REL homology domain of Dorsal is shared by the mammalian protein nuclear factor kappa B (NF-κB) (Steward 1987). At the time, NF-κB was known to be a transcription factor expressed in lymphocytes (Baeuerle and Henkel 1994), and its drosophila paralog Dif was known to activate transcription of cecropin, an antimicrobial peptide (Ip et al. 1993), suggesting an inducible role for the NF-κB family of transcription factors in immunity. Later, NF-κB was demonstrated to be activated by ligands of Toll and IL-1R1 (Belvin and Anderson 1996; Whitham et al. 1996). Notably, several mammalian proteins were identified which were found to exhibit an even greater degree of homology with Toll than did IL-1R1, sharing the Toll-Interleukin-1 receptor (TIR) domain. Medzhitov and colleagues explored the function of the so-called human Toll (hToll), discovering that hToll activates NF-κB and thus expression of NF-κB-dependent genes (Medzhitov et al. 1997). Shortly thereafter, five additional mammalian Toll homologs were identified and collectively referred to as Toll-like receptors (TLRs). The hToll identified in the previous study is now referred to as TLR4 which will be further explored in following sections.

Today we recognize that TLRs are among the most famous innate immune regulators and are present in all animals from insects to humans, with 10 TLRs conserved in humans and 13 TLRs in mice. Coincident with Charles Janeway's proposed pattern recognition theory, TLRs are the founding members of an expansive family of pattern recognition receptors, yielding an elegant system capable of recognizing and responding to potentially hazardous cues derived from microorganisms (PAMPs) or endogenous host cells (DAMPs). Given that all organisms appear to be at risk of infection, the selective pressure to evolve mechanisms to defend against exogenous and endogenous threats is, and always has been, immense. Thus, PRRs are posited to have early in the history of life, and thus are present in all living things (Janeway 2013; Janeway Jr. 1989).

8.2 TLR Signaling in Immune Cells

Supporting their long evolutionary history and functional significance, all TLRs have a highly conserved structure. They are Type I transmembrane glycoproteins composed of an N-terminal extracellular domain that is leucine-rich, a transmembrane domain, and a C-terminal intracellular TIR domain (Harvey Lodish et al. 2000; Rock et al. 1998). The leucine-rich repeats that make up the ectodomain fold into a solenoid, horseshoe-shaped structure that is inactive until cleaved by endosomal proteases, which are stimulated following TLR-specific ligand interactions. This cleavage of the TLR ectodomain yields functional ability of the TLR to act as a PRR (Botos et al. 2011; Fitzgerald and Kagan 2020; Latz et al. 2007; Ohto et al. 2018; Tanji et al. 2016). All mammalian TLRs examined thus far form direct contact with their ligands through interactions between these ectodomains and their specific ligands. The single transmembrane domain leads to the cytosolic TIR domain, which functions to trigger the downstream signaling of TLRs through interactions with various adaptor proteins.

In accordance with the critical role of TLRs in innate immunity, they are highly expressed by a wide variety of immune cells, with some degree of cell-type specificity (Zuany-Amorim et al. 2002). TLRs also exhibit differential patterns of membrane localization (Fitzgerald and Kagan 2020; Liu et al. 2010a; Randow and Seed 2001). In naïve conditions, TLRs recognizing microbial membrane components such as proteins, lipids, or lipoproteins (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) are present on the cell surface where they typically encounter their natural agonists (Fig. 8.1). In contrast, nucleic acid sensing TLRs (TLR3, TLR7, TLR8, and TLR9) are typically localized within endosomal compartments, which poises them to detect aberrant intracellular nucleic acids while limiting the degree to which self-derived ligands released by dying cells in the extracellular space promote autoimmune responses (Alexopoulou et al. 2001; Hemmi et al. 2002, 2000) (Fig. 8.1). Cell surface-localized TLRs are typically monomeric, forming homo- and/or heterodimers with other monomers upon ligand binding (Jin et al. 2007; Jin and Lee 2008; Park et al. 2009), whereas endosomal TLRs have been shown to exist as pre-formed homodimers (Ohto et al. 2018; Tanji et al. 2016). Unlike their surface-localized counterparts, the ectodomains of endosomal TLRs must be cleaved by cathepsins to enable dimerization and signal transduction (Ewald et al. 2011; Fukui et al. 2018).

For both cell surface- and endosome-localized TLRs, ligand binding causes conformational changes which bring the cytosolic TIR domains into close proximity. The subsequent detection of dimerized TIR domains by the membrane adaptor proteins TIRAP and TRAM leads to the assembly of an oligomerized scaffold of cytosolic proteins known as supramolecular organizing centers (SMOCs). TIRAP prompts the assembly of the myddosome, which is characterized by the presence of myeloid differentiation primary response 88 (MyD88) oligomers, interleukin-1 receptor-associated kinases (IRAKs), and TNF receptor associated factor-6 (TRAF6), leading to downstream signaling. Loss of MyD88 or other myddosome

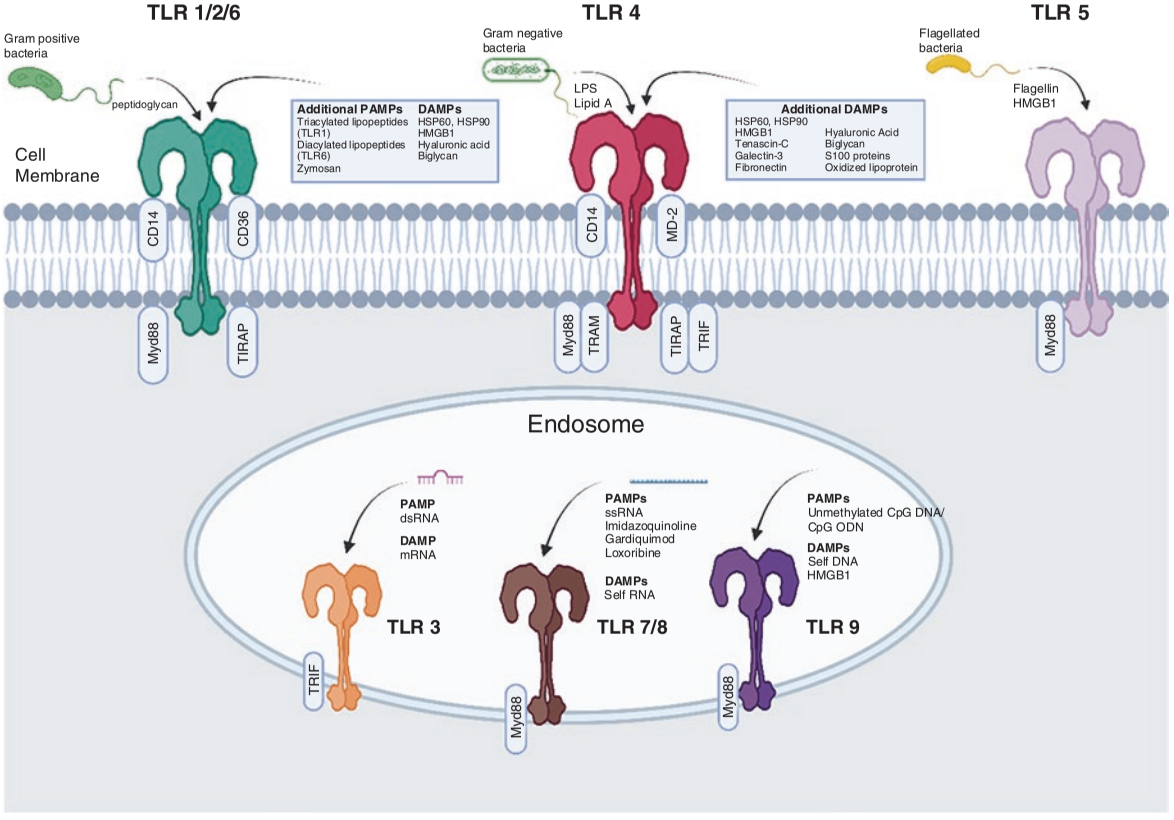

Fig. 8.1 Canonical ligands for Toll-like receptors. TLR 1, 2, 4, 5, and 6 are known as cell surface receptors shown by their placement on the cell membrane. TLRs 3, 7, 8, and 9 are referred to as endosomal receptors as represented by their location within a cellular endosome. It is important to note that while several PAMPs and DAMPs are shown for each TLR, the figure is not comprehensive. PAMPs for TLR 1, 2, and 6 consist of peptidoglycan as shown above, in addition to triacylated lipopeptides (with TLR1), diacylated lipopeptides (with TLR6), and zymosan. DAMPS for these three TLRs consists of HSP60, HSP90, HMGB1, hyaluronic acid, and biglycan. In addition to LPS, Lipid A also serves as a PAMP for TLR4, while HSP60, HSP90, HMGB1, Tenascin-C, Galectin-3, Fibronectin, Hyaluronic acid, Biglycan, S100 proteins, and oxidized lipoproteins function as DAMPs. HMGB1 and Self RNA are DAMPs for TLR5 and TLR7/8, respectively. In addition to the ligands shown for TLR9, HMGB1 also serves as a DAMP for TLR9 (Lacagnina et al. 2018)

components impairs signaling by all TLRs except TLR3 and TLR4 (Akira and Hoshino 2003), and thus, myddosome-mediated signaling is critical for most TLR-mediated functions. Within the myddosome, TRAF6 activates the kinase, TAK1, which stimulates IKK-mediated NF-kB and MAPK/AP-1-mediated transcriptional responses (Emmerich et al. 2013; Wang et al. 2001), as well as recruitment of tank-binding kinase-1 (TBK1). While activation of the transcription factors NF-kB and AP-1 are responsible for the upregulation of inflammatory mediators, myddosome-dependent TBK1 is critical for induction of rapid metabolic changes such as induction of glycolysis (Everts et al. 2014; Fitzgerald and Kagan 2020; Tan and Kagan 2019).

MyD88-independent signaling mediated by TLR4 requires the membrane protein TRAM, which recognizes the homodimers of TLR4, prompting binding to TIR-domain-containing adapter-inducing interferon-β (TRIF) and subsequent TNF receptor associated factor-3 (TRAF3)-dependent activation of the kinase tank-binding kinase-1 (TBK1). This TRIF-dependent, myddosome-independent pathway has been referred to as the triffosome (Fitzgerald and Kagan 2020). TBK1 phosphorylation drives transcription of type-I interferons (IFN-I) and other interferon stimulated genes (ISG) (Fitzgerald et al. 2003a; McWhirter et al. 2004). Although TBK1 is an element of both the myddosome and triffosome, MyD88 lacks a pLxIS motif present within TRIF, and phosphorylation of this motif enables TRIF to interact with the interferon regulatory factor-3 (IRF3) which is critical for TBK1-mediated IFN-I responses. Thus, IFN-I induction is a feature largely restricted to myddosome-independent TLR signaling mediated by the triffosome. Interestingly, it is worth noting that TRAM does not bind to TLR3, and it remains unclear whether a presently unknown adaptor protein with a function analogous to TRAM is required for MyD88-independent TLR3 signaling (Fitzgerald et al. 2003b; Kawai et al. 2001; Navarro and David 1999; Yamamoto et al. 2003a, b).

In summary, TLR activation by PAMPs or DAMPs in immune cells leads to activation of a downstream signaling cascade that varies depending on the TLR in question, but can be broadly divided into two main categories: (1) Myd88/myddosome-mediated TLR signaling, which is employed by all TLRs except TLR3 and is associated with NF-kB and AP-1-dependent transcriptional induction of inflammatory mediators and TRAF6/TBK1-dependent metabolic changes; and (2) TRIF/triffosome-mediated TLR3 and TLR4 signaling, which activates NF-kB, AP-1, and TBK1, yielding an inflammatory response which also features IFN-I (Fig. 8.2). While initially TLR signaling was studied primarily in innate and adaptive immune cells, a plethora of literature has emerged to suggest that other cell types are also capable of participating in inflammation and immunity via canonical TLR signaling (Karikó et al. 2004; Lafon et al. 2006; McKimmie and Fazakerley 2005).

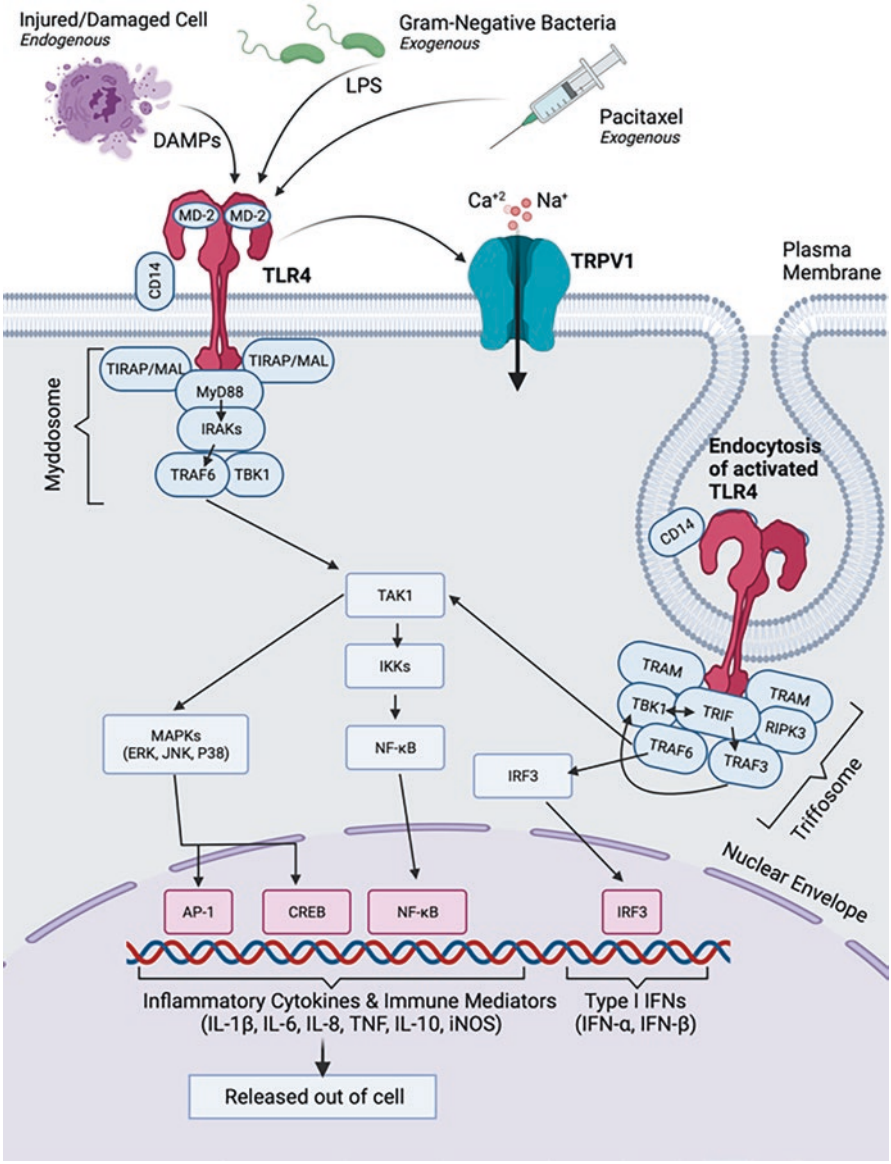

Fig. 8.2 TLR4 signaling in-depth. TLR4 is the most significant and well-known TLR when it comes to pain. This figure displays the downstream effectors of TLR4 activation from DAMPs, LPS, and PTX. There are two SMOCs for TLR4: the myddosome (which forms around most activated TLRs except TLR3) and the MyD88-independent Triffosome (which only forms around TLR4 and TLR3). Activation by a DAMP or PAMP causes formation of a TLR4 dimer on the cell surface, leading to formation of the myddosome, composed of TIRAP, TBK1, TRAF6, and MyD88, which forms around the base of the receptor. This can then activate TNF receptor-associated factor 3 (TRAF3) and downstream kinases such as IRAKs/TRAF6 which come together to form the triffosome. CD14 induces endocytosis upstream of TLR4 signaling and the combined work of CD14 and TLR4 promotes the TRIF signaling in the triffosome (Gay et al. 2014;

8.3 Detecting Danger Is a Shared Responsibility of Immune Cells and Neurons

Hydra, a freshwater metazoan in the Cnidaria phylum, is among the first organisms known to have evolved a nervous system. Notably, Hydra lacks a mesoderm, and thus lacks a defined immune system (Augustin et al. 2017). Given the strong evolutionary selection for organisms to develop host defense machinery, the apparent lack of an immune system is a curious finding and implies other cell types likely contribute to host defense. Interestingly, emerging evidence suggests that the Hydra nervous system plays a direct and active role in host defense, producing antimicrobial peptides and shaping the microbial communities that coat its external body surface (Augustin et al. 2017; Fraune and Bosch 2007). Notably, single cell sequencing analysis of Hydra neurons indicates that they possess elements of TLRs and other pattern recognition receptors, such as NOD-like receptors (NLRs) and C-type lectin receptors (CLRs), and a large proportion of neuronal genes encode antimicrobial peptides (Klimovich et al. 2020). In C. elegans, many components of the innate immune system are missing, and neurons appear to compensate for their loss (Irazoqui et al. 2010a, b; Pujol et al. 2001). For example, nematode sensory neurons mediate avoidance behaviors in response to pathogenic bacteria (Cao and Aballay 2016) and utilizes neuronal GPCR-mediated signaling for the induction or repression of protective immunity in other tissues through many transcriptional mechanisms (Wani et al. 2020). These real-world examples illustrate the highly overlapping role of the nervous system and immune system in protecting organisms from potentially hazardous stimuli and raise questions as to the evolutionary origins of both systems. Appreciating that the very concept of the immune system and nervous system is in some ways an artifact of artificial classification systems, we recognize vertebrates as possessing a well-defined immune system which is separate and distinct in many ways from the nervous system, but an intricate coupling between the two systems exists. Neuronal detection of physical, thermal, and chemical stimuli on a timescale of milliseconds enables rapid behavioral modification to avoid acute threats. In addition, immune cell-derived inflammatory mediators can modulate sensory neuron excitability to draw the attention of an organism to an existing injury or threat leading to protective behavioral responses. In this section we will highlight how TLR signaling in immune cells and PNS- or CNS-resident glial cells contributes to pain through the production of inflammatory mediators. We will also discuss how neuronal TLR signaling is similar and divergent compared to canonical TLR signaling in immune cells.

Fig. 8.2 (continued) Kieser and Kagan 2017). CD14 is an essential component for the subsequent internalization of the TLR and PAMP through endocytosis (Zanoni et al. 2011). Of note, MD2 is the only protein required specifically for TLR4 endocytosis and it has no ability to signal independently of TLR4 (Tan et al. 2015). This process leads to downstream activation of interferon regulatory factors (IRF3), NF-κB, and various MAP kinases and eventually cytokines. Simultaneously TLR4 can directly activate TRPV1 channels to cause cation influx which increases excitability in neurons. TLR4 is an important example of conserved aspects of TLR signaling and non-canonical ion channel coupling (Fitzgerald and Kagan 2020)

8.4 TLR Signaling in Immune Cells Is Indirectly Coupled to Pain via Release of Inflammatory Mediators

The classical view of TLRs signaling in immune cells is coupled to pain through the secretion of inflammatory mediators that sensitize nociceptors. Common among all TLRs, as their canonical immune response function, is to produce inflammatory cytokines which in turn activate resident immune cells, mast cells, and macrophages. Within minutes these immune cells release more proinflammatory mediators and chemokines in addition to other injury response mediators. These activated immune cells and their mediators contribute to peripheral nociceptive sensitization through these factors and interacting directly with nociceptors (Ren and Dubner 2010). All TLRs, except TLR3, use MyD88 as an adaptor in their mechanism which then mainly activates NFκB family members and MAP kinases which then tend to produce a pro-inflammatory response through cytokines like IL-6 and IL-12. TLR3 only uses TRIF which mainly activates IRF family members to induce anti-viral interferon responses (Liu and Ding 2016; Liu et al. 2017; Takeuchi and Akira 2010). Nociceptors express many receptors for these proinflammatory cytokines and chemokines such as IL-1R, TNFR1, TrkA, gp140, and IL-5R which respond to the following TLR-induced upregulated and exocytosed 1L-1β, TNF, NGF, IL-6, and IL-5, respectively. These released IFMs can induce phosphorylation of ligand-gated channels, such as TRPV1 and TRPA1, or modification of voltage-gated sodium channels which leads to increased excitability through changes in neuronal membrane properties, increased firing, and heightened sensitivity to thermal or mechanical stimuli (Pinho-Ribeiro et al. 2017). As an example, one study using a rat L5 spinal nerve ligation model showed that upregulation of TLR3, by way of Poly(I:C) administration, promoted neuropathic pain through promoted expression of inflammatory mediators. Knockdown of TLR3 was able to inhibit SNL-induced microglia autophagy along with relieving mechanical and cold hypersensitivity (Chen and Lu 2017). Additionally, TLR7-mediated recognition of single stranded RNA from both influenza virus and nonviral origins induces production of inflammatory cytokines of the innate immune system, including interferons which have been shown to directly activate sensory neurons in DRG and trigeminal ganglia (Barragán-Iglesias et al. 2020; Diebold Sandra et al. 2004). Painful conditions and disease have been well documented as a result of TLR-mediated proinflammatory processes increase analgesia through direct interaction with nociceptors (Aravalli et al. 2007; Liu and Ding 2016; Nicotra et al. 2012). Thus, TLR activation in immune cells can not only lead to general inflammation but sensitization of nociceptors and pain.

8.5 TLR Signaling in Pain via Neuro-Immune and Neuro-Glia Interactions

While TLRs are classically thought as being expressed by innate and adaptive immune cells, TLRs are also expressed by cell types throughout the peripheral and central nervous systems, including Schwann cells, oligodendrocytes, microglia, astrocytes, and various peripheral and central neuron populations (Bruno et al. 2018). Among peripheral sensory neurons, expression of various TLRs and MyD88 has been demonstrated using multiple experimental methods, including transcriptomic analysis, immunohistochemistry and in situ hybridization, and functional approaches such as electrophysiology and behavioral experiments (Donnelly et al. 2020; Goethals et al. 2010; Liu et al. 2014). However, unlike immune cells, peripheral sensory neurons are electrically excitable cells, bearing ion channels which are coupled, either directly or indirectly, to receptors for inflammatory mediators (IFMs) as well as TLRs. This coupling enables IFMs and TLR ligands to rapidly alter neuronal excitability, leading to behavioral outputs such as pain, analgesia, or itch (Donnelly et al. 2020).

Under homeostatic conditions, DRG-resident satellite glial cells (SGCs) express relatively high levels of *Tlr3* and low levels of *Tlr2* and *Tlr6*. After intraplantar CFA administration, murine *Tlr2* and *Tlr6* are upregulated in SGCs, and agonists of the TLR2/6 heterodimer drive IL-33 production, which directly sensitizes nociceptors (Huang et al. 2020). The contribution of TLR3 in SGCs is unknown. Interestingly, expression of TLR4 in SGCs has been reported to be dependent on neuronal contact, as SGCs isolated from DRG neurons dramatically upregulate Tlr4 (Tse et al. 2014).

Sensory ganglia-infiltrating macrophages are another important contributor to pain pathogenesis after injury, and DRG-infiltrating macrophages have been shown to express several TLRs including TLR2, TLR4, TLR9 (Bruno et al. 2018; Kim et al. 2011; Chen et al. 2020; Luo et al. 2019). Following nerve injury, mice lacking Tlr2 exhibited reduced accumulation of DRG-infiltrating macrophages (Kim et al. 2011; Shi et al. 2011). Given that numerous cell types in the DRG and spinal dorsal horn express TLR4, it has been difficult to identify the specific contribution of TLR4 in macrophages. In rodent models of chemotherapy-induced peripheral neuropathy (CIPN)-associated pain, TLR4 signaling in DRG sensory neurons and spinal microglia are each regarded to contribute to pain pathogenesis (Li et al. 2014). Interestingly, a recent study found that the gut microbiota contributes to CIPN-induced pain, and bone marrow transplant experiments suggested this was through a mechanism involving TLR4 signaling in hematopoietic cells, including macrophages (Shen et al. 2017). TLR9 also contributes to CIPN-induced neuropathic pain, and reportedly does so in male but not female mice (Luo et al. 2019). According to a recent single cell RNA-seq study evaluating several different injury models, a multitude of additional TLRs are induced in neuronal and non-neuronal cell types following injury or insults, including Tlr1, Tlr2, Tlr3, Tlr4, Tlr7, and Tlr9 (Renthal et al. 2020). Thus, it will be interesting to determine how additional TLRs as well as TLR signaling in additional cell types may contribute to acute and chronic pain conditions.

Within the spinal cord, glial cells also express TLRs, and their activation has been shown to increase excitability and sensitization of peripheral sensory neurons through neuro–immune interactions. Microglia, a CNS-resident immune cell which shares many features with peripheral macrophages, are known to express most TLRs, which enables them to detect a variety of PAMPs and DAMPs and initiate protective immunity (Bsibsi et al. 2002; Olson and Miller 2004). Following nerve injury, murine spinal cord glial cells isolated from Tlr2 knockout (KO) mice exhibit reduced expression of TNF, IL-1β, IL-6, and iNOS genes comparison to WT glial cells, and Tlr2 KO mice exhibit reduced mechanical allodynia and thermal hyperalgesia, suggesting glial Tlr2-mediated IFMs are an important contributor to neuronal sensitization and neuropathic pain after injury (Kim et al. 2007). In both rodents and humans, the TLR4 ligand LPS is regarded as one of the best-known activators of microglia, inducing robust production and release of IFMs and causing marked microglial activation and persistent pain (Clark et al. 2010; Saito et al. 2010). Tlr4 KO mice exhibit reduced neuropathic pain behaviors following nerve injury and a concomitant reduction in microglia proliferation and pro-inflammatory cytokine induction in the spinal cord (Tanga et al. 2005). Intrathecal injection of TLR4 antagonists reverses nerve injury-induced mechanical allodynia and thermal hyperalgesia in mice and rats (Bettoni et al. 2008; Nicotra et al. 2012). In microglia, TLR4-mediated activation of p38 MAPK is critical for the release of TNF, IL-1β, BDNF, prostaglandin E2 (PGE2), and nitric oxide, all of which contribute to pain hypersensitivity (Chen et al. 2018; Saito et al. 2010).

Compared to microglia, astrocytes express a more limited repertoire of TLRs, and fewer studies exist which have studied the role of TLR signaling in astrocytes in pain pathogenesis. In rodents, TLR2, TLR3, and TLR4 regulate spinal astrocyte activation following nerve injury (Kim et al. 2007; Tanga et al. 2005) and appear to be the main TLRs expressed in astrocytes. A recent single cell RNA-seq analysis of murine brain astrocytes found that Tlr3 is expressed most abundantly, with more modest levels of Tlr2. This study failed to detect Tlr4, although more sensitive methods have shown low levels of constitutive expression of Tlr4 in astrocytes (Hasel et al. 2021). TLR3 activation by extracellular poly I:C treatment prompts astrocytes to produce pro-inflammatory mediators (Scumpia et al. 2005). LPS stimulation of astrocytes induces proinflammatory cytokine production (Carpentier et al. 2005; Gorina et al. 2011). TLR4 signaling in astrocytes contributes to the pathogenesis of CIPN-associated neuropathic pain in mice and rats (Li et al. 2014).

8.6 Non-canonical TLR Signaling in Sensory Neurons via Coupling to Ion Channels

The transient receptor potential (TRP) channel family is the largest and most widely studied family of noxious stimulus detectors (Patapoutian et al. 2009). This family is composed of 28 structurally similar non-selective ligand-gated cation channels divided into multiple subfamilies. As discussed in previous chapters, TRP channels

are critical for sensing various thermal and cold stimuli, and can be activated by naturally occurring chemical ligands (e.g., capsaicin for TRPV1, menthol for TRPM8, and mustard oil for TRPA1) (Moore et al. 2018). TRPV1 and TRPA1 are well-documented to play a critical roles in neurogenic inflammation, neuronal sensitization, acute and chronic pain, and itch (Bautista et al. 2006; Caterina et al. 2000; Donnelly et al. 2020; Liu et al. 2016, 2010b; Patapoutian et al. 2009). Notably, RNA-Seq studies have demonstrated DRGs expression TLR1, TLR2, TLR3, TLR4, and TLR5 in both mice and non-human primates (Kupari et al. 2021; Usoskin et al. 2015; Yang et al. 2019; Zeisel et al. 2018). With one noteworthy exception which we discuss later (TLR5), most TLRs are selectively enriched in nociceptors. Interestingly, many studies have demonstrated that TLRs are co-expressed with TRPV1 or TRPA1, and TLR activation by PAMPs or DAMPs can rapidly modulate neuronal excitability through functional coupling to TRPV1 or TRPA1 (Barajon et al. 2009; Fitzgerald and Kagan 2020; Lacagnina et al. 2018; Liu et al. 2012a; Materazzi et al. 2012; Park et al. 2014; Qi et al. 2011).

Among TLRs exhibiting expression in sensory neurons, TLR4 is the most widely studied. TLR4 is important for the initiation and establishment of chronic pain as well as other inflammatory pathologies (Bruno et al. 2018; Gao et al. 2017; Mohammad Hosseini et al. 2015; Wu et al. 2010; Zuany-Amorim et al. 2002). While much of this has been attributed to TLR4 signaling in non-neuronal cells, contributing to pain via neuro–immune or neuro–glia interactions, TLR4 is also highly expressed in DRG nociceptors (Bruno et al. 2018; Donnelly et al. 2020; Zuany-Amorim et al. 2002). The prototypical PAMP, lipopolysaccharide (LPS, also known as endotoxin) signals through TLR4 by binding its co-receptor myeloid differentiation protein-2 (MD-2) in the TLR4 binding cavity. TLR4 also responds to various DAMPs, including heat shock proteins, extracellular matrix degradation products, and high-mobility group box-1 (HMGB1) (Fig. 8.3) (Beutler and Rietschel 2003; Botos et al. 2011; Kelly et al. 2006). Acute application of LPS to dissociated trigeminal ganglion (TG) neurons evokes inward currents and increased Ca2+ influx within a timescale of seconds to minutes (Diogenes et al. 2011). Rapid capsaicin-induced pain behaviors are reduced in global TLR4 knockout mice, and capsaicin-induced Ca2+ influx is reduced in Tlr4 deficient DRG neurons (Min et al. 2018). Notably, co-IP experiments suggest that TLR4 and TRPV1 physically interact via the TIR domain of TLR4, which prevents internalization of TRPV1. TLR4 signaling in peripheral sensory neurons was also recently reported to contribute to the early onset of nerve injury-induced neuropathic pain behaviors in female mice, but not male mice, via HMGB1-mediated TLR4 activation in nociceptors (Szabo-Pardi et al. 2021). This study did not explore whether these effects were TRPV1-dependent. Thus, TLR4 signaling in nociceptors contributes to pain, possibly via multiple cellular mechanisms, including direct coupling to TPRV1.

Although the central dogma is that nucleic acid sensing TLRs such as TLR3 and TLR7 are localized to endosomal membranes, recent studies have suggested surface localization can occur in sensory neurons and some immune cell types (Kanno et al. 2015; Liu et al. 2010b; Mielcarska et al. 2021; Park et al. 2014). In DRG neurons, TLR7 was reported to be expressed by small-diameter nociceptors and colocalized

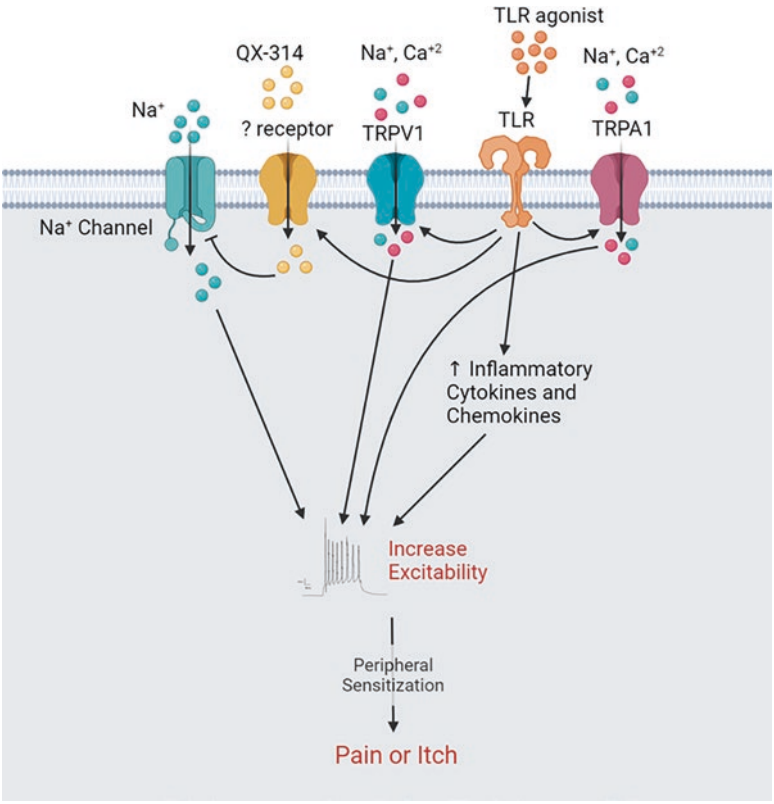

Fig. 8.3 Overview of TLR mechanisms in neurons TLRs are well known to increase inflammatory cytokines and chemokines yet these factors have various effects beyond innate and adaptive immunity. Upon activation of a TLR it can lead to activation of TRPV1, TRPA1, or an unknown receptor of QX-314 in addition to canonical upregulation of pro-inflammatory cytokines and chemokines. The production of IFMs as well as TRPV1 and TRPA1 activation leads to increased neuronal excitability and in turn causes peripheral sensitization of sensory neurons, experienced as pain or itch. The activation of the receptor of QX-314, such as from TLR5 activation, will inhibit sodium ion influx which would otherwise increase the neuronal excitability

on the cell surface with TPRV1 (Liu et al.). Intraplantar administration of the microRNA (miRNA) let-7b elicits rapid pain-related behaviors in mice, and miR-let-7b perfusion rapidly induces inward currents and action potentials in DRG nociceptors. These actions were reported to be dependent on functional coupling between TLR7 and TRPA1, wherein TLR7-mediated detection of let-7b modulates neuronal excitability via TRPA1 (Park et al. 2014). These studies suggest that damaged host cells may release miRNAs, which act as DAMPs, activating TLR7 in nociceptors to rapidly promote pain. Tlr3 is also expressed in TRPV1+ nociceptors (Barajon et al. 2009; Liu et al. 2012a) and appears to be dispensable for acute pain, but important for central sensitization-mediated pain hypersensitivity. The TLR3 agonists Poly(I:C) and dsDNA rapidly induce inward currents and action potentials

in DRG neurons, an effect which is abolished in *Tlr3* KO mice. Recordings from lamina II neurons of the spinal dorsal horn in slice preparations found that Poly(I:C) induced a TLR3-dependent increase in the frequency of spontaneous excitatory post-synaptic currents (sEPSCs), and tetanic stimulation of C-fibers induced long-term potentiation (LTP) of the field potential in WT, but not Tlr3-deficient mice (Liu et al. 2012a). Activation of TLR3 was also shown to upregulate TRPV1 and rapidly enhanced TRPV1-mediated Ca^{2+} flux (Qi et al. 2011). Thus, activation of TLR3 in nociceptors promotes central sensitization through coupling to TRPV1, although the precise mechanism remains unknown.

Historically, TLR8 has been less studied than its colleagues TLR7 and TLR3 since it was believed to be non-functional in mice; however, studies showing its role in cell apoptosis and upregulation in the CNS in response to microbial activation have since brought it more attention (Ma et al. 2007; Olson and Miller 2004). One such recent study demonstrated that while acute itch, pain sensation, and inflammatory pain were not impaired by TLR8-/-, chronic pain in a spinal nerve ligation (SNL) model was significantly reduced in the TLR8 knockout mice. Subsequent imaging showed that not only was TLR8 highly expressed in small-diameter, mainly nonpeptidergic IB4+, neurons, but the intensity of TLR8 was increased post-SNL at all timepoints (3, 20, and 21 days) (Zhang et al. 2018). TLR8 has been shown to play a similar role in the other collection of primary sensory neurons, trigeminal ganglia (TG). In a model of partial infraorbital nerve ligation (pIONL), similar to SNL, TLR8 has enduring increased expression in TG neurons. Genetic deletion of TLR8 with pIONL not only attenuated mechanical allodynia but the expected effects of reduced activation of ERK and p38-MAPK and reduced pro-inflammatory cytokines, for example, TNF-α, Il-1β (Zhao et al. 2021). In both the TG and DRG models of nerve ligation, timing of TLR8 knockdown was an important factor that contributed to the conclusion that TLR8 is required for the maintenance of neuropathic pain. Additionally, TLR8 agonists, VTX-2337, in naïve mice alone is enough to produce mechanical allodynia based on nocifensive behavior (for TG-directed pain) and paw-withdrawal (for DRG-directed pain) (Zhang et al. 2018; Zhao et al. 2021).

8.7 TLR5 and Mechanical Allodynia

TLR5 is unique in that it is one of the few TLRs that recognizes a protein PAMP, bacterial flagellin, which is a critical component of bacterial flagella in both Gram-negative and -positive bacteria (Hayashi et al. 2001). While it is highly expressed in the intestinal mucosal epithelium, immunohistochemistry, in situ hybridization, and RNA-sequencing studies demonstrate that TLR5 is also highly expressed by large-diameter neurofilament-200 (NF-200)-positive DRG sensory neurons (Gewirtz et al. 2001; Xu et al. 2015; Yang et al. 2019). In fact, in situ hybridization showed that nearly all (91%) of Tlr5+ neurons exhibit NF200-positive immunoreactivity, and that the vast majority (78%) of NF200-positive neurons co-express Tlr5+.

NF200 is a marker for large-diameter, low-threshold, myelinated A-fiber neurons which are responsible for mechanical allodynia (Ahn et al. 2012; Campbell et al. 1988; Ohsawa et al. 2013; Ossipov et al. 2002). Application of the TLR5 ligand, flagellin, induces calcium responses in mouse DRG neurons. Additionally, co-application of flagellin with QX-314, a positively charged membrane-impermeable lidocaine derivative, suppresses mechanical allodynia in animal models of chemotherapy-induced peripheral neuropathy (CIPN), sciatic nerve injury, and diabetic neuropathy-induced neuropathic pain. This co-application selectively blocks action potentials by suppressing sodium currents, thereby suppressing Aβ-fiber conduction to attenuate neuropathic pain behaviors in both naive and CIPN mice. However, the effect is lost in *Tlr5* deficient mice, emphasizing that it is TLR5-mediated (Xu et al. 2015). This has since been followed up in a chronic constriction injury (CCI) model of neuropathic pain in rats where activation of TLR5, via flagellin administration, relieved mechanical hyperalgesia and mechanical allodynia for up to 6 h post-injection (Chang et al. 2021). In the CNS, TLR5 is expressed in microglia and has been shown to modulate their activity in response to flagellin or brain injury. However, in alignment with the canonical view of TLRs, microglial activation of TLR5 in the brain appears to exacerbate nerve injury-induced neuroinflammation and neuropathic pain (Ifuku et al. 2020).

8.8 TLR Signaling in Itch Pathogenesis

As discussed in previous chapters, pruriception, or the sensation of itch, serves as an additional sensory mechanism to aid in the detection of potential environmental hazards or threats. Scratching behavior activates reward circuits, which in turn evoke pleasurable feelings along with an increased desire to scratch (Su et al. 2019). There are several distinct subtypes of itch, including: touch-evoked itch (mechanical itch), chemical itch, opioid-induced itch, and chronic itch, a condition in which itching lasts for longer than 6 weeks Isaac M. Chiu (2018). Chronic itch can occur as a consequence of many conditions beyond dermatological diseases, including neoplasms (cutaneous T-cell lymphoma), systemic diseases (e.g., chronic liver disease, end-stage kidney diseases), and metabolic disorders (Chen et al. 2021; Kremer et al. 2020; Kurban et al. 2008). As discussed in previous chapters, the peripheral and central neural circuits responsible for itch is a rapidly developing field that has primarily emerged within the last 15 years (Dong and Dong 2018). In peripheral sensory ganglia, itch-sensing neurons are regarded as a subpopulation of nociceptors, termed pruriceptors, although TRPV1 and TRPA1 are expressed in both populations (Ji 2015). In this section we will review how TLRs contribute to itch pathogenesis via neuro–immune interactions or via sensory neuron-intrinsic mechanisms involving coupling with ion channels.

TLRs are expressed by several cell types within the skin, including keratinocytes and Langerhans cells in the epidermis, and macrophages, dendritic cells, and mast cells within the dermis (Miller 2008). Keratinocytes, the major epidermal cell type,

are frequently the first responders to exogenous pathogens or injury. In keratinocytes obtained from psoriatic skin lesions, expression of several TLRs is elevated leading to proinflammatory cytokine signaling which exacerbates skin inflammation (Cristina Lebre et al. 2003; Lebre et al. 2007). In chronic itch patients with atopic dermatitis, prurigo nodularis, and psoriasis, protein levels of TLR3 are increased in lesional skin compared to non-lesional skin or healthy skin. Human keratinocytes treated with the TLR3 agonist poly(I:C) show increased expression of endothelin-1 (ET-1), which stimulates mouse DRG neurons to release of B-type natriuretic peptide (Szöllősi et al. 2019), a known neuropeptide uniquely involved in pruritus (Shimizu et al. 2014). Notably, ET-1 is also elevated in lesional skin in prurigo nodularis patients (Kido-Nakahara et al. 2014), suggesting this mechanism may be conserved in humans. As mentioned in the previous section, small-diameter DRG neurons, usually considered nociceptors, express TLR3, which contributes to central sensitization after an acute pain stimulus. Itch-sensing sensory neurons, or pruriceptors, are a subpopulation of nociceptors, and TLR3 is expressed within a subset of TRPV1+, GRP+ pruriceptors. Interestingly, intradermal injection of the TLR3 agonist poly(I:C) induces robust scratching behaviors in mice, which are abolished in Tlr3 KO mice. Scratching behaviors induced by intradermal pruritogens were also attenuated in mice lacking Tlr3, and TLR3-deficient mice did not exhibit dry skin-induced scratching behaviors unlike WT controls. This appears to be directly dependent on TLR3 signaling in pruriceptors, as acute perfusion with RNA extracted from skin or poly(I:C) induces inward currents in TLR3+ DRG neurons (Ji 2015).

Similar to TLR3, TLR7 has been demonstrated to play important roles in both pain and itch. TLR7 is expressed in a subpopulation of pruriceptors, only some of which express the GPCR MrgprA3, which is responsible for histamine-independent, chloroquine-dependent itch behaviors. TLR7 agonists induce dose-dependent scratching behaviors in mice, and dose-dependent inward currents in DRG neurons, both of which are abolished in Tlr7 KO mice (Liu et al. 2012b). Interestingly, pruritus is a common side effect of the TLR7 agonist imiquimod when applied topically in human cancer patients (Madan et al. 2010) Similar to TLR3, TLR2 is expressed in mouse DRG and TG neurons, and mice lacking TLR2 exhibit attenuated acute and chronic itch behaviors, as well as reduced formalin-induced inflammatory pain. Activation of TLR1/2 heterodimers using the agonist Pam3CSK4 evoked both pain and itch, whereas activation of TLR2/6 heterodimers using lipoteichoic acid and zymosan produced only pain (Wang et al. 2020). These effects were attributed to direct activation of DRG neurons, as Pam3CSK4 and zymosan increased Ca2+ signals in dissociated DRG neurons, although it is possible other cell types contribute in vivo.

Several studies have investigated the role of TLR4 in itch. Human β-defensin 2 (hBD2) is an antimicrobial peptide highly upregulated in keratinocytes in psoriasis patients (Jansen et al. 2009). Intradermal hBD2 injection prompted itch behaviors in WT mice, but not in mice lacking Tlr4 globally or mice lacking Tlr4 selectively in myeloid cells (LysM-Cre; Tlr4fx/fx mice) Similarly, TRPV1 KO mice exhibited reduced scratching behaviors, suggesting both TLR4 and TRPV1 are important in

this process. Calcium imaging experiments revealed that hBD2 induced Ca2+ signals in keratinocytes, but not DRG sensory neurons, and this effect was TLR4-dependent. Thus, hBD2 appears to induce itch via neuro–immune interactions, accomplished via TLR4 activation in cutaneous immune cells, likely leading to induction of an unidentified pruriceptive mediator that acts, in part, via TRPV1+ in nociceptors (Feng et al. 2017). Liu et al. (2016) found that TLR4 does not contribute to acute itch behaviors induced by intradermal administration of pruritogens, but TLR4 is critical in the pathogenesis of dry skin-induced chronic itch (Tong Liu et al. 2016). Interestingly, this was attributed to TLR4 signaling in astrocytes, which contribute to central sensitization in chronic pain and chronic itch conditions (Ji et al. 2019; Liu et al. 2016).

8.9 Conclusion

Over the course of the past 40 years, our knowledge of TLRs has significantly expanded. Ample evidence now exists which demonstrates that canonical TLR signaling in immune cells is a critical regulator of pain and itch, accomplished by TLR-mediated induction of IFMs which themselves alter the excitability of peripheral nociceptors. In addition, TLR signaling in peripheral sensory neurons, including nociceptors (TLR3, TLR4, TLR7) and mechanoreceptors (TLR5), can directly modulate neuronal excitability through a unique, non-canonical, and transcription-independent mechanism involving coupling with ion channels. The plethora of research focused on how TLR signaling controls sensory neuron activity has led to a deeper understanding of the intricate coupling between sensory neurons and immune cells and the inextricable link between pain and inflammation. While less extensive research exists regarding the role of TLRs in itch, there has nevertheless been significant findings to further understand how TLRs affect acute and chronic itch conditions. Understanding how exogenous PAMPs or endogenous DAMPs signal via TLRs via canonical and non-canonical mechanisms has also altered our conceptual understanding of the role of peripheral sensory neurons as detectors of danger signals, expanding the repertoire beyond just the detection of physical and thermal stimuli. This has opened the door to an emerging, but at present undeveloped field of research focused on interactions between neurons and microorganisms. Thus, from the discovery of Toll in drosophila to recent studies investigating the role of TLRs beyond immunity and in nociception, there have been important advancements made toward a deeper understanding of TLRs in neuro–immune interactions.

References

Ahn H, Kim JM, Lee K, Kim H, Jeong D. Extracellular acidosis accelerates bone resorption by enhancing osteoclast survival, adhesion, and migration. Biochem Biophys Res Commun. (1090–2104 (Electronic)). 2012;418:144–8.

Akira S, Hoshino K. Myeloid differentiation factor 88—dependent and—independent pathways in toll-like receptor signaling. J Infect Dis. 2003;187(Supplement_2):S356–63.

Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by toll-like receptor 3. Nature. 2001;413(6857):732–8.

Anderson KV, Bokla L, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the drosophila embryo: the induction of polarity by the toll gene product. Cell. 1985a;42(3):791–8.

Anderson KV, Jürgens G, Nüsslein-Volhard C. Establishment of dorsal-ventral polarity in the drosophila embryo: genetic studies on the role of the toll gene product. Cell. 1985b;42(3):779–89.

Aravalli RN, Peterson PK, Lokensgard JR. Toll-like receptors in defense and damage of the central nervous system. J Neuroimmune Pharmacol. 2007;2(4):297–312.

Augustin R, Schröder K, Murillo Rincón AP, Fraune S, Anton-Erxleben F, Herbst E-M, Wittlieb J, Schwentner M, Grötzinger J, Wassenaar TM, et al. A secreted antibacterial neuropeptide shapes the microbiome of hydra. Nat Commun. 2017;8(1):698.

Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol. 1994;12(1):141–79.

Barajon I, Serrao G, Arnaboldi F, Opizzi E, Ripamonti G, Balsari A, Rumio C. Toll-like receptors 3, 4, and 7 are expressed in the enteric nervous system and dorsal root ganglia. J Histochem Cytochem. 2009;57(11):1013–23.

Barragán-Iglesias P, Franco-Enzástiga Ú, Jeevakumar V, Shiers S, Wangzhou A, Granados-Soto V, Campbell ZT, Dussor G, Price TJ. Type I interferons act directly on nociceptors to produce pain sensitization: implications for viral infection-induced pain. J Neurosci. 2020;40(18):3517–32.

Bautista DM, Jordt S-E, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell. 2006;124(6):1269–82.

Belvin MP, Anderson KV. A conserved signaling pathway: the drosophila toll-dorsal pathway. Annu Rev Cell Dev Biol. 1996;12(1):393–416.

Bettoni I, Comelli F, Rossini C, Granucci F, Giagnoni G, Peri F, Costa B. Glial TLR4 receptor as new target to treat neuropathic pain: efficacy of a new receptor antagonist in a model of peripheral nerve injury in mice. Glia. 2008;56(12):1312–9.

Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. Nat Rev Immunol. 2003;3(2):169–76.

Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, et al. Toll-like receptor 4-dependent activation of dendritic cells by β-defensin 2. Science. 2002;298(5595):1025.

Botos I, Segal DM, Davies DR. The structural biology of toll-like receptors. Structure. 2011;19(4):447–59.

Bruno K, Woller SA, Miller YI, Yaksh TL, Wallace M, Beaton G, Chakravarthy K. Targeting toll-like receptor-4 (TLR4)-an emerging therapeutic target for persistent pain states. Pain. 2018;159(10):1908–15.

Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol. 2002;61(11):1013–21.

Burnet FM. A modification of Jerne's theory of antibody production using the concept of clonal selection. CA Cancer J Clin. 1976;26(2):119–21.

Campbell JN, Raja SN, Meyer RA, Mackinnon SE. Myelinated afferents signal the hyperalgesia associated with nerve injury. Pain. 1988;32(1):89–94.

Cao X, Aballay A. Neural inhibition of dopaminergic signaling enhances immunity in a cell-non-autonomous manner. Curr Biol. 2016;26(17):2329–34.

Carpentier PA, Begolka WS, Olson JK, Elhofy A, Karpus WJ, Miller SD. Differential activation of astrocytes by innate and adaptive immune stimuli. Glia. 2005;49(3):360–74.
Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 2000;288(5464):306.
Chang C, Liu H-K, Yeh C-B, Yang M-L, Liao W-C, Liu C-H, Tseng T-J. Cross-talk of toll-like receptor 5 and mu-opioid receptor attenuates chronic constriction injury-induced mechanical hyperalgesia through a protein kinase C alpha-dependent signaling. Int J Mol Sci. 2021;22(4):1891.
Chen W, Lu Z. Upregulated TLR3 promotes neuropathic pain by regulating autophagy in rat with l5 spinal nerve ligation model. Neurochem Res. 2017;42(2):634–43.
Chen G, Zhang Y-Q, Qadri YJ, Serhan CN, Ji R-R. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100(6):1292–311.
Chen O, Donnelly CR, Ji R-R. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol. 2020;62:17–25.
Chen Y, Wang Z-L, Yeo M, Zhang Q-J, López-Romero AE, Ding H-P, Zhang X, Zeng Q, Morales-Lázaro SL, Moore C, et al. Epithelia-sensory neuron cross talk underlies cholestatic itch induced by lysophosphatidylcholine. Gastroenterology. 2021;161(1):301–17.e316.
Chiu IM. Infection, pain, and itch. Neurosci Bull. 2018;34(1):109–19.
Clark AK, Staniland AA, Marchand F, Kaan TKY, McMahon SB, Malcangio M. P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci. 2010;30(2):573–82.
Cristina Lebre M, Antons JC, Kalinski P, Schuitemaker JHN, van Capel TMM, Kapsenberg ML, de Jong EC. Double-stranded RNA-exposed human keratinocytes promote Th1 responses by inducing a type-1 polarized phenotype in dendritic cells: Role of keratinocyte-derived tumor necrosis factor α, type I interferons, and interleukin-18. J Investig Dermatol. 2003;120(6):990–7.
Diebold Sandra S, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 2004;303(5663):1529–31.
Diogenes A, Ferraz CC, Akopian AN, Henry MA, Hargreaves KM. LPS sensitizes TRPV1 via activation of TLR4 in trigeminal sensory neurons. J Dent Res. 2011;60:759–64. (1544-0591 (Electronic))
Dong X, Dong X. Peripheral and central mechanisms of itch. Neuron. 2018;98(3):482–94.
Donnelly CR, Chen O, Ji RR. How do sensory neurons sense danger signals? Trends in Neuroscience. 2020;43(10):822–38. (1878-108X (Electronic))
Emmerich CH, Ordureau A, Strickson S, Arthur JSC, Pedrioli PGA, Komander D, Cohen P. Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. Proc Natl Acad Sci U S A. 2013;110(38):15247–52.
Everts B, Amiel E, Huang SC-C, Smith AM, Chang C-H, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJW, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. Nat Immunol. 2014;15(4):323–32.
Ewald SE, Engel A, Lee J, Wang M, Bogyo M, Barton GM. Nucleic acid recognition by toll-like receptors is coupled to stepwise processing by cathepsins and asparagine endopeptidase. J Exp Med. 2011;208(4):643–51.
Feng J, Luo J, Mack MR, Yang P, Zhang F, Wang G, Gong X, Cai T, Mei Z, Kim BS, et al. The antimicrobial peptide human beta-defensin 2 promotes itch through toll-like receptor 4 signaling in mice. J Allergy Clin Immunol. 2017;140(3):885–8.e886.
Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. Cell. 2020;180(6):1044–66.
Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao S-M, Maniatis T. IKKε and TBK1 are essential components of the IRF3 signaling pathway. Nat Immunol. 2003a;4(5):491–6.
Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT. LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. J Exp Med. 2003b;198(7):1043–55.

Fraune S, Bosch TCG. Long-term maintenance of species-specific bacterial microbiota in the basal metazoan Hydra. Proc Natl Acad Sci. 2007;104(32):13146.

Fukui R, Yamamoto C, Matsumoto F, Onji M, Shibata T, Murakami Y, Kanno A, Hayashi T, Tanimura N, Yoshida N, et al. Cleavage of toll-like receptor 9 ectodomain is required for in vivo responses to single strand DNA. Front Immunol. 2018;9:1491.

Gao W, Xiong Y, Li Q, Yang H. Inhibition of toll-like receptor signaling as a promising therapy for inflammatory diseases: a journey from molecular to nano therapeutics. Front Physiol. 2017;8:508.

Gay NJ, Keith FJ. Drosophila toll and IL-1 receptor. Nature. 1991;351(6325):355–6.

Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of toll-like receptor signalling complexes. Nat Rev Immunol. 2014;14(8):546–58.

Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J Immunol. 2001;167(4):1882.

Goethals S, Ydens E, Timmerman V, Janssens S. Toll-like receptor expression in the peripheral nerve. Glia. 2010;58(14):1701–9. (1098-1136 (Electronic))

Gorina R, Font-Nieves M, Márquez-Kisinousky L, Santalucia T, Planas AM. Astrocyte TLR4 activation induces a proinflammatory environment through the interplay between MyD88-dependent NFκB signaling, MAPK, and Jak1/Stat1 pathways. Glia. 2011;59(2):242–55.

Harvey Lodish AB, Lawrence Zipursky S, Matsudaira P, Baltimore D, Darnell J. Molecular cell biology. 4th ed. New York: W.H. Freeman; 2000.

Hasel P, Rose IVL, Sadick JS, Kim RD, Liddelow SA. Neuroinflammatory astrocyte subtypes in the mouse brain. Nat Neurosci. 2021;24:1475–87.

Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A. The innate immune response to bacterial flagellin is mediated by toll-like receptor 5. Nature. 2001;410:1099–103. (0028-0836 (Print))

Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, et al. A toll-like receptor recognizes bacterial DNA. Nature. 2000;408(6813):740–5.

Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol. 2002;3(2):196–200.

Hodgkin PD, Heath WR, Baxter AG. The clonal selection theory: 50 years since the revolution. Nat Immunol. 2007;8(10):1019–26.

Huang J, Gandini MA, Chen L, M'Dahoma S, Stemkowski PL, Chung H, Muruve DA, Zamponi GW. Hyperactivity of innate immunity triggers pain via TLR2-IL-33-mediated neuroimmune crosstalk. Cell Rep. 2020;33(1):108233.

Ifuku M, Hinkelmann L, Kuhrt LD, Efe IE, Kumbol V, Buonfiglioli A, Krüger C, Jordan P, Fulde M, Noda M, et al. Activation of toll-like receptor 5 in microglia modulates their function and triggers neuronal injury. Acta Neuropathol Commun. 2020;8(1):159.

Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, González-Crespo S, Tatei K, Levine M. Dif, a dorsal-related gene that mediates an immune response in drosophila. Cell. 1993;75(4):753–63.

Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, Ausubel FM. Distinct pathogenesis and host responses during infection of C. elegans by p. aeruginosa and s. aureus. PLOS Pathog. 2010a;6(7):e1000982.

Irazoqui JE, Urbach JM, Ausubel FM. Evolution of host innate defence: insights from caenorhabditis elegans and primitive invertebrates. Nat Rev Immunol. 2010b;10(1):47–58.

Janeway CA. Pillars article: approaching the asymptote? Evolution and revolution in immunology. Cold spring harb symp quant biol. J Immunol. 2013;191(9):4475.

Janeway CA Jr. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol. 1989;54(Pt 1):1–13. (0091-7451 (Print))

Jansen PAM, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jongh GJ, van Vlijmen-Willems IMJJ, Bergboer JGM, van Rossum MM, de Jong EMGJ, et al. Beta-defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS One. 2009;4(3):e4725.

Ji R-R. Neuroimmune interactions in itch: do chronic itch, chronic pain, and chronic cough share similar mechanisms? Pulm Pharmacol Ther. 2015;35:81–6.
Ji R-R, Donnelly CR, Nedergaard M. Astrocytes in chronic pain and itch. Nat Rev Neurosci. 2019;20(11):667–85.
Jin MS, Lee J-O. Structures of the toll-like receptor family and its ligand complexes. Immunity. 2008;29(2):182–91.
Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik S-G, Lee H, Lee J-O. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007;130(6):1071–82.
Kanno A, Tanimura N, Ishizaki M, Ohko K, Motoi Y, Onji M, Fukui R, Shimozato T, Yamamoto K, Shibata T, et al. Targeting cell surface TLR7 for therapeutic intervention in autoimmune diseases. Nat Commun. 2015;6(1):6119.
Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for toll-like receptor 3. J Biol Chem. 2004;279(13):12542–50.
Kawai T, Takeuchi O, Fujita T, Inoue J-i, Mühlradt PF, Sato S, Hoshino K, Akira S. Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol. 2001;167(10):5887.
Kelly MG, Alvero AB, Chen R, Silasi D-A, Abrahams VM, Chan S, Visintin I, Rutherford T, Mor G. Tlr-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res. 2006;66(7):3859.
Kido-Nakahara M, Buddenkotte J, Kempkes C, Ikoma A, Cevikbas F, Akiyama T, Nunes F, Seeliger S, Hasdemir B, Mess C, et al. Neural peptidase endothelin-converting enzyme 1 regulates endothelin 1-induced pruritus. J Clin Invest. 2014;124(6):2683–95.
Kieser KJ, Kagan JC. Multi-receptor detection of individual bacterial products by the innate immune system. Nat Rev Immunol. 2017;17(6):376–90.
Kim D, Kim MA, Cho I-H, Kim MS, Lee S, Jo E-K, Choi S-Y, Park K, Kim JS, Akira S, et al. A critical role of toll-like receptor 2 in nerve injury-induced spinal cord glial cell activation and pain hypersensitivity. J Biol Chem. 2007;282(20):14975–83.
Kim D, You B, Lim H, Lee SJ. Toll-like receptor 2 contributes to chemokine gene expression and macrophage infiltration in the dorsal root ganglia after peripheral nerve injury. Mol Pain. 2011;7:74.
Klimovich A, Giacomello S, Björklund Å, Faure L, Kaucka M, Giez C, Murillo-Rincon AP, Matt A-S, Willoweit-Ohl D, Crupi G, et al. Prototypical pacemaker neurons interact with the resident microbiota. Proc Natl Acad Sci. 2020;117(30):17854.
Kremer AE, Mettang T, Weisshaar E. Non-dermatological challenges of chronic itch. Acta Derm Venereol. 2020;100:adv00025. (1651-2057 (Electronic))
Kupari J, Usoskin D, Parisien M, Lou D, Hu Y, Fatt M, Lönnerberg P, Spångberg M, Eriksson B, Barkas N, et al. Single cell transcriptomics of primate sensory neurons identifies cell types associated with chronic pain. Nat Commun. 2021;12(1):1510.
Kurban MS, Boueiz A, Kibbi A-G. Cutaneous manifestations of chronic kidney disease. Clin Dermatol. 2008;26(3):255–64.
Lacagnina MJ, Watkins LR, Grace PM. Toll-like receptors and their role in persistent pain. Pharmacol Ther. 2018;184:145–58.
Lafon M, Megret F, Lafage M, Prehaud C. The innate immune facet of brain. J Mol Neurosci. 2006;29(3):185–94.
Latz E, Verma A, Visintin A, Gong M, Sirois CM, Klein DCG, Monks BG, McKnight CJ, Lamphier MS, Duprex WP, et al. Ligand-induced conformational changes allosterically activate toll-like receptor 9. Nat Immunol. 2007;8(7):772–9.
Lebre MC, van der Aar AMG, van Baarsen L, van Capel TMM, Schuitemaker JHN, Kapsenberg ML, de Jong EC. Human keratinocytes express functional toll-like receptor 3, 4, 5, and 9. J Investig Dermatol. 2007;127(2):331–41.
Li Y, Zhang H, Zhang H, Kosturakis AK, Jawad AB, Dougherty PM. Toll-like receptor 4 signaling contributes to paclitaxel-induced peripheral neuropathy. J Pain. 2014;15(7):712–25.

Liu Q, Ding JL. The molecular mechanisms of TLR-signaling cooperation in cytokine regulation. Immunol Cell Biol. 2016;94(6):538–42.
Liu B, Yang Y, Qiu Z, Staron M, Hong F, Li Y, Wu S, Li Y, Hao B, Bona R, et al. Folding of toll-like receptors by the HSP90 paralogue gp96 requires a substrate-specific cochaperone. Nat Commun. 2010a;1(1):79.
Liu T, Xu Z-Z, Park C-K, Berta T, Ji R-R. Toll-like receptor 7 mediates pruritus. Nat Neurosci. 2010b;13(12):1460–2.
Liu T, Berta T, Xu ZZ, Park CK, Zhang L, Lü N, Liu Q, Liu Y, Gao YJ, Liu YC, et al. TLR3 deficiency impairs spinal cord synaptic transmission, central sensitization, and pruritus in mice. J Clin Investig. 2012a;122(6):2195–207.
Liu T, Gao YJ, Ji R-R. Emerging role of toll-like receptors in the control of pain and itch. Neurosci Bull. 2012b;28(2):131–44. (1995-8218 (Electronic))
Liu X-J, Zhang Y, Liu T, Xu Z-Z, Park C-K, Berta T, Jiang D, Ji R-R. Nociceptive neurons regulate innate and adaptive immunity and neuropathic pain through myd88 adapter. Cell Res. 2014;24(11):1374–7.
Liu T, Han Q, Chen G, Huang Y, Zhao L-X, Berta T, Gao Y-J, Ji R-R. Toll-like receptor 4 contributes to chronic itch, alloknesis, and spinal astrocyte activation in male mice. Pain. 2016;157(4):806–17.
Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023.
Luo X, Huh Y, Bang S, He Q, Zhang L, Matsuda M, Ji R-R. Macrophage toll-like receptor 9 contributes to chemotherapy-induced neuropathic pain in male mice. J Neurosci. 2019;39(35):6848–64.
Ma Y, Haynes RL, Sidman RL, Vartanian T. TLR8: an innate immune receptor in brain, neurons and axons. Cell Cycle. 2007;6(23):2859–68.
Madan V, Lear JT, Szeimies R-M. Non-melanoma skin cancer. Lancet. 2010;375(9715):673–85.
Materazzi S, Fusi C, Benemei S, Pedretti P, Patacchini R, Nilius B, Prenen J, Creminon C, Geppetti P, Nassini R. TRPA1 and TRPV4 mediate paclitaxel-induced peripheral neuropathy in mice via a glutathione-sensitive mechanism. Pflugers Arch. 2012;463(4):561–9.
McKimmie CS, Fazakerley JK. In response to pathogens, glial cells dynamically and differentially regulate toll-like receptor gene expression. J Neuroimmunol. 2005;169(1):116–25.
McWhirter SM, Fitzgerald KA, Rosains J, Rowe DC, Golenbock DT, Maniatis T. IFN-regulatory factor 3-dependent gene expression is defective in TBK1-deficient mouse embryonic fibroblasts. Proc Natl Acad Sci. 2004;101(1):233.
Medzhitov R, Preston-Hurlburt P, Janeway CA. A human homologue of the drosophila toll protein signals activation of adaptive immunity. Nature. 1997;388(6640):394–7.
Mielcarska MB, Bossowska-Nowicka M, Toka FN. Cell surface expression of endosomal toll-like receptors-A necessity or a superfluous duplication? Front Immunol. 2021;11:620972.
Miller LS. Toll-like receptors in skin. Adv Dermatol. 2008;24:71–87.
Min H, Cho W-H, Lee H, Choi B, Kim Y-J, Lee HK, Joo Y, Jung SJ, Choi S-Y, Lee S, et al. Association of TRPV1 and TLR4 through the TIR domain potentiates TRPV1 activity by blocking activation-induced desensitization. Mol Pain. 2018;14:1744806918812636.
Modlin RL. Innate immunity: ignored for decades, but not forgotten. J Invest Dermatol. 2012;132(3 Pt 2):882–6.
Mohammad Hosseini A, Majidi J, Baradaran B, Yousefi M. Toll-like receptors in the pathogenesis of autoimmune diseases. Adv Pharm Bull. 2015;5(Suppl 1):605–14.
Moore C, Gupta R, Jordt S-E, Chen Y, Liedtke WB. Regulation of pain and itch by TRP channels. Neurosci Bull. 2018;34(1):120–42.
Navarro L, David M. P38-dependent activation of interferon regulatory factor 3 by lipopolysaccharide. J Biol Chem. 1999;274(50):35535–8.
Nicotra L, Loram LC, Watkins LR, Hutchinson MR. Toll-like receptors in chronic pain. Exp Neurol. 2012;234(2):316–29.
Ohsawa M, Miyabe Y, Katsu H, Yamamoto S, Ono H. Identification of the sensory nerve fiber responsible for lysophosphatidic acid-induced allodynia in mice. Neuroscience. 2013;247:65–74.

Ohto U, Ishida H, Shibata T, Sato R, Miyake K, Shimizu T. Toll-like receptor 9 contains two DNA binding sites that function cooperatively to promote receptor dimerization and activation. Immunity. 2018;48(4):649–58.e644.

Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol. 2004;173(6):3916.

Ossipov MH, Zhang E-T, Carvajal C, Gardell L, Quirion R, Dumont Y, Lai J, Porreca F. Selective mediation of nerve injury-induced tactile hypersensitivity by neuropeptide y. J Neurosci. 2002;22(22):9858–67.

Park BS, Song DH, Kim HM, Choi B-S, Lee H, Lee J-O. The structural basis of lipopolysaccharide recognition by the TLR4–MD-2 complex. Nature. 2009;458(7242):1191–5.

Park C-K, Xu Z-Z, Berta T, Han Q, Chen G, Liu X-J, Ji R-R. Extracellular microRNAs activate nociceptor neurons to elicit pain via TLR7 and TRPA1. Neuron. 2014;82(1):47–54.

Parkin J, Cohen B. An overview of the immune system. Lancet. 2001;357(9270):1777–89.

Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. Nat Rev Drug Discov. 2009;8(1):55–68.

Pinho-Ribeiro FA, Verri WA Jr, Chiu IM. Nociceptor sensory neuron-immune interactions in pain and inflammation. Trends Immunol. 2017;38(1):5–19.

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan M-W, Ray KP, Solari R, Johnson CD, Ewbank JJ. A reverse genetic analysis of components of the toll signaling pathway in caenorhabditis elegans. Curr Biol. 2001;11(11):809–21.

Qi J, Buzas K, Fan H, Cohen JI, Wang K, Mont E, Klinman D, Oppenheim JJ, Howard OMZ. Painful pathways induced by TLR stimulation of dorsal root ganglion neurons. J Immunol. 2011;186(11):6417–26.

Randow F, Seed B. Endoplasmic reticulum chaperone gp96 is required for innate immunity but not cell viability. Nat Cell Biol. 2001;3(10):891–6.

Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010;16(11):1267–76.

Renthal W, Tochitsky I, Yang L, Cheng Y-C, Li E, Kawaguchi R, Geschwind DH, Woolf CJ. Transcriptional reprogramming of distinct peripheral sensory neuron subtypes after axonal injury. Neuron. 2020;108(1):128–44.e129.

Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to drosophila toll. Proc Natl Acad Sci U S A. 1998;95(2):588–93.

Saito O, Svensson CI, Buczynski MW, Wegner K, Hua XY, Codeluppi S, Schaloske RH, Deems RA, Dennis EA, Yaksh TL. Spinal glial TLR4-mediated nociception and production of prostaglandin E(2) and TNF. Br J Pharmacol. 2010;160(7):1754–64.

Scumpia PO, Kelly KM, Reeves WH, Stevens BR. Double-stranded RNA signals antiviral and inflammatory programs and dysfunctional glutamate transport in TLR3-expressing astrocytes. Glia. 2005;52(2):153–62.

Seong S-Y, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. Nat Rev Immunol. 2004;4(6):469–78.

Shen S, Lim G, You Z, Ding W, Huang P, Ran C, Doheny J, Caravan P, Tate S, Hu K, et al. Gut microbiota is critical for the induction of chemotherapy-induced pain. Nat Neurosci. 2017;20(9):1213–6.

Shi XQ, Zekki H, Zhang J. The role of TLR2 in nerve injury-induced neuropathic pain is essentially mediated through macrophages in peripheral inflammatory response. Glia. 2011;59(2):231–41.

Shimizu Y, Sonoda A, Nogi C, Ogushi Y, Kanda R, Yamaguchi S, Nohara N, Aoki T, Yamada K, Nakata J, et al. B-type (brain) natriuretic peptide and pruritus in hemodialysis patients. Int J Nephrol Renovasc Dis. 2014;7:329–35.

Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM, et al. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science. 1988;241(4865):585.

Steward R. Dorsal, an embryonic polarity gene in drosophila, is homologous to the vertebrate proto-oncogene, c-rel. Science. 1987;238(4827):692.
Su X-Y, Chen M, Yuan Y, Li Y, Guo S-S, Luo H-Q, Huang C, Sun W, Li Y, Zhu MX, et al. Central processing of itch in the midbrain reward center. Neuron. 2019;102(4):858–72.e855.
Szabo-Pardi TA, Barron LR, Lenert ME, Burton MD. Sensory neuron TLR4 mediates the development of nerve-injury induced mechanical hypersensitivity in female mice. Brain Behav Immun. 2021;97:42–60.
Szöllősi AG, McDonald I, Szabó IL, Meng J, van den Bogaard E, Steinhoff M. TLR3 in chronic human itch: a keratinocyte-associated mechanism of itch sensitization. J Investig Dermatol. 2019;139(11):2393–6.e2396.
Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805–20.
Tan Y, Kagan JC. Innate immune signaling organelles display natural and programmable signaling flexibility. Cell. 2019;177(2):384–98.e311.
Tan Y, Zanoni I, Cullen TW, Goodman AL, Kagan JC. Mechanisms of toll-like receptor 4 endocytosis reveal a common immune-evasion strategy used by pathogenic and commensal bacteria. Immunity. 2015;43(5):909–22.
Tanga FY, Nutile-McMenemy N, DeLeo JA. The CNS role of toll-like receptor 4 in innate neuroimmunity and painful neuropathy. Proc Natl Acad Sci U S A. 2005;102(16):5856–61.
Tanji H, Ohto U, Motoi Y, Shibata T, Miyake K, Shimizu T. Autoinhibition and relief mechanism by the proteolytic processing of toll-like receptor 8. Proc Natl Acad Sci. 2016;113(11):3012.
Tse KH, Chow KBS, Leung WK, Wong YH, Wise H. Primary sensory neurons regulate toll-like receptor-4-dependent activity of glial cells in dorsal root ganglia. Neuroscience. 2014;279:10–22.
Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, Hjerling-Leffler J, Haeggström J, Kharchenko O, Kharchenko PV, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci. 2015;18(1):145–53.
Wang C, Deng L, Hong M, Akkaraju GR, Inoue J-i, Chen ZJ. Tak1 is a ubiquitin-dependent kinase of MKK and IKK. Nature. 2001;412(6844):346–51.
Wang T-T, Xu X-Y, Lin W, Hu D-D, Shi W, Jia X, Wang H, Song N-J, Zhang Y-Q, Zhang L. Activation of different heterodimers of TLR2 distinctly mediates pain and itch. Neuroscience. 2020;429:245–55.
Wani KA, Goswamy D, Irazoqui JE. Nervous system control of intestinal host defense in C. elegans. Curr Opin Neurobiol. 2020;62:1–9.
Whitham S, McCormick S, Baker B. The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc Natl Acad Sci U S A. 1996;93(16):8776–81.
Wu F-x, Bian J-j, Miao X-r, Huang S-d, Xu X-w, Gong D-j, Sun Y-m, Lu Z-j, Yu W-f. Intrathecal siRNA against toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. Int J Med Sci. 2010;7(5):251–9.
Xu Z-Z, Kim YH, Bang S, Zhang Y, Berta T, Wang F, Oh SB, Ji R-R. Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. Nat Med. 2015;21(11):1326–31.
Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science. 2003a;301(5633):640.
Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O, Takeda K, Akira S. Tram is specifically involved in the toll-like receptor 4–mediated MyD88-independent signaling pathway. Nat Immunol. 2003b;4(11):1144–50.
Yang Z, Liu P, Bai L, Trimmer JS, Bean BP, Ginty DD. Deep sequencing of somatosensory neurons reveals molecular determinants of intrinsic physiological properties. Neuron. 2019;103(4):598–616.e597.
Zanoni I, Ostuni R, Marek Lorri R, Barresi S, Barbalat R, Barton Gregory M, Granucci F, Kagan Jonathan C. CD14 controls the LPS-induced endocytosis of toll-like receptor 4. Cell. 2011;147(4):868–80.

Zeisel A, Hochgerner H, Lönnerberg P, Johnsson A, Memic F, van der Zwan J, Häring M, Braun E, Borm LE, La Manno G, et al. Molecular architecture of the mouse nervous system. Cell. 2018;174(4):999–1014.e1022.

Zhang Z-J, Guo J-S, Li S-S, Wu X-B, Cao D-L, Jiang B-C, et al. TLR8 and its endogenous ligand miR-21 contribute to neuropathic pain in murine DRG. J Exp Med. 2018;215(12):3019–3037. https://doi.org/10.1084/jem.20180800. PMID 30455267.

Zhao L-X, Jiang M, Bai X-Q, Cao D-L, Wu X-B, Zhang J, et al. (2021). TLR8 in the trigeminal ganglion contributes to the maintenance of trigeminal neuropathic pain in mice. Neurosci Bull. 2021;37(4):550–562. https://doi.org/10.1007/s12264-020-00621-4. PMID 33355900.

Zuany-Amorim C, Hastewell J, Walker C. Toll-like receptors as potential therapeutic targets for multiple diseases. Nat Rev Drug Discov. 2002;1(10):797–807. https://doi.org/10.1038/nrd914. PPMID 12360257.

Chapter 9
Immunotherapy and Pain

Junli Zhao, Alexus Roberts, Yul Huh, and Ru-Rong Ji

Abstract Immunotherapy was initially developed as a method to treat cancer through the use of the host's immune system. Now, immunotherapy is used as a treatment for a wide variety of diseases. The connection between the nervous system and the immune system in chronic pain and neurological disease has given a new facet to immunotherapy research. This chapter provides an overview of the most common forms of immunotherapy and the emerging potential of immunotherapy in the treatment and management of various neurological diseases, including brain tumors, Alzheimer's disease, multiple sclerosis, stroke, spinal cord injury, and pain. We will particularly highlight pain-related immunotherapy mechanisms that target the programmed cell death protein 1 (PD-1) and stimulator of interferon gene (STING) pathways, as well as cytokine pathways, immune cell ablation, and adoptive cell transfer.

Keywords Adoptive cell transfer · Bone cancer pain · Cytokines · PD-1 · PD-L1 · Immune checkpoint inhibitor · Immune cell ablation · Immunotherapy · Macrophages · Osteoclast · type-I interferons

9.1 Introduction

Immunotherapy is often perceived to be a relatively recent medical advancement despite its historical background across several cultures. The prevalence of disease in human populations has been a problem across time and efforts to prevent various diseases emerged early in human history. One of the first recorded instances of a possible immunotherapy treatment occurred in the third century BC China, during the Qin dynasty, where inoculation with the variola minor virus prevented smallpox

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infection (Decker et al. 2017; Dobosz and Dzieciatkowski 2019). This chapter will cover modern advancements in immunotherapy, with a particular focus on cancer treatment research from the initial studies through to the discovery of cancer immunotherapy by James P. Allison and Tasuku Honjo, who were awarded the Nobel Prize in 2018 (Fig. 9.1).

"Immunotherapy" as a term encompasses a wide variety of methods and therapies, including cytokines, vaccines, oncolytic viruses, adoptive cell therapy, and antibody-based immunotherapy. Cytokines are small proteins produced and secreted by immune cells and are essential for immune cell signaling. Cytokines were initially recognized as systemic soluble factors that regulate lymphocyte function and inflammatory responses and were recognized as "non-specific immunotherapies" (Berraondo et al. 2019). The first cytokine type I interferon (IFN) was discovered in 1957 (Isaacs and Lindenmann 1957). The FDA approved the use of type I interferon

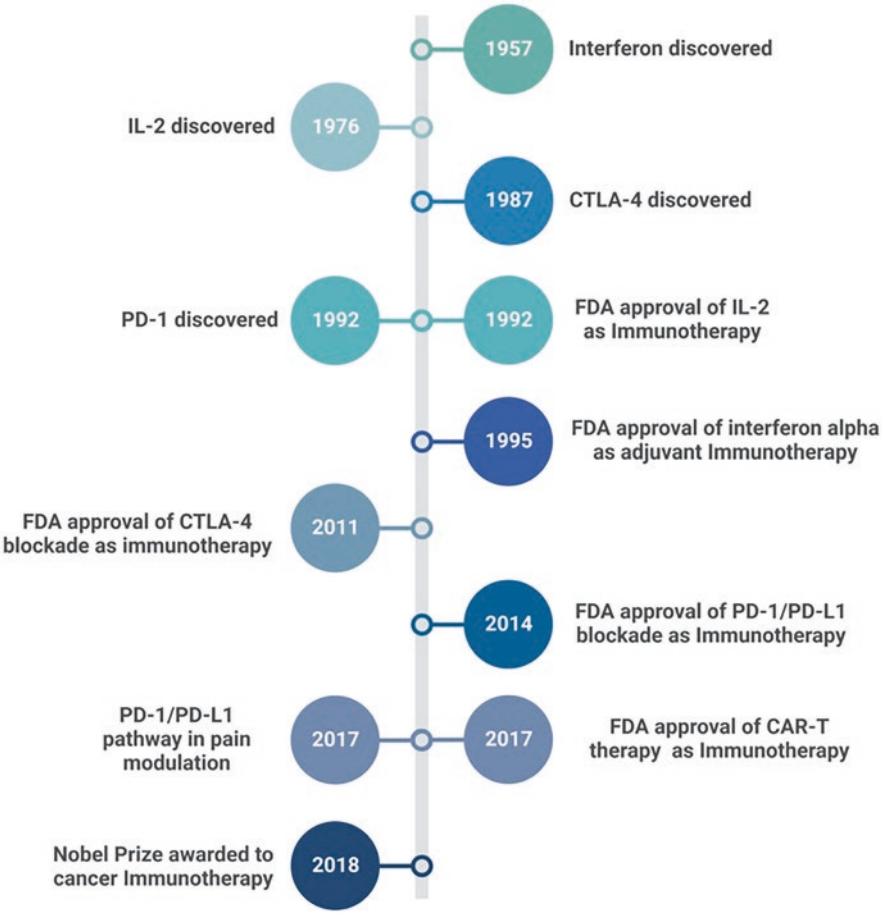

Fig. 9.1 Timeline for major events leading to the development of immunotherapy

as an adjuvant immunotherapy in 1995. The T-cell growth factor interleukin 2 (IL-2) was discovered in 1976. IL-2 is a key cytokine with pleiotropic effects on the immune system and is considered as an immunostimulatory cytokine (Morgan et al. 1976). The discovery of IL-2 revolutionized the fields of basic immunology, immunotherapy for human cancers since IL-2 was an early candidate for cancer immunotherapy, and FDA approved it for the treatment of metastatic renal cell carcinoma in 1992 and metastatic melanoma in 1998. In addition to these two cytokines, other pro-inflammatory cytokines including IL-1β and tumor necrosis factor α (TNFα) and anti-inflammatory cytokines such as IL-10 and IL-6 have also been used in immunotherapy.

Antibodies to immune checkpoint molecules have become the most promising form of immunotherapy for the treatment of cancer due to their low toxicity profile and the ease by which they can be prepared and administered to patients. The first immune checkpoint molecule cytotoxic T-lymphocyte antigen number 4 (CTLA-4) was discovered in 1987 (Brunet et al. 1987). The function of CTLA-4 as a crucial immune checkpoint and target for anticancer therapy was discovered in 1995, after which the first anti-CTLA-4 antibody was immediately developed and tested in animal models in 1996 (Krummel and Allison 1995; Leach et al. 1996). The first checkpoint inhibitor used in cancer patients was ipilimumab, which was approved by the FDA in 2011 for the advanced melanoma treatment. Another very notable immune checkpoint molecule is programmed cell death protein 1 (PD-1), which was discovered by Dr. Tasuku Honjo's group at Kyoto University in 1992 (Ishida et al. 1992). After many clinical trials, the FDA approved the first anti-PD-1 inhibitor, nivolumab, in 2014.

Even though immunotherapy is primarily known as a cancer treatment, it has significant potential for the treatment of a variety of additional diseases and conditions (Fig. 9.2). This potential has become the focus of immunotherapy research. The role of the immune system in chronic and neuropathic pain has been well-documented in the literature, but the implications of these findings are still being studied. Significant pain augmentation or reduction immunotherapy regimens have been demonstrated across numerous molecular mechanisms, including immune checkpoint blockade, adoptive immunotherapy, and various cytokines. These examples illustrate the breadth of research topics within immunotherapy, which is bound to expand further. Notably, many immunotherapy treatments have not been studied in the context of pain management. Thus, future research efforts hold great promise for the development of novel immunotherapy-based clinical treatments for chronic pain.

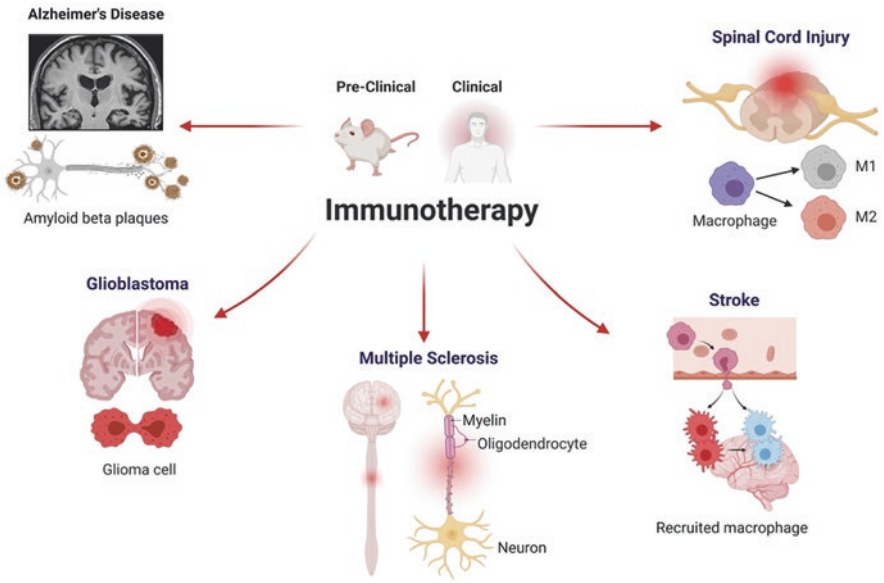

Fig. 9.2 Immunotherapy in CNS diseases. Immunotherapy is widely used in various CNS disorders in both animal models and patients, including Alzheimer's disease, glioblastoma, multiple sclerosis, stroke, and spinal cord injury

9.2 Immunotherapy for Neurological Disease

9.2.1 Immunotherapy in Brain Tumors and Brain Metastases

Gliomas are the most common type of primary brain tumors and are categorized based on their cell of origin, which can include astrocytes, oligodendrocytes, and ependymal cells (Gladson et al. 2010). Gliomas are further graded from grade I to IV based on the malignancy of the tumor as determined by WHO guidelines (Louis et al. 2016). Glioblastoma multiforme (GBM) has been a central focus of current research efforts due to its aggressive nature, high lethality, and the fact that it is the most common malignant brain tumor diagnosed in adults. GBM is a grade IV glioma that forms from astrocytes, which are a type of glial cell found in the central nervous system.

Recent research into treating GBM has focused primarily on immune checkpoint blockade, in which anti-PD-1 therapy induces a pro-inflammatory environment to increase the infiltration of immune cells into the tumor (Wang et al. 2021b). However, clinical studies have yet to show consistent and efficient results from treatment due to challenges posed by the nature of GBM tumors. The main complication of GBM treatment is the blood-brain barrier (BBB) which prevents the successful trafficking of anti-PD-1 drugs to the tumor site, and recent research has focused on methods to overcome the obstruction posed by the BBB through the use

of peptide shuttles (Cavaco et al. 2020). Additionally, the immunosuppressive microenvironment of GBM tumors poses another hurdle for anti-PD-1 therapy, which likely contributes to the inconsistent outcomes of clinical studies (Sampson et al. 2020).

Chimeric antigen receptor-T, or CAR-T, cell immunotherapy is a form of adoptive immunotherapy that takes T-cells from patients and modifies them to add the chimeric antigen receptor, which allows these engineered T-cells to bind to and attack cancer cells (Sterner and Sterner 2021). This form of immunotherapy has successfully treated blood cancers and is currently being explored as a potential treatment for GBM. Although clinical studies have shown promising results, complications remain when treating solid tumors, with particular concerns about T-cell trafficking, tumor infiltration, and the immunosuppressive microenvironment of GBM tumors.

9.2.2 Immunotherapy in Alzheimer's Disease

Alzheimer's disease (AD) is a common neurodegenerative disease primarily found in older individuals, and it is the most common cause of dementia worldwide. The clinical symptoms of AD include a decline in cognitive abilities in two or more areas, such as memory, language, and behavior. The cellular mechanisms of this disorder have been a central focus of research, with two mechanisms garnering the most interest, namely, the extracellular accumulation of β-amyloid (Aβ) plaques and the formation of neurofibrillary tangles, which are composed of hyperphosphorylated tau proteins (Weller and Budson 2018). The Aβ plaques have been studied extensively as a potential point of intervention for treatments under development.

The PD-1/PD-L1 pathway has been an area of interest in Alzheimer's research, though studies have shown mixed results. Alzheimer's patients have been observed to have lower expression of PD-1 on T-cells and lower expression of PD-L1 on monocytes and macrophages. These findings indicate the clinical significance of the PD-1/PD-L1 pathway in the development of Alzheimer's disease (Saresella et al. 2012).

The efficacy of anti-PD-1 treatments in the reduction of AD symptoms and pathology has been well demonstrated with the mechanisms of action well-documented. In a study published by Rosenzweig et al. and supported by additional studies by Baruch et al., anti-PD-1 treatment was found to improve memory and led to increased Aβ plaque clearance (Baruch et al. 2016; Rosenzweig et al. 2019). PD-1/PD-L1 blockade boosts immune cell activity with the mobilization of monocyte-derived macrophages to the brain, which results in the reduction of Aβ plaque loads.

PD-1 signaling has also been associated with the production of IL-10, which has been found to inhibit inflammatory responses and reduce AD pathology in animal models (Guillot-Sestier et al. 2015; Koronyo-Hamaoui et al. 2009). This indicates that increasing PD-1 expression in AD patients may have a positive effect through re-establishing immune homeostasis.

9.2.3 Immunotherapy in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disease of the CNS that is characterized by lesions of the brain and spinal cord due to the degradation of myelin, which is the protective covering of nerves and particularly the axons. The physical symptoms of MS include neuropathic pain, bladder dysfunction, and fatigue, and patients experience episodes that worsen over time. MS is the most common cause of disability in young adults, and its incidence and prevalence are increasing (Dobson and Giovannoni 2019).

Research examining the expression of a wide variety of co-stimulatory molecules in MS patients found a significant increase in cells expressing PD-1 (Trabattoni et al. 2009), which indicated the potential for the treatment of MS through the PD-1/PD-L1 pathway. Animal studies found that anti-PD-1 treatment or deletion of the *Pdcd1* gene worsened MS pathology due to alterations of cytokine expression by T-cells (IFN-γ, IL-17) and B-cells (IL-10), suggesting the possibility of an MS treatment based on the enhancement of PD-1/PD-L1 signaling (Carter et al. 2007; Salama et al. 2003).

The use of interferon beta (IFN-β) to treat relapsing-remitting multiple sclerosis has been for over 20 years and remains an important treatment despite a lack of understanding of its mechanisms (Jakimovski et al. 2018). One mechanism discovered through clinical studies is the apparent suppression of certain pro-inflammatory cytokines, specifically interleukin-17 (IL-17) and interleukin-23 (IL-23) (Kurtuncu et al. 2012).

The STING (stimulator of interferon gene) agonist cGAMP was also reported as a therapy for MS. Studies showed that cGAMP mitigated MS by stimulating type I interferon dependent and independent immune-regulatory pathways. cGAMP induces IL-10 expression in both APCs and $CD4^+$ T-cells in a process that is ERK and CREB dependent. IL-10 induction by cGAMP is primarily IL-27 dependent, and cGAMP induction of IL-10 and IL-27 is crucial for protection against MS (Johnson et al. 2021). Meanwhile, splenic myeloid DCs may be another pivotal cell population that senses ingested DNA and cGAMP to generate robust tolerogenic responses to prevent MS progression in animal models (Lemos et al. 2020).

9.2.4 Immunotherapy in Stroke

Stroke is a severe neurological event characterized by a disruption in the flow of blood to the brain, which results in cell death and brain damage. The initial clinical symptoms of stroke include difficulty with speech and speech comprehension, paralysis, numbness, and loss of coordination. In the acute phase of stroke, there is a significant increase in neuroinflammation, which has certain beneficial effects but also significant detrimental effects.

PD-1 is expressed by activated microglia and macrophages after stroke. PD-1 knockout leads to larger brain infarcts and exacerbated neurological deficits. PD-1 expression by B-cells can lead to the inhibition of inflammatory responses of other immune effector cells. B-cells can also produce IL-10 and increase PD-1 expression by T-cells, providing neuroprotection against stroke (Bodhankar et al. 2013; Ren et al. 2011a; Ren et al. 2011b). T regulatory cells could lead to the inhibition of neutrophils through PD-1/PD-L1 signaling and protect the BBB by suppressing the expression of matrix metalloproteinase-9 (MMP-9). Thus, activation of the PD-1/PD-L1 pathway can serve a protective role during a stroke (Qin et al. 2019; Ren et al. 2011a).

Inflammatory processes that occur after the initial onset of the event worsen the negative impacts of a stroke. This suggests that the upregulation of anti-inflammatory cytokines could prove beneficial in decreasing long-term negative effects following a stroke. The connection between expression of anti-inflammatory cytokines and positive stroke outcomes has been the focus of several studies, in which upregulating specific interferons, especially IFN-β, has been shown to reduce neuroinflammation in the brain after stroke. Studies show that systemically administrated IFN-β could attenuate brain infarct progression after stroke (Veldhuis et al. 2003). Immune cells like mast cells, macrophages, and neutrophils from the circulation release the inflammatory cytokines after stroke, including IL-6, IL-4, IL-1β, IL-23p9, and TNFα, which lead to CNS inflammation and result in infarct formation in the brain. IFN-β treatment may suppress these overexpressed inflammatory cytokines and reduce brain infarcts caused by strokes (Inacio et al. 2015; Kuo et al. 2016). Meanwhile, microglia switch from a resting to a reactive state during a stroke, and this change of phenotype leads to the release of inflammatory cytokines. IFN-β treatment may inhibit microglia activation and protect the brain from inflammatory cytokines after stroke (Kuo et al. 2016).

9.2.5 Immunotherapy in Spinal Cord Injury

Spinal cord injury (SCI) is a debilitating condition where significant damage to the spinal cord occurs from a traumatic or nontraumatic injury. Traumatic SCI has a primary and secondary stage of injury. The primary stage includes the physical injury to the spinal cord, and the secondary stage occurs in response to the injury event of the primary stage. This secondary stage response is characterized by inflammation and inflammatory cascades (Zha et al. 2014). The inflammatory response is the stage of SCI that can be addressed and treated with immunotherapy treatment, with anti-PD-1 therapies and IFN-β among the most well researched.

It was found that PD-1 expression is significantly upregulated in chronic SCI, which then impairs T-cell cytokine production. The use of anti-PD-1 therapy could potentially ameliorate these changes and rescue T-cell function in the spinal cord (Zha et al. 2014). PD-1 expression also plays an important role in the modulation of macrophage and microglial phenotypes after SCI. There are two primary

polarization types: the M1 "classically activated" phenotype and the M2 "alternatively activated" phenotype. The M1 phenotype promotes neuroinflammation through an increase in pro-inflammatory cytokines while the M2 phenotype suppresses neuroinflammation and encourages axonal regeneration. Upregulation of PD-1 signaling promotes the M2 phenotype after SCI, which in turn leads to better disease outcomes (Yao et al. 2014).

IFN-β has been reported to have potential therapeutic effects in acute SCI. Administration of IFN-β after SCI reduces myeloperoxidase activity, lipid peroxidation, and expression of inflammatory cytokines (mainly IL-6), with improved motor recovery (Gok et al. 2007; Sandrow-Feinberg et al. 2010). Nishimura et al. engineered neural stem cells (NSC) to constitutively secrete large amounts of IFN-β within the spinal cord injury site (Nishimura et al. 2013). Animals treated with these IFN-β-secreting neural stem cells had a significant reduction in astrocyte proliferation and enhanced preservation of axons, ultimately resulting in improved motor performance after SCI. Thus, IFN-β is beneficial in reducing SCI-related tissue damage and injury.

9.3 PD-1-Based Immunotherapy and Pain

9.3.1 Immunotherapy Targeting PD-1/PD-L1 in Physiological and Pathological Pain

Immune checkpoint mechanisms and their blockade are one of the most well-studied immunotherapy treatments. The primary immune checkpoint explored in this chapter is the PD-1/PD-L1 axis, which consists of the cell surface receptor PD-1 and its corresponding ligand PD-L1. This pathway inhibits the function of T-cells, so the disruption or blockade of these pathways leads to a stronger immune response, which has been utilized extensively in cancer treatments. Recent research has demonstrated a strong link between PD-1/PD-L1 axis and acute and chronic pain (Zhao et al. 2021).

"No pain, no gain" is true with anti-PD-1 immunotherapy. PD-1/PD-L1 expression has been shown to inhibit nociceptive neuron excitability, producing significant analgesic effects in both mouse and human studies. Conversely, blocking of the PD-1/PD-L1 pathway activation induces spontaneous pain and hypersensitivity. PD-1/PD-L1 pathway suppresses neuronal excitability in both mouse and human DRG neurons through the modulation of sodium and potassium channels (Chen et al. 2017). Furthermore, PD-1/PD-L1 signaling potentiates the TREK2 potassium channel. These modifications of sodium and potassium channels are regulated by SHP-1, which is activated by PD-L1 in DRG neurons via phosphorylation. Thus, the PD-1/PD-L1 pathway produces antinociception through neuromodulation (Fig. 9.3).

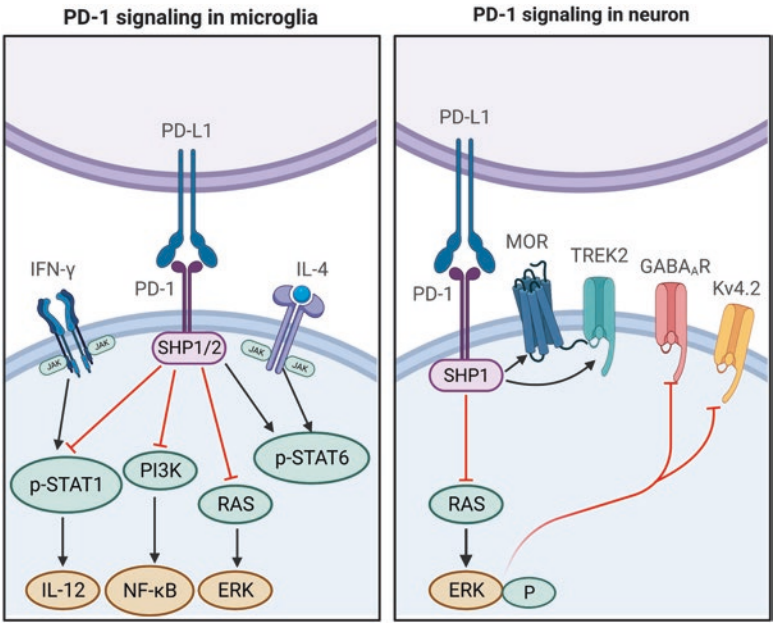

Fig. 9.3 PD-1 signaling in microglia and neurons. Black arrows indicate positive regulation and red lines with bars represent inhibitory regulation. Abbreviations: $GABA_AR$, gamma-aminobutyric acid A receptor; Kv4.2, potassium voltage-gated channel subfamily D member 2; MOR, mu-opioid receptor; PD-1, programmed cell death protein 1; PD-L1, PD-1 ligand; RAS, a small GTPase encoding RAS (retrovirus-associated DNA sequences); SHP, Src homology 2 domain-containing protein tyrosine phosphatases; TREK2, TWIK-related potassium channel-2

9.3.2 Immunotherapy Targeting PD-1 in Melanoma

Immunotherapies targeting PD-1 have revolutionized the clinical treatment of melanoma (Carlino et al. 2021). Given the important role of PD-1/PD-L1 signaling in melanoma, Chen et al. examined the contribution of PD-1/PD-L1 signaling to pain sensitivity in a mouse model of melanoma (Chen et al. 2017). The authors used several approaches, including pharmacological (soluble PD-1), genetic therapy (PD-1 siRNA), and anti-PD-1 antibody (nivolumab) treatments as means to block the PD-1/PD-L1 pathway in a melanoma animal model. The treatments elicited marked spontaneous pain and mechanical allodynia. *In vivo* recordings of mouse sciatic nerve showed that nivolumab treatment significantly increased spontaneous firing of nerve fibers, indicating that anti-PD-1 treatment can unmask pain by increasing the excitability of primary afferent fibers. Even though PD-1 blockade increased pain sensitivity in melanoma-bearing mice, the increased levels of mRNAs encoding T-cell markers (CD2, CD3), a macrophage marker (CD68), and inflammatory cytokine markers (TNF, IL-1β, IL-6, IFN-γ, CCL2) did not change, indicating that blocking PD-1/PD-L1 signaling induces pain via nonimmune modulation in the acute phase (first 3 hours).

9.3.3 Immunotherapy Targeting PD-1 in Morphine Analgesia

PD-1 co-localizes with opioid receptors in DRG sensory neurons in mouse and human DRG tissues. PD-1 receptor and opioid receptor interaction modulates the function of opioid receptors in DRG sensory neurons (Wang et al. 2020b). PD-1 knockout or blockade by anti-PD-1 antibody could impair morphine-mediated analgesia in both rodents and nonhuman primates. Morphine could produce antinociception via suppression of calcium currents in DRG neurons and synaptic transmission in the spinal cord, which could be reversed by PD-1 knockout or blockade. Additionally, PD-1 deficiency enhances opioid-induced hyperalgesia, tolerance, and long-term potentiation in the spinal cord.

9.3.4 Immunotherapy Targeting PD-1 in Bone Cancer Pain

Bone cancer pain usually results from tumor metastases reaching the bone from late-stage metastatic cancers, which form osteolytic bone lesions and fractures. The evidence of potent effects of anti-PD-1 immunotherapy in reducing metastatic tumors led Wang et al. to investigate whether anti-PD-1 blockade can reduce primary or metastatic bone cancer pain. They used a bone cancer pain animal model that received an inoculation of Lewis lung cancer cells (LLC) into the intramedullary canal of the femur (Wang et al. 2020a). PD-1 knockout mice or WT mice undergoing a PD-1 blockade by repeated administration of anti-PD-1 antibody (nivolumab) exhibited remarkable protection against bone destruction in the mentioned pain model. Mechanistically, PD-L1 promoted RANKL-induced osteoclastogenesis through JNK activation and chemokine C-C motif ligand 2 (CCL2) secretion. Moreover, PD-L1 can also activate SHP-1 to downregulate TRPV1 in DRG neurons and delay the development of bone cancer pain in mice. Thus, immunotherapy targeting PD-1/PD-L1 signaling could produce long-term benefits by preserving the bone structure and alleviating bone cancer pain through the suppression of osteoclastogenesis (Fig. 9.4).

Notably, anti-PD-1 treatment with nivolumab initially (early phase) causes an increase in bone cancer pain due to neuronal modulation in the animal model (Wang et al. 2020a). In contrast, nivolumab reduced bone cancer pain in the late phase of treatment through modulation of osteoclasts and protection from bone destruction. While the PD-1/PD-L1 pathway produces acute antinociception through neuromodulation, the delayed effects of this pathway may also depend on immunomodulation (Wang et al. 2020a). Thus, anti-PD-1 treatment's initial increase of cancer pain before the later phase reduction of cancer pain is a result of both neuromodulation and immunomodulation.

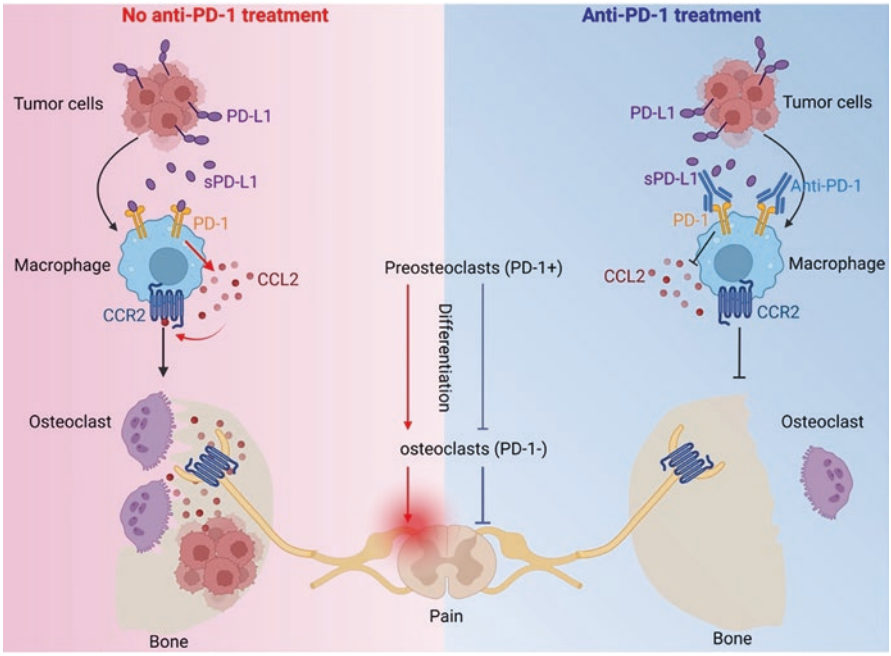

Fig. 9.4 Neuroimmune interactions mediated by the PD-L1 and PD-1 axis in bone cancer pain. Activation of this immune checkpoint pathway results in osteoclast differentiation, bone destruction, and bone cancer pain. Immunotherapy with anti-PD-1 treatment (nivolumab) can inhibit the differentiation of preosteoclasts into osteoclasts, thus protecting against bone destruction and cancer pain

9.4 Interferon-Based Immunotherapy and Pain

9.4.1 Immunotherapy Targeting IFN-β in Pain

Increasing evidence suggests that type-I interferons (IFNs) regulate pain via neuroimmune and neuro-glial interactions (Tan et al. 2021). Intrathecal injection of IFN-β increases paw withdrawal threshold in naïve mice and provides analgesia in inflammatory and neuropathic pain animal models. IFN-β treatment relieves mechanical allodynia induced by intrathecal injection of TLR2 or TLR4 ligands (Stokes et al. 2013). A single intrathecal IFN-β administration attenuates nerve injury-induced mechanical allodynia for several days in mice, which may be mediated by an inhibition of MAPK activation and the induction of interferon-stimulated gene 15 (ISG-15) (Liu et al. 2020). Intrathecal injection of IFN-β also showed a significant transient dose-dependent inhibition of CFA-induced inflammatory pain and this analgesic effect is reversed by intrathecal naloxone, suggesting that IFN-β analgesia occurs through central opioid receptor-mediated signaling (Liu et al. 2021).

9.4.2 Immunotherapy Targeting STING in Physiological and Neuropathic Pain

STING, as an innate immune regulator, is a critical sensor of self and pathogen-derived DNA. DNA sensed by STING leads to the induction of type-I interferons and other cytokines, which promote immune cell-mediated eradication of pathogens and neoplastic cells (Ishikawa and Barber 2008; Woo et al. 2014). In addition to the important role of STING in the immune system, STING has been shown to be a critical regulator of nociception through IFN-I signaling in DRG sensory neurons. Donnelly et al. demonstrated that administration of synthetic (DMXAA and ADU-S100) or biological (cGAMP) STING agonists can increase mechanical pain thresholds in both naïve and neuropathic pain model mice. More importantly, STING agonists produce analgesia in nonhuman primates. In contrast, mice lacking STING, including global (*Sting1*$^{gt/gt}$) and sensory neuron-selective (*Sting1*$^{fl/fl}$; *Nav1.8-Cre*) knockout mice, showed enhanced pain hypersensitivity and nociceptor excitability (Donnelly et al. 2021).

Activation of STING drives the production of IFN-I family members IFN-α and IFN-β. Mice lacking IFNAR1, including global (*Ifnar1*$^{gt/gt}$) and sensory neuron-selective (*Ifnar1*$^{fl/fl}$; *Nav1.8-Cre*) knockout mice, exhibited robust hypersensitivity to mechanical and cold stimuli as well as nociceptive activity. Thus, STING signaling with IFN-I serves as a critical regulator of physiological nociception and a promising target for treating neuropathic pain (Donnelly et al. 2021).

Another study showed that the spinal cord microglial STING/TBK1/NF-κB pathway contributes to pain initiation via IL-6 signaling. Pharmacological blockade of STING with the antagonist C-176 may be a promising target in preventing or limiting the initiation of neuropathic pain (Sun et al. 2022).

9.4.3 Immunotherapy Targeting STING in Bone Cancer Pain

STING is a robust driver of anti-tumor immunity, which has led to the development of STING activators and small-molecule agonists as adjuvants for cancer immunotherapy (Kwon and Bakhoum 2020). Activation of the STING pathway by the synthetic agonists DMXAA or ADU-S100 potently enhances anti-tumor immunity and promotes bone formation in a murine bone autoimmune disease model (Baum et al. 2017; Kwon et al. 2019). In addition, STING agonists can also serve as a therapeutic strategy for metastatic bone cancer pain and its comorbidities, including tumor-induced bone destruction and functional impairment. Wang et al. demonstrated that STING-mediated IFN-I signaling has direct effects on bone cancer pain in several different animal models via direct suppression of nociceptive excitability (neuromodulation). In addition, the potent analgesic effects of STING agonists are likely due to a combination of their direct effects on DRG sensory neurons (neuromodulation) and suppression of tumor burden and bone destruction (immune modulation) (Wang et al. 2021a).

9.5 Immunotherapy Targeting Pro-Inflammatory Cytokines in Pain

Cytokines are the main signaling molecules of immune system activation and have been shown to facilitate and increase pain. Cytokines are grouped into either pro-inflammatory or anti-inflammatory. The pro-inflammatory cytokines, including TNFα, IL-1β, IL-6, and IL-17, have been found to be elevated in pain animal models. Meanwhile, anti-inflammatory cytokines, including IL-4, IL-10, and TGF-β, are associated with a reduction in pain symptoms. Current studies have shown that anti-cytokine immunotherapies in the clinic were focused on pro-inflammatory cytokines and their receptors (Kalpachidou et al. 2022), and the following will serve as an introduction to these cytokines.

Tumor necrosis factor α (TNFα) was discovered in 1975 by Carswell et al. and the initial studies on TNFα primarily focused on its potential as a treatment for cancer (Carswell et al. 1975). However, anti-TNFα treatments have also been extensively explored in the context of neuropathic pain. Several studies have shown a correlation between neuropathic pain and TNFα upregulation (Leung and Cahill 2010; Wagner and Myers 1996). This correlation was also confirmed in humans through clinical studies in which elevated levels of TNFα were observed in patients with chronic neuropathic pain conditions (Alexander et al. 2005; Brisby et al. 2002). These findings suggest a clinical benefit to reducing the expression of TNFα, which was validated in animal models and clinical studies that found anti-TNFα treatment reduced neuropathic pain (Monaco et al. 2015). TNFα inhibitors, including infliximab, etanercept, adalimumab, certolizumab pegol, and golimumab, are currently FDA-approved for painful disorders such as inflammatory bowel disease, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis (Hung et al. 2017). With its safety profile and tolerability with long-term use, TNFα blockade may have significant value in the clinical treatment of chronic pain.

Interleukin 1β (IL-1β) is another pro-inflammatory cytokine that lowers pain thresholds, which has been explored as a potential target to reduce pain. The expression and upregulation of IL-1β have been extensively documented in various neuropathic pain conditions. Treatment with exogenous IL-1β has been shown to induce both mechanical and thermal hyperalgesia (Xiang et al. 2019; Zelenka et al. 2005). Treatment with anti-IL-1β antibody can reduce pain symptoms in animal models (Schafers et al. 2001). The mechanisms behind the hyperalgesia effects of IL-1β were studied in a variety of clinical studies and animal models. One important mechanism is the pro-inflammatory cascade, in which other pronociceptive mediators are significantly upregulated in response to IL-1β expression. IL-1β acts on both the peripheral and central nervous system, establishing the importance of IL-1β as a pro-inflammatory cytokine as well as an attractive target for anti-cytokine therapies developed to reduce neuropathic pain. Three IL-1-targeting agents have been approved for the treatment of inflammatory diseases: the IL-1 receptor antagonist anakinra for rheumatoid arthritis, the soluble decoy receptor rilonacept for cryopyrin-associated periodic syndromes, and the monoclonal anti-IL-1β antibody

canakinumab for systemic juvenile idiopathic arthritis (Alten et al. 2011; Botsios et al. 2007; Norheim et al. 2012). Several other drugs targeting IL-1β are in clinical trials. These anti-IL-1β treatments will need more studies with regard to pain conditions.

As a pro-inflammatory cytokine, IL-17 is upregulated in several animal pain models. IL-17 levels also increase with time during the development of chronic pain (Kim and Moalem-Taylor 2011; Meng et al. 2013; Noma et al. 2011). Exogenous IL-17 injection can induce neuropathic pain while anti-IL-17 antibody or IL-17 genetic knockout can decrease pain (Day et al. 2014). The mechanism underlying IL-17 involvement in neuropathic pain is related to the increased activity of transient receptor protein vanilloid 4 (TRPV4), an ion channel that has been found to mediate mechanical allodynia (Segond von Banchet et al. 2013). IL-17 also regulates neuron-glial interactions and synaptic transmission in neuropathic pain (Luo et al. 2019). The IL-23/IL-17A/TRPV1 axis plays an important role in neuropathic pain via macrophage-sensory neuron interactions (Luo et al. 2021). Currently, there are two IL-17A antibodies secukinumab and ixekizumab which are approved for the treatment of plaque psoriasis and also in clinical trials for other inflammatory diseases (Zwicky et al. 2020).

9.6 Immunotherapy to Ablate Immune Cell Populations in Pain

The immune system includes the innate immune system and the adaptive immune system. The innate immune system (T-cells, macrophages, neutrophils, microglia) and pro-inflammatory cytokines contribute to the pain transition from acute to chronic phase (Baral et al. 2019; Ji et al. 2016). Recent studies are pointing toward a potential role of immune cells in pain management.

T-cells are the main regulators of the immune response that contribute to the initiation and resolution of pain. T-cells can be divided into several subsets based on the pattern of cytokine production and specific expression of characteristic transcription factors (Zhu and Paul 2008). Thus, different subsets of T-cells may play different roles in pain. Many studies evaluated the contribution of T-cells to pain by comparing pain behaviors in WT mice and different types of T-cell deficiency mice, including *Rag1*$^{-/-}$, *Rag2*$^{-/-}$, nude, and SCID mice, as well as the mice reconstituted with specific populations of T-cells. At baseline, there is no significant difference in pain sensitivity between WT and T-cell-deficient mice (Cao and DeLeo 2008; Moalem et al. 2004; Vicuna et al. 2015). In different pain animal models, researchers did find varying contributions from different subsets of T-cells to the transition from acute to chronic pain. In neuropathic pain models, T-cells infiltrated the nervous system after nerve injury, mainly in DRGs, and contributed to pain hypersensitivity (Austin et al. 2012; Du et al. 2018). Ablation of T-cells in neuropathic pain models (CCI, SNI, and PSNL) reduced pain sensitivity compared with control mice (Costigan et al. 2009; Kleinschnitz et al. 2006; Kobayashi et al. 2015). The

development of pain hypersensitivity was completely prevented in *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mice in the SNI model and the reintroduction of T-cells was able to reverse the results, which indicate a detrimental role for T-cells in chronic pain induced by nerve injury (Cao and DeLeo 2008; Costigan et al. 2009; Vicuna et al. 2015). Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment and is often associated with pain (Krukowski et al. 2016). In the CIPN model induced by injection of paclitaxel or cisplatin, *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mice had similar pain hypersensitivity as WT mice but their resolution of pain was significantly delayed (Krukowski et al. 2016; Laumet et al. 2019). Reconstitution of CD8^{+} T-cells restored pain resolution in these T-cell-deficient CIPN model mice. In an inflammatory pain model induced by injection of Complete Freund's Adjuvant (CFA), the severity of mechanical allodynia was identical in WT and several different subsets of T-cell-deficient mice, including nude, *Tcrb*$^{-/-}$, *Tcrd*$^{-/-}$, *Rag1*$^{-/-}$, and *Rag2*$^{-/-}$ mice (Ghasemlou et al. 2015; Laumet et al. 2018; Petrovic et al. 2019; Sorge et al. 2015). However, pain resolution was significantly delayed in these T-cell-deficient mice, and reconstitution with wild-type T-cells was able to restore pain resolution (Basso et al. 2016). In summary, T-cells are evidently detrimental in nerve injury models and beneficial in CIPN and CFA models. This differentiation may be derived from the engagement of different T-cell subsets. Thus, immunotherapy targeting T-cells should select for different T-cell subsets in different pain conditions.

Crosstalk between microglia and infiltrating macrophages or tissue-resident macrophages with primary afferent neurons contributes to the induction and maintenance of different chronic pain conditions (Bang et al. 2018; Chen et al. 2018). Macrophage number is significantly increased after nerve injury, indicating pronociceptive neurons and macrophages interact during pain. The effects of macrophages on pain modulation were examined by pharmacological and genetic ablations of macrophages in neuropathic pain models. Studies showed that clodronate ablation of DRG macrophages reduced mechanical allodynia in a nerve injury model and CIPN model (Cobos et al. 2018; Old et al. 2014). CSF1R inhibitors have the ability to cross the BBB and deplete CNS microglia and macrophages. As a result, blocking CSF1R signaling effectively attenuates injury-triggered neuropathic pain behavior. In addition, transgenic mouse lines that express a drug-inducible suicide gene, for example, herpes simplex virus type 1 thymidine kinase (CD11b-TK) or diphtheria toxin receptor (CD11b-DTR, LysM-DTR, and Cx3cr1-DTR) in both microglia and macrophages, or *Cx3cr1* global knockout mice, can be used to ablate microglia and macrophages. Thus, the ablation of microglia and macrophages can attenuate the allodynia and pain hypersensitivity from neuropathic pain.

9.7 Adoptive Immunotherapy in Pain

This section will cover the adoptive transfer of macrophages and T-cells for resolution of inflammatory pain, neuropathic pain, and infection-induced pain. The role of bone marrow stromal (stem) cells in pain control will be covered in Chap. 10.

9.7.1 *Adoptive Transfer of Macrophages for the Resolution of Pain*

Even though the mechanisms of pain induction have been extensively studied, the mechanisms of pain resolution are still not fully understood. Macrophages have been reported as key players in the resolution of pain. One study pointed toward macrophages as key players in the resolution of pain whose activity is blunted during nerve injury based on data from single-cell RNA sequencing (Niehaus et al. 2021). They demonstrated that the macrophages from superficially injured animals had increased expression of the anti-inflammatory mediator CD163 and alleviated nociceptive hypersensitivity via the cytokine IL-10. The hypersensitivity could be reduced in nerve injury animal models by increasing macrophage CD163 expression.

GPR37 expressed in macrophages is also a critical contributor to the resolution of inflammatory pain and infection-induced pain. Bang et al. demonstrated that GPR37 knockout (*Gpr37*$^{-/-}$) mice exhibited deficits in macrophage phagocytic activity, dysregulation of pro- or anti-inflammatory cytokines, and delayed resolution of zymosan-induced inflammatory pain (Bang et al. 2018). Macrophage depletion delays the resolution of inflammatory pain while the adoptive transfer of macrophages from WT mice promotes the resolution of inflammatory pain. Neuroprotectin D1 (NPD1) and artesunate (ARU) act as ligands of GPR37. Activation of GPR37 by NPD1 and ARU promotes the resolution of inflammatory and bacterial infection-induced pain. In addition, the adoptive transfer of macrophages primed with GPR37 activators could confer protection against malaria and listeria infection (Bang et al. 2021; Bang et al. 2018). Thus, specific targeting of macrophage-bound GPR37 by NPD1 or ARU could help promote inflammatory pain resolution and alleviate other inflammation-related disorders (Fig. 9.5).

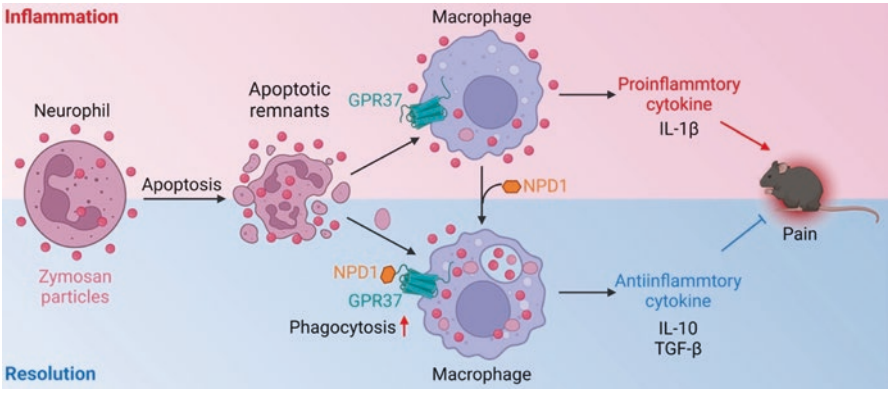

Fig. 9.5 GPR37 regulates macrophage phenotype and phagocytosis in the resolution of inflammatory pain. Neuroprotectin D1 (NPD1) treatment can increase the phagocytosis of macrophages and thus relieve pain

Furthermore, recent research efforts into inflammatory pain resolution point toward macrophage mitochondrial transfer as a major mechanism. Vlist et al. found that macrophages actively control the resolution of inflammatory pain outside of the site of inflammation by transferring mitochondria to sensory neurons (van der Vlist et al. 2022). During inflammatory pain resolution, M2-like macrophages infiltrate the DRG containing the somata of sensory neurons, correlating with the recovery of oxidative phosphorylation in sensory neurons. The resolution of pain by mitochondrial transfer requires the expression of the CD200 receptor (CD200R) on macrophages and the non-canonical CD200R-ligand iSec1 on sensory neurons (van der Vlist et al. 2022). Thus, the restoration of mitochondrial homeostasis in neurons and perhaps enhanced mitochondria transferred from macrophages could be a novel therapeutic strategy for chronic pain resolution.

9.7.2 Adoptive Transfer of T-Cells for the Resolution of Pain

Adoptive transfer of T-cells, either from the patient or from other sources, to treat various conditions is known as adoptive immunotherapy. Although this treatment was initially developed as a cancer treatment, it has been found to be useful in treating neurological diseases and more recently in pain management. In the context of pain management, a recent clinical study demonstrated the effectiveness of adoptive immunotherapy to reduce pain in advanced cancer patients. One recent study showed that transfusion of immune cells was reportedly associated with reductions in opioid consumption and pain intensity among patients with advanced cancer and suggested increased production of endogenous opioids due to the activation of $CD4^+$ T-lymphocytes (Zhou et al. 2020).

The administration of mesenchymal stem cells (MSCs) or bone marrow stromal cells (BMSCs) is another strategy to relieve bone cancer pain, neuropathic pain, or osteoarthritis. Injection of autologous MSCs into the joint alleviated pain in patients with osteoarthritis (Harrell et al. 2019). Intrathecal injection of BMSCs has been shown to exert antinociceptive effects in animal models of bone cancer pain and neuropathic pain (Chen et al. 2015; Huh et al. 2017; Sun et al. 2017). BMSC transplantation may have the potential to treat painful diseases in patients. Thus, cell-based pain therapies will represent a promising strategy to treat chronic pain.

9.8 Concluding Remarks

Although immunotherapy is perceived to be a recent medical advancement in clinical cancer therapy, increasing evidence highlights the emerging role of immunotherapy in treating a range of diseases, including pain. The mechanisms of pain induction and resolution are complex, and the potential of immunotherapy as a novel approach to pain management will be a research area of increasing interest to

address the multifaceted mechanisms of chronic pain. Ongoing and future research in connecting immunotherapy and the treatment of pain will thus hold promise in novel clinical applications.

References

Alexander GM, van Rijn MA, van Hilten JJ, Perreault MJ, Schwartzman RJ. Changes in cerebrospinal fluid levels of pro-inflammatory cytokines in CRPS. Pain. 2005;116:213–9.

Alten R, Gomez-Reino J, Durez P, Beaulieu A, Sebba A, Krammer G, Preiss R, Arulmani U, Widmer A, Gitton X, Kellner H. Efficacy and safety of the human anti-IL-1beta monoclonal antibody canakinumab in rheumatoid arthritis: results of a 12-week, phase II, dose-finding study. BMC Musculoskelet Disord. 2011;12:153.

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.

Bang S, Xie YK, Zhang ZJ, Wang Z, Xu ZZ, Ji RR. GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. J Clin Invest. 2018;128:3568–82.

Bang S, Donnelly CR, Luo X, Toro-Moreno M, Tao X, Wang Z, Chandra S, Bortsov AV, Derbyshire ER, Ji RR. Activation of GPR37 in macrophages confers protection against infection-induced sepsis and pain-like behaviour in mice. Nat Commun. 2021;12:1704.

Baral P, Udit S, Chiu IM. Pain and immunity: implications for host defence. Nat Rev Immunol. 2019;19:433–47.

Baruch K, Deczkowska A, Rosenzweig N, Tsitsou-Kampeli A, Sharif AM, Matcovitch-Natan O, Kertser A, David E, Amit I, Schwartz M. PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease. Nat Med. 2016;22:135–7.

Basso L, Boue J, Mahiddine K, Blanpied C, Robiou-du-Pont S, Vergnolle N, Deraison C, Dietrich G. Endogenous analgesia mediated by CD4(+) T lymphocytes is dependent on enkephalins in mice. J Neuroinflammation. 2016;13:132.

Baum R, Sharma S, Organ JM, Jakobs C, Hornung V, Burr DB, Marshak-Rothstein A, Fitzgerald KA, Gravallese EM. STING contributes to abnormal bone formation induced by deficiency of DNase II in mice. Arthritis Rheumatol. 2017;69:460–71.

Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Perez-Gracia JL, Rodriguez-Ruiz ME, Ponz-Sarvise M, Castanon E, Melero I. Cytokines in clinical cancer immunotherapy. Br J Cancer. 2019;120:6–15.

Bodhankar S, Chen YX, Vandenbark AA, Murphy SJ, Offner H. IL-10-producing B-cells limit CNS inflammation and infarct volume in experimental stroke. Metab Brain Dis. 2013;28:375–86.

Botsios C, Sfriso P, Furlan A, Ostuni P, Biscaro M, Fiocco U, Todesco S, Punzi L. [Anakinra, a recombinant human IL-1 receptor antagonist, in clinical practice. Outcome in 60 patients with severe rheumatoid arthritis]. Reumatismo. 2007;59:32–37.

Brisby H, Olmarker K, Larsson K, Nutu M, Rydevik B. Proinflammatory cytokines in cerebrospinal fluid and serum in patients with disc herniation and sciatica. Eur Spine J. 2002;11:62–6.

Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P. A new member of the immunoglobulin superfamily--CTLA-4. Nature. 1987;328:267–70.

Cao L, DeLeo JA. CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. Eur J Immunol. 2008;38:448–58.

Carlino MS, Larkin J, Long GV. Immune checkpoint inhibitors in melanoma. Lancet. 2021;398:1002–14.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A. 1975;72:3666–70.

Carter LL, Leach MW, Azoitei ML, Cui J, Pelker JW, Jussif J, Benoit S, Ireland G, Luxenberg D, Askew GR, Milarski KL, Groves C, Brown T, Carito BA, Percival K, Carreno BM, Collins M, Marusic S. PD-1/PD-L1, but not PD-1/PD-L2, interactions regulate the severity of experimental autoimmune encephalomyelitis. J Neuroimmunol. 2007;182:124–34.

Cavaco M, Gaspar D, Arb Castanho M, Neves V. Antibodies for the treatment of brain metastases, a dream or a reality? Pharmaceutics. 2020;12:62.
Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-beta secretion. J Clin Invest. 2015;125:3226–40.
Chen G, Kim YH, Li H, Luo H, Liu DL, Zhang ZJ, Lay M, Chang W, Zhang YQ, Ji RR. PD-L1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via PD-1. Nat Neurosci. 2017;20:917–26.
Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR. Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron. 2018;100:1292–311.
Cobos EJ, Nickerson CA, Gao F, Chandran V, Bravo-Caparros I, Gonzalez-Cano R, Riva P, Andrews NA, Latremoliere A, Seehus CR, Perazzoli G, Nieto FR, Joller N, Painter MW, Ma CHE, Omura T, Chesler EJ, Geschwind DH, Coppola G, Rangachari M, Woolf CJ, Costigan M. Mechanistic differences in neuropathic pain modalities revealed by correlating behavior with global expression profiling. Cell Rep. 2018;22:1301–12.
Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert TA, Barrett L, Brenner GJ, Vardeh D, Woolf CJ, Fitzgerald M. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J Neurosci. 2009;29:14415–22.
Day YJ, Liou JT, Lee CM, Lin YC, Mao CC, Chou AH, Liao CC, Lee HC. Lack of interleukin-17 leads to a modulated micro-environment and amelioration of mechanical hypersensitivity after peripheral nerve injury in mice. Pain. 2014;155:1293–302.
Decker WK, da Silva RF, Sanabria MH, Angelo LS, Guimaraes F, Burt BM, Kheradmand F, Paust S. Cancer immunotherapy: historical perspective of a clinical revolution and emerging preclinical animal models. Front Immunol. 2017;8:829.
Dobosz P, Dzieciatkowski T. The intriguing history of cancer immunotherapy. Front Immunol. 2019;10:2965.
Dobson R, Giovannoni G. Multiple sclerosis – a review. Eur J Neurol. 2019;26:27–40.
Donnelly CR, Jiang C, Andriessen AS, Wang K, Wang Z, Ding H, Zhao J, Luo X, Lee MS, Lei YL, Maixner W, Ko MC, Ji RR. STING controls nociception via type I interferon signalling in sensory neurons. Nature. 2021;591:275–80.
Du B, Ding YQ, Xiao X, Ren HY, Su BY, Qi JG. CD4+ alphabeta T cell infiltration into the leptomeninges of lumbar dorsal roots contributes to the transition from acute to chronic mechanical allodynia after adult rat tibial nerve injuries. J Neuroinflammation. 2018;15:81.
Ghasemlou N, Chiu IM, Julien JP, Woolf CJ. CD11b+Ly6G- myeloid cells mediate mechanical inflammatory pain hypersensitivity. Proc Natl Acad Sci U S A. 2015;112:E6808–17.
Gladson CL, Prayson RA, Liu WM. The pathobiology of glioma tumors. Annu Rev Pathol. 2010;5:33–50.
Gok B, Okutan O, Beskonakli E, Palaoglu S, Erdamar H, Sargon MF. Effect of immunomodulation with human interferon-beta on early functional recovery from experimental spinal cord injury. Spine (Phila Pa 1976). 2007;32:873–80.
Guillot-Sestier MV, Doty KR, Gate D, Rodriguez J Jr, Leung BP, Rezai-Zadeh K, Town T. Il10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. Neuron. 2015;85:534–48.
Harrell CR, Markovic BS, Fellabaum C, Arsenijevic A, Volarevic V. Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives. Biomed Pharmacother. 2019;109:2318–26.
Huh Y, Ji RR, Chen G. Neuroinflammation, bone marrow stem cells, and chronic pain. Front Immunol. 2017;8:1014.
Hung AL, Lim M, Doshi TL. Targeting cytokines for treatment of neuropathic pain. Scand J Pain. 2017;17:287–93.
Inacio AR, Liu Y, Clausen BH, Svensson M, Kucharz K, Yang Y, Stankovich T, Khorooshi R, Lambertsen KL, Issazadeh-Navikas S, Deierborg T. Endogenous IFN-beta signaling exerts anti-inflammatory actions in experimentally induced focal cerebral ischemia. J Neuroinflammation. 2015;12:211.

Isaacs A, Lindenmann J. Virus interference. I. the interferon. Proc R Soc Lond B Biol Sci. 1957;147:258–67.

Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11:3887–95.

Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature. 2008;455:674–8.

Jakimovski D, Kolb C, Ramanathan M, Zivadinov R, Weinstock-Guttman B. Interferon beta for multiple sclerosis. Cold Spring Harb Perspect Med. 2018;8

Ji RR, Chamessian A, Zhang YQ. Pain regulation by non-neuronal cells and inflammation. Science. 2016;354:572–7.

Johnson BM, Uchimura T, Gallovic MD, Thamilarasan M, Chou WC, Gibson SA, Deng M, Tam JW, Batty CJ, Williams J, Matsushima GK, Bachelder EM, Ainslie KM, Markovic-Plese S, Ting JP. STING agonist mitigates experimental autoimmune encephalomyelitis by stimulating type I IFN-dependent and -independent immune-regulatory pathways. J Immunol. 2021;206:2015–28.

Kalpachidou T, Riehl L, Schöpf CL, Ucar B, Kress M. Proinflammatory cytokines and their receptors as druggable targets to alleviate pathological pain. Pain. 2022;163(Suppl 1):S79–98.

Kim CF, Moalem-Taylor G. Interleukin-17 contributes to neuroinflammation and neuropathic pain following peripheral nerve injury in mice. J Pain. 2011;12:370–83.

Kleinschnitz C, Hofstetter HH, Meuth SG, Braeuninger S, Sommer C, Stoll G. T cell infiltration after chronic constriction injury of mouse sciatic nerve is associated with interleukin-17 expression. Exp Neurol. 2006;200:480–5.

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.

Koronyo-Hamaoui M, Ko MK, Koronyo Y, Azoulay D, Seksenyan A, Kunis G, Pham M, Bakhsheshian J, Rogeri P, Black KL, Farkas DL, Schwartz M. Attenuation of AD-like neuropathology by harnessing peripheral immune cells: local elevation of IL-10 and MMP-9. J Neurochem. 2009;111:1409–24.

Krukowski K, Eijkelkamp N, Laumet G, Hack CE, Li Y, Dougherty PM, Heijnen CJ, Kavelaars A. CD8+ T cells and endogenous IL-10 are required for resolution of chemotherapy-induced neuropathic pain. J Neurosci. 2016;36:11074–83.

Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J Exp Med. 1995;182:459–65.

Kuo PC, Scofield BA, Yu IC, Chang FL, Ganea D, Yen JH. Interferon-beta modulates inflammatory response in cerebral ischemia. J Am Heart Assoc. 2016;5:e002610.

Kurtuncu M, Tuzun E, Turkoglu R, Petek-Balci B, Icoz S, Pehlivan M, Birisik O, Ulusoy C, Shugaiv E, Akman-Demir G, Eraksoy M. Effect of short-term interferon-beta treatment on cytokines in multiple sclerosis: significant modulation of IL-17 and IL-23. Cytokine. 2012;59:400–2.

Kwon J, Bakhoum SF. The cytosolic DNA-sensing cGAS-STING pathway in cancer. Cancer Discov. 2020;10:26–39.

Kwon Y, Park OJ, Kim J, Cho JH, Yun CH, Han SH. Cyclic dinucleotides inhibit osteoclast differentiation through STING-mediated interferon-beta signaling. J Bone Miner Res. 2019;34:1366–75.

Laumet G, Edralin JD, Chiang AC, Dantzer R, Heijnen CJ, Kavelaars A. Resolution of inflammation-induced depression requires T lymphocytes and endogenous brain interleukin-10 signaling. Neuropsychopharmacology. 2018;43:2597–605.

Laumet G, Edralin JD, Dantzer R, Heijnen CJ, Kavelaars A. Cisplatin educates CD8+ T cells to prevent and resolve chemotherapy-induced peripheral neuropathy in mice. Pain. 2019;160:1459–68.

Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271:1734–6.

Lemos H, Mohamed E, Ou R, McCardle C, Zheng X, McGuire K, Homer NZM, Mole DJ, Huang L, Mellor AL. Co-treatments to boost IDO activity and inhibit production of downstream catabolites induce durable suppression of experimental autoimmune encephalomyelitis. Front Immunol. 2020;11:1256.

Leung L, Cahill CM. TNF-alpha and neuropathic pain – a review. J Neuroinflammation. 2010;7:27.

Liu S, Karaganis S, Mo RF, Li XX, Wen RX, Song XJ. IFNbeta treatment inhibits nerve injury-induced mechanical allodynia and MAPK signaling by activating ISG15 in mouse spinal cord. J Pain. 2020;21:836–47.

Liu CC, Lu IC, Wang LK, Chen JY, Li YY, Yang CP, Liu PH, Cheng WJ, Tan PH. Interferon-beta suppresses inflammatory pain through activating micro-opioid receptor. Mol Pain. 2021;17:17448069211045211.

Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016;131:803–20.

Luo H, Liu HZ, Zhang WW, Matsuda M, Lv N, Chen G, Xu ZZ, Zhang YQ. Interleukin-17 regulates neuron-glial communications, synaptic transmission, and neuropathic pain after chemotherapy. Cell Rep. 2019;29:2384–2397 e2385.

Luo X, Chen O, Wang Z, Bang S, Ji J, Lee SH, Huh Y, Furutani K, He Q, Tao X, Ko MC, Bortsov A, Donnelly CR, Chen Y, Nackley A, Berta T, Ji RR. IL-23/IL-17A/TRPV1 axis produces mechanical pain via macrophage-sensory neuron crosstalk in female mice. Neuron. 2021;109:2691–2706 e2695.

Meng X, Zhang Y, Lao L, Saito R, Li A, Backman CM, Berman BM, Ren K, Wei PK, Zhang RX. Spinal interleukin-17 promotes thermal hyperalgesia and NMDA NR1 phosphorylation in an inflammatory pain rat model. Pain. 2013;154:294–305.

Moalem G, Xu K, Yu L. T lymphocytes play a role in neuropathic pain following peripheral nerve injury in rats. Neuroscience. 2004;129:767–77.

Monaco C, Nanchahal J, Taylor P, Feldmann M. Anti-TNF therapy: past, present and future. Int Immunol. 2015;27:55–62.

Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science. 1976;193:1007–8.

Niehaus JK, Taylor-Blake B, Loo L, Simon JM, Zylka MJ. Spinal macrophages resolve nociceptive hypersensitivity after peripheral injury. Neuron. 2021;109:1274–1282 e1276.

Nishimura Y, Natsume A, Ito M, Hara M, Motomura K, Fukuyama R, Sumiyoshi N, Aoki I, Saga T, Lee HJ, Wakabayashi T, Kim SU. Interferon-beta delivery via human neural stem cell abates glial scar formation in spinal cord injury. Cell Transplant. 2013;22:2187–201.

Noma N, Khan J, Chen IF, Markman S, Benoliel R, Hadlaq E, Imamura Y, Eliav E. Interleukin-17 levels in rat models of nerve damage and neuropathic pain. Neurosci Lett. 2011;493:86–91.

Norheim KB, Harboe E, Goransson LG, Omdal R. Interleukin-1 inhibition and fatigue in primary Sjogren's syndrome--a double blind, randomised clinical trial. PLoS One. 2012;7:e30123.

Old EA, Nadkarni S, Grist J, Gentry C, Bevan S, Kim KW, Mogg AJ, Perretti M, Malcangio M. Monocytes expressing CX3CR1 orchestrate the development of vincristine-induced pain. J Clin Invest. 2014;124:2023–36.

Petrovic J, Silva JR, Bannerman CA, Segal JP, Marshall AS, Haird CM, Gilron I, Ghasemlou N. Gammadelta T cells modulate myeloid cell recruitment but not pain during peripheral inflammation. Front Immunol. 2019;10:473.

Qin C, Zhou LQ, Ma XT, Hu ZW, Yang S, Chen M, Bosco DB, Wu LJ, Tian DS. Dual functions of microglia in ischemic stroke. Neurosci Bull. 2019;35:921–33.

Ren X, Akiyoshi K, Vandenbark AA, Hurn PD, Offner H. Programmed death-1 pathway limits central nervous system inflammation and neurologic deficits in murine experimental stroke. Stroke. 2011a;42:2578–83.

Ren XF, Akiyoshi K, Dziennis S, Vandenbark AA, Herson PS, Hurn PD, Offner H. Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. J Neurosci. 2011b;31:8556–63.

Rosenzweig N, Dvir-Szternfeld R, Tsitsou-Kampeli A, Keren-Shaul H, Ben-Yehuda H, Weill-Raynal P, Cahalon L, Kertser A, Baruch K, Amit I, Weiner A, Schwartz M. PD-1/PD-L1 checkpoint blockade harnesses monocyte-derived macrophages to combat cognitive impairment in a tauopathy mouse model. Nat Commun. 2019;10:465.

Salama AD, Chitnis T, Imitola J, Ansari MJ, Akiba H, Tushima F, Azuma M, Yagita H, Sayegh MH, Khoury SJ. Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. J Exp Med. 2003;198:71–8.

Sampson JH, Gunn MD, Fecci PE, Ashley DM. Brain immunology and immunotherapy in brain tumours. Nat Rev Cancer. 2020;20:12–25.

Sandrow-Feinberg HR, Zhukareva V, Santi L, Miller K, Shumsky JS, Baker DP, Houle JD. PEGylated interferon-beta modulates the acute inflammatory response and recovery when combined with forced exercise following cervical spinal contusion injury. Exp Neurol. 2010;223:439–51.

Saresella M, Calabrese E, Marventano I, Piancone F, Gatti A, Farina E, Alberoni M, Clerici M. A potential role for the PD1/PD-L1 pathway in the neuroinflammation of Alzheimer's disease. Neurobiol Aging. 2012;33(624):e611–22.

Schafers M, Brinkhoff J, Neukirchen S, Marziniak M, Sommer C. Combined epineurial therapy with neutralizing antibodies to tumor necrosis factor-alpha and interleukin-1 receptor has an additive effect in reducing neuropathic pain in mice. Neurosci Lett. 2001;310:113–6.

Segond von Banchet G, Boettger MK, Konig C, Iwakura Y, Brauer R, Schaible HG. Neuronal IL-17 receptor upregulates TRPV4 but not TRPV1 receptors in DRG neurons and mediates mechanical but not thermal hyperalgesia. Mol Cell Neurosci. 2013;52:152–60.

Sorge RE, Mapplebeck JC, Rosen S, Beggs S, Taves S, Alexander JK, Martin LJ, Austin JS, Sotocinal SG, Chen D, Yang M, Shi XQ, Huang H, Pillon NJ, Bilan PJ, Tu Y, Klip A, Ji RR, Zhang J, Salter MW, Mogil JS. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18:1081–3.

Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J. 2021;11:69.

Stokes JA, Corr M, Yaksh TL. Spinal toll-like receptor signaling and nociceptive processing: regulatory balance between TIRAP and TRIF cascades mediated by TNF and IFNbeta. Pain. 2013;154:733–42.

Sun Y, Tian Y, Li H, Zhang D, Sun Q. Antinociceptive effect of intrathecal injection of genetically engineered human bone marrow stem cells expressing the human proenkephalin gene in a rat model of bone cancer pain. Pain Res Manag. 2017;2017:7346103.

Sun J, Zhou YQ, Xu BY, Li JY, Zhang LQ, Li DY, Zhang S, Wu JY, Gao SJ, Ye DW, Mei W. STING/NF-kappaB/IL-6-mediated inflammation in microglia contributes to spared nerve injury (SNI)-induced pain initiation. J Neuroimmune Pharmacol. 2022;17:453–69.

Tan PH, Ji J, Yeh CC, Ji RR. Interferons in pain and infections: emerging roles in neuro-immune and neuro-glial interactions. Front Immunol. 2021;12:783725.

Trabattoni D, Saresella M, Pacei M, Marventano I, Mendozzi L, Rovaris M, Caputo D, Borelli M, Clerici M. Costimulatory pathways in multiple sclerosis: distinctive expression of PD-1 and PD-L1 in patients with different patterns of disease. J Immunol. 2009;183:4984–93.

van der Vlist M, Raoof R, Willemen H, Prado J, Versteeg S, Martin Gil C, Vos M, Lokhorst RE, Pasterkamp RJ, Kojima T, Karasuyama H, Khoury-Hanold W, Meyaard L, Eijkelkamp N. Macrophages transfer mitochondria to sensory neurons to resolve inflammatory pain. Neuron. 2022;110:613–626 e619.

Veldhuis WB, Derksen JW, Floris S, Van Der Meide PH, De Vries HE, Schepers J, Vos IM, Dijkstra CD, Kappelle LJ, Nicolay K, Bar PR. Interferon-beta blocks infiltration of inflammatory cells and reduces infarct volume after ischemic stroke in the rat. J Cereb Blood Flow Metab. 2003;23:1029–39.

Vicuna L, Strochlic DE, Latremoliere A, Bali KK, Simonetti M, Husainie D, Prokosch S, Riva P, Griffin RS, Njoo C, Gehrig S, Mall MA, Arnold B, Devor M, Woolf CJ, Liberles SD, Costigan

M, Kuner R. The serine protease inhibitor SerpinA3N attenuates neuropathic pain by inhibiting T cell-derived leukocyte elastase. Nat Med. 2015;21:518–23.
Wagner R, Myers RR. Schwann cells produce tumor necrosis factor alpha: expression in injured and non-injured nerves. Neuroscience. 1996;73:625–9.
Wang K, Gu Y, Liao Y, Bang S, Donnelly CR, Chen O, Tao X, Mirando AJ, Hilton MJ, Ji RR. PD-1 blockade inhibits osteoclast formation and murine bone cancer pain. J Clin Invest. 2020a;130:3603–20.
Wang Z, Jiang C, He Q, Matsuda M, Han Q, Wang K, Bang S, Ding H, Ko MC, Ji RR. Anti-PD-1 treatment impairs opioid antinociception in rodents and nonhuman primates. Sci Transl Med. 2020b;12:eaaw6471.
Wang K, Donnelly CR, Jiang C, Liao Y, Luo X, Tao X, Bang S, McGinnis A, Lee M, Hilton MJ, Ji RR. STING suppresses bone cancer pain via immune and neuronal modulation. Nat Commun. 2021a;12:4558.
Wang X, Lu J, Guo G, Yu J. Immunotherapy for recurrent glioblastoma: practical insights and challenging prospects. Cell Death Dis. 2021b;12:299.
Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. F1000Research. 2018;7. https://doi.org/10.12688/f1000research.14506.1
Woo SR, Fuertes MB, Corrales L, Spranger S, Furdyna MJ, Leung MY, Duggan R, Wang Y, Barber GN, Fitzgerald KA, Alegre ML, Gajewski TF. STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors. Immunity. 2014;41:830–42.
Xiang HC, Lin LX, Hu XF, Zhu H, Li HP, Zhang RY, Hu L, Liu WT, Zhao YL, Shu Y, Pan HL, Li M. AMPK activation attenuates inflammatory pain through inhibiting NF-kappaB activation and IL-1beta expression. J Neuroinflammation. 2019;16:34.
Yao A, Liu F, Chen K, Tang L, Liu L, Zhang K, Yu C, Bian G, Guo H, Zheng J, Cheng P, Ju G, Wang J. Programmed death 1 deficiency induces the polarization of macrophages/microglia to the M1 phenotype after spinal cord injury in mice. Neurotherapeutics. 2014;11:636–50.
Zelenka M, Schafers M, Sommer C. Intraneural injection of interleukin-1beta and tumor necrosis factor-alpha into rat sciatic nerve at physiological doses induces signs of neuropathic pain. Pain. 2005;116:257–63.
Zha J, Smith A, Andreansky S, Bracchi-Ricard V, Bethea JR. Chronic thoracic spinal cord injury impairs CD8+ T-cell function by up-regulating programmed cell death-1 expression. J Neuroinflammation. 2014;11:65.
Zhao J, Roberts A, Wang Z, Savage J, Ji RR. Emerging role of PD-1 in the central nervous system and brain diseases. Neurosci Bull. 2021;37:1188–202.
Zhou X, Qiao G, Ren J, Wang X, Wang S, Zhu S, Yuan Y, Morse MA, Hobeika A, Lyerly HK. Adoptive immunotherapy with autologous T-cell infusions reduces opioid requirements in advanced cancer patients. Pain. 2020;161:127–34.
Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood. 2008;112:1557–69.
Zwicky P, Unger S, Becher B. Targeting interleukin-17 in chronic inflammatory disease: a clinical perspective. J Exp Med. 2020;217:e20191123.

Chapter 10
Cell Therapy and Regenerative Pain Medicine: Preclinical Studies

Toby Chen, Yul Huh, and Andrew Breglio

Abstract Mesenchymal stem cells (MSCs) and bone marrow stem cells (BMSCs) represent a novel therapy for chronic pain due to their immunomodulatory and neuroprotective properties. MSCs have shown long-lasting analgesic effects in preclinical models of pain that have been historically difficult to treat clinically. These conditions include neuropathic pain and cancer pain. The analgesic effects conferred by MSCs are mediated by a multitude of secreted mediators, including anti-inflammatory cytokines and growth factors, which can reduce neuroinflammation and pain signaling via neuroimmune interactions and further protect neurodegeneration. Extracellular vesicles secreted from MSCs are increasingly understood to mediate anti-inflammatory, pro-survival, regenerative, and analgesic effects previously attributed to MSCs themselves. These MSC-EVs may hold several advantages as therapeutics as compared to MSC cell therapy, though much remains to be learned. Importantly, there are multiple routes of administration for MSCs that can be catered to clinical needs. Overall, MSCs and their secretome provide a highly promising treatment modality to resolve some of the most prevalent and debilitating chronic pain conditions.

Keywords Bone marrow stromal cells (BMSCs) · Extracellular vesicles (EVs) · Mesenchymal stem cells (MSCs) · Neuroimmune modulation · Neuropathic pain · Transforming growth factor beta 1 (TGF-β1)

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10.1 Introduction: Defining Mesenchymal Stem Cells and Bone Marrow Stromal Cells

Nonhematopoietic stem cell populations within the bone marrow were first observed by German pathologist Julius Connheim in the late nineteenth century (Prockop 1997). In the 1980s, Friedenstein and fellow researchers discovered that a particular portion of whole bone marrow filtrates possesses the ability to adhere to plastic culture dishes, while non-adherent hematopoietic cells could be washed away. These cells were termed mesenchymal stem cells (MSCs) by Arnold Caplan (Caplan 1991) or bone marrow stromal cells (BMSCs) (Chamberlain et al. 2007). In culture, adherent MSCs multiply rapidly within 2–4 days, and the resulting colonies of cells demonstrate multipotency, differentiating into colonies of osteoblasts, chondrocytes, adipocytes, and myoblasts (Castro-Malaspina et al. 1980).

MSCs have been isolated from a variety of different sources including bone marrow, adipose tissue, amniotic fluid, periosteum, and fetal tissues such as the umbilical cord (Campagnoli et al. 2001; In 't Anker et al. 2003). These cells are characterized by specific cell markers. Human MSCs are negative for the hematopoietic markers CD45, CD34, CD14, and CD11; the costimulatory markers CD80, CD86, and CD40; and the adhesion molecules CD31(PECAM-1), CD18 (LFA-1), and CD56. MSCs do express CD105 (SH2), CD73 (SH3/4), CD44, CD90 (Thy-1), CD71, and Stro-1, as well as adhesion molecules CD106 (VCAM-1), CD166 (ALCAM), ICAM-1, and CD29 (Haynesworth et al. 1992). Furthermore, adult human MSCs have been shown to express intermediate levels of MHC class I, but do not express MHC class II (Le Blanc and Ringden 2005).

Therapeutic applications have been explored in a variety of diseases, such as autoimmune diseases, degenerative diseases, neurological diseases, and cardiovascular diseases. Many routes of delivery have been investigated to optimize the therapeutic potential of these cells (Fig. 10.1).

10.2 Neuroimmune Modulation by MSCs

MSCs show various effects on innate immunity processes. For instance, MSCs have been shown to inhibit the maturation of monocytes (Jiang et al. 2005) and to impair the antigen-presenting function of dendritic cells (DCs) via a decrease of MHC class II, CD11c, CD83 expression, and decreased IL-12 production, in addition to inhibiting DC production of inflammatory tumor necrosis factor (TNF) (Aggarwal and Pittenger 2005). Furthermore, MSCs can inhibit resting NK cell cytotoxic activity and can also inhibit NK-cell proliferation and IFN-γ production (Spaggiari et al. 2006). Interestingly, IFN-γ protects MSCs from NK-cell-mediated lysis, and thus an inflammatory environment rich in IFN-γ may increase survival in favor of MSCs (Uccelli et al. 2008). MSCs have also been shown to reduce neutrophil respiratory bursts and extend the life span of neutrophils by inhibiting IL-6-mediated neutrophil

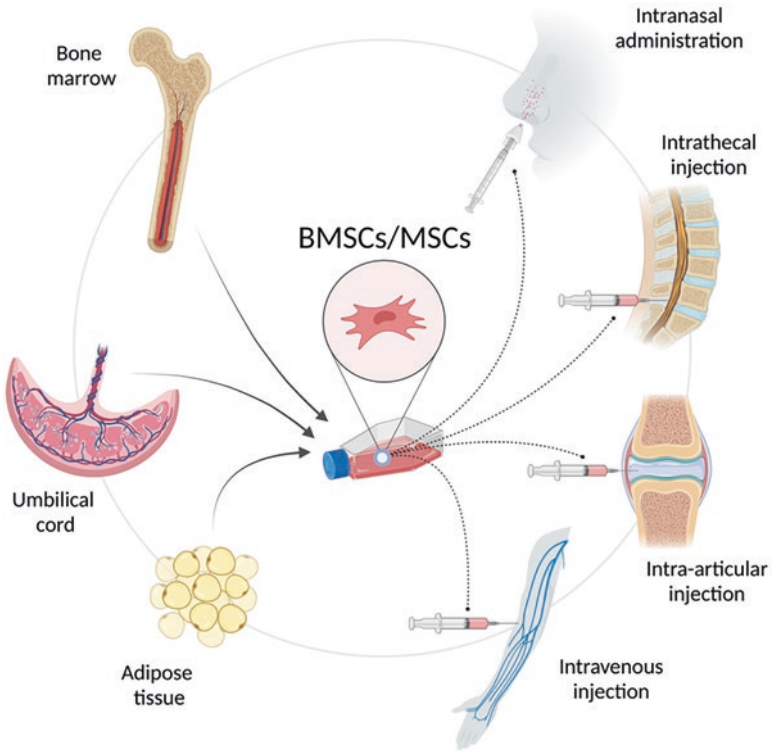

Fig. 10.1 BMSC/MSC routes of administration. These routes include intranasal, intrathecal, intra-articular, and intravenous delivery

apoptosis (Raffaghello et al. 2008). The MSC secretome enhances the immunoregulatory and pro-resolving properties of regulatory macrophages by increasing CD206 expression and decreasing expression of MMP2 (Holopainen et al. 2020).

With regard to adaptive immunity, MSCs have been shown to inhibit T cell proliferation, though T cell apoptosis is not promoted by MSCs. Rather, the antiproliferative effect on T cells is associated with converting T cells to a state of quiescence and survival (Zappia et al. 2005). Decreased proliferation leads to decreased IFN-γ production, shifting T cells from a pro-inflammatory state to an anti-inflammatory state marked by increased IL-4 production by T helper 2 cells (Aggarwal and Pittenger 2005). MSCs have also been found to cause IL-10 production by plasmacytoid dendritic cells, which then stimulates the generation of additional regulatory T cells, leading to additional suppression of immune system activation (Maccario et al. 2005). B cell proliferation and differentiation has been found to be inhibited by MSCs in vitro, from the release of soluble factors by MSCs, as well as cell-cell contact possibly mediated by PD1 interactions (Uccelli et al. 2008).

Several soluble immunosuppressive factors are secreted by MSCs. IFN-γ can trigger MSCs to release nitric oxide and indoleamine 2,3-dioxygenase (IDO)

(Krampera et al. 2006). IDO specifically can inhibit lymphocyte proliferation by depleting the lymphocyte-essential amino acid tryptophan (Uccelli et al. 2008). Nitric oxide is involved in the inhibition of T cell activation (Ren et al. 2008). Additional soluble factors are constitutively secreted by MSCs and include TGF-β1, HGF, IL-10, PGE2 (prostaglandin E2), heme oxygenase-1 (HO-1), IL-6, and soluble HLA-G5 (Jiang and Xu 2020). The production of these various molecules creates a redundant system of MSC-mediated immunoregulation.

MSCs can also exhibit effects on surrounding neurons and neural precursor cells. Trophic and anti-apoptotic molecules secreted by MSCs can rescue injured neurons and oligodendrocytes from apoptosis (Li et al. 2002). The anti-inflammatory and antiproliferative effects that MSCs have on microglia and astrocytes form a neuroprotective environment (Uccelli et al. 2008). And notably, local neural precursor cells are promoted to proliferate and mature by MSCs, enabling differentiation of mature neurons and oligodendrocytes (Munoz et al. 2005). Thus, MSCs have wide neuroimmune-modulatory effects that can be harnessed to treat neuroinflammation and pain (Fig. 10.2).

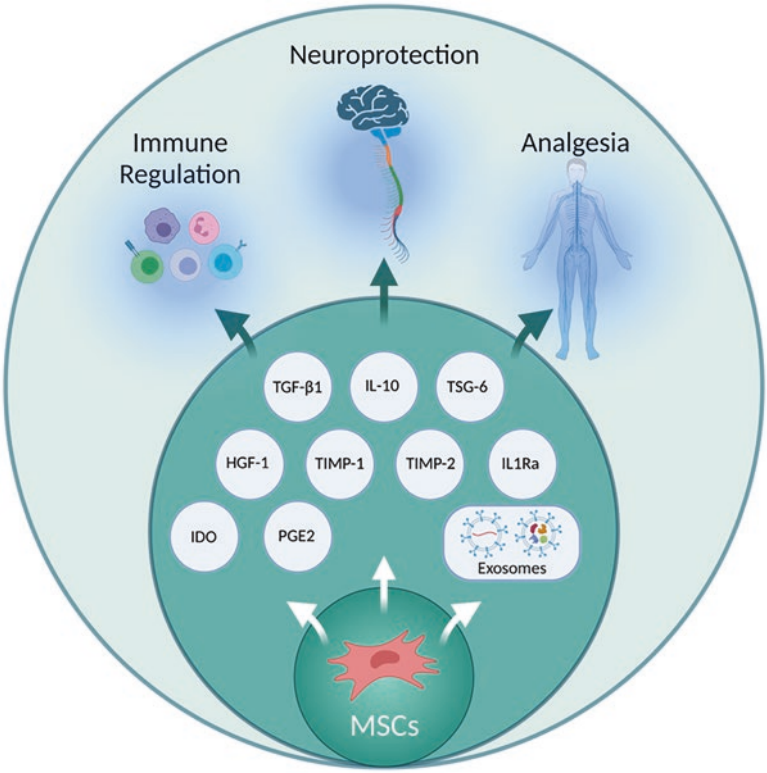

Fig. 10.2 BMSCs/MSCs secrete anti-inflammatory and pro-resolution factors as well as neurotrophic factors

10.3 Preclinical Models of MSC/BMSC-Mediated Pain Relief

MSCs have attracted interest in treating chronic pain conditions that have been particularly difficult to treat clinically, including degeneration/inflammation-related pain, neuropathic pain, cancer pain, opioid tolerance, and opioid-induced hyperalgesia. Preclinical studies using cellular therapies to treat animal pain models have enabled researchers to evaluate the safety and efficacy of MSC treatment. BMSCs have been utilized in several studies to determine the efficacy of treatment via intravenous injection as well as local injection at the site of injury (Huh et al. 2017). These studies found BMSCs sourced from mouse, rat, and human bone marrow conferred analgesic effects in rodent models of neuropathic pain including nerve injury, spinal cord injury, diabetic neuropathy induced by streptozotocin, and arthritis (Huh et al. 2017).

MSCs exhibit potent immunosuppressive properties which make them promising candidates for autologous and allogeneic transplantation, as their use may not necessitate the need for immunosuppressive regimens (Giordano et al. 2007). Although MSCs were originally thought to possess the ability to replace diseased or injured tissue due to their differentiation into various types of tissue in vitro (Pittenger et al. 1999), the secreted factors from MSCs including cytokines, chemokines, and trophic factors have been found to mediate profound immunomodulatory effects (Caplan and Correa 2011). Thus, MSCs are a promising treatment for many pain conditions, and many preclinical studies have shown their ability to provide pain relief (Table 10.1).

10.3.1 Degenerative/Inflammatory Pain

One of the most encountered chronic pain conditions in the clinic is osteoarthritis (OA). Patients often report a significant impact on their quality of life due to OA despite current therapies that are intended to provide pain relief, reduction of inflammation, and improvements in joint function (Martel-Pelletier et al. 2016). There have been several studies using autologous or allogeneic MSCs to treat animal models of OA, including in rodents, rabbits, sheep, and horses (Alfaqeh et al. 2008; Grigolo et al. 2009; Horie et al. 2012; Schnabel et al. 2013). These initial studies examined the ability of transplanted MSCs to serve as a regenerative therapy for cartilage content at the articular surface (Freitag et al. 2016; Wyles et al. 2015).

More recently, the paracrine activity of MSCs and the resulting major benefits conferred by these secreted products on inflammatory pain have come under focus (Pers et al. 2015). Although intra-articular MSC treatments have shown joint tissue regeneration, studies found that the transplanted MSCs represented a smaller number of cells in the regenerated tissue versus the host's native cells (Murphy et al. 2003). Additional studies found that MSC transplantation leads to a change in the

Table 10.1 Preclinical studies of BMSC/MSC treatments for chronic pain conditions

Reference	Pain model	Species	Delivery site	Effects on pain
Musolino et al.	SLNC	Rat	Intraganglionic	Prevention of mechanical and thermal allodynia
Klass et al.	CCI	Rat	Intravenous	Improvement of mechanical allodynia and thermal hyperalgesia
Shibata et al.	STZ-induced diabetes	Rat	Injection in the hind limb skeletal muscle	Improvement of hypoalgesia
Abrams et al.	Spinal cord injury	Rat	Injury site	Improvement of mechanical allodynia, no effect on thermal hyperalgesia
Siniscalco et al.	SNI	Mouse	Lateral cerebral ventricle	Improvement of mechanical allodynia and thermal hyperalgesia
Siniscalco et al.	SNI	Mouse	Intravenous	Improvement of mechanical allodynia and thermal hyperalgesia
Orozco et al.	Degenerative disk disease	Human	Intradiscal injection	Decrease in pain
Guo et al.	Chronic orofacial pain	Rat	Intravenous injury site	Reversed mechanical hypersensitivity
Naruse et al.	STZ-induced diabetes	Rat	Injection in the hind limb skeletal muscle	Improves mechanical hyperalgesia, cold allodynia
van Buul et al.	Osteoarthritis	Rat	Intra-articular injection	Decrease in pain
Zhang, et al.	SNL	Rat	Intrathecal injection	Improvement of mechanical allodynia
Chen et al.	CCI, SNI, mouse	Mouse	Intrathecal injection	Suppress neuropathic pain
Pettine et al.	Degenerative disk disease	Human	Intrathecal injection	Decrease in pain
Yousefifard et al.	Spinal cord injury	Rat	Injury site	Improvement of mechanical and cold allodynia; mechanical and thermal hyperalgesia
Guo et al	TL, SNL, CCI-ION	Rat and mice	Intravenous; injury site	Improvement of mechanical and thermal hyperalgesia; suppress aversive behavior
Li et al.	SNL	Rat	Intrathecal injection	Improvement of mechanical allodynia and thermal hyperalgesia
Fischer et al.	TNI	Rat	Intrathecal injection	Improvement of mechanical hyperalgesia

(continued)

Table 10.1 (continued)

Reference	Pain model	Species	Delivery site	Effects on pain
Hua et al.	Opioid tolerance and opioid-induced hyperalgesia	Rat and mouse	Intrathecal injection and intravenous injection	Prevention and reversal of opioid tolerance; prevention and reversal of opioid-induced hyperalgesia
Liu et al.	CCI	Rat	Intrathecal injection and intravenous injection	Improvement of mechanical allodynia and thermal hyperalgesia
Boukelmoune et al.	Cisplatin and paclitaxel-induced CIPN	Mouse	Intranasal administration	Improvement of mechanical allodynia and spontaneous pain

cell signaling environment, which then leads to increases in the collagen production in the host (Horie et al. 2012). Thus, these findings suggest that MSC treatments lead to considerable shifts in the inflammatory milieu to encourage the repair of injured tissue versus serving as replacement tissue, which further suggests a major immunomodulatory role that is carried out by MSCs.

A rat tendon injury model was treated with a single intravenous or local injection of rat MSCs by Guo et al. in 2011, and they found that the MSC treatment was able to reverse mechanical allodynia in this pain model. Furthermore, the group also found that the anti-allodynic effect of MSCs was blocked by the opioid receptor antagonist naloxone, which suggests the involvement of endogenous opioids (Guo et al. 2011). A follow-up study by the group additionally demonstrated that long-term pain relief from MSC treatment was mediated by immune interactions and the activation of monocytes along with the activation of μ-opioid receptors in the brain stem and CXCL1/CXCR2 chemokine signaling (Guo et al. 2017).

Chronic low back pain is a common clinical condition that is often caused by intervertebral disk degeneration. Cytokines contained in degenerative disk tissue can directly cause pain by upregulating protease activity on surrounding nerve tissue (Risbud and Shapiro 2014). Current treatments for chronic back pain are unable to completely treat the condition without major side effects, including pharmacological therapies that provide limited long-term efficacy and surgical treatments that may be beneficial but carry the risk of subsequent disk injury adjacent to the surgical site. MSC therapies are being investigated as an alternative approach to repair injured vertebral disks (Sakai and Andersson 2015). In a similar mechanistic model as MSC treatment for OA, MSCs may serve as immune modulatory cells that can signal through paracrine activity to shift the inflammatory environment of the injured disk toward an anti-inflammatory and pro-resolution environment to stimulate healing processes for the injured tissue (Orozco et al. 2011).

MSCs have also been studied for their ability to treat conditions related to inflammatory bowel disease (IBD). IBD carries significant morbidity and mortality risks and major effects on the quality of life of a patient due to significant symptoms including abdominal pain, gastrointestinal bleeding, and impaired gastrointestinal

function (Srinath et al. 2014). Current treatments are limited to treating the symptoms of IBD and do not address the underlying causes currently under investigation. A recent study utilized intravenous injections of MSCs in a rat model of IBD, and the treatment was able to lead to a significant reduction of intestinal damage caused by TNF-α-mediated inflammatory processes, ischemia-reperfusion injury, and tight junction disruption related to ZO-1 downregulation (Shen et al. 2013).

10.3.2 Neuropathic Pain

Neuropathic pain is caused by a disease of, or direct injury to, the somatosensory nervous system or sensory nervous system (Cheng 2021), and the resulting pain is often refractory to treatments. Since current clinical treatments for neuropathic pain are limited in their efficacy, there is a pressing need for novel, more effective treatments. Preclinical neuropathic pain models have been utilized to investigate various MSC treatments including intraganglionic (Musolino et al. 2007), intraspinal (Abrams et al. 2009), intracranial (Siniscalco et al. 2010), intramuscular (Shibata et al. 2008), and intrathecal administration (Fischer et al. 2017), as well as systemic administration via intravenous and intranasal injections.

Intravenous MSC treatments were found to reduce both mechanical allodynia and thermal hyperalgesia in several rodent chronic pain models including the sciatic nerve injury model (Klass et al. 2007), infraorbital nerve injury model (Guo et al. 2016), and the spared nerve injury model (SNI) (Siniscalco et al. 2011). Intramuscular injections of MSCs effectively reduced mechanical allodynia and cold allodynia in the STZ diabetic neuropathy model (Naruse et al. 2011). In spinal cord injury rat and mice models, intraspinal injection of MSCs reduced mechanical allodynia and thermal hyperalgesia (Abrams et al. 2009; Watanabe et al. 2015). Intraganglionic injection of MSCs in a rat sciatic nerve chronic constriction injury model led to reduced mechanical allodynia and thermal hyperalgesia (Musolino et al. 2007). Similar effects were observed in a rat spinal nerve ligation model after intrathecal injections of MSCs (Zhang et al. 2014b).

A group from Duke University found that a single intrathecal injection of 2.5×10^5 BMSCs provided long-term analgesia in mouse models of neuropathic pain including chronic constriction injury (CCI) of the sciatic nerve as well as a spared nerve injury (SNI) model (Chen et al. 2015). This treatment was able to provide analgesic effects for early- and late-phase neuropathic pain and the significance of the effect was measured for several weeks following the single intrathecal injection. Upon examination of the dorsal root ganglia (DRG), the BMSCs provided protection for DRG neurons from damage to their axons, and furthermore, both DRG and spinal cord tissues taken from CCI model mice treated with intrathecal BMSCs exhibited reduced neuroinflammation.

The group found that upon injection into the intrathecal space, the BMSCs specifically migrated to the DRGs by a CXCL12/CXCR4 chemokine interaction, where injured DRGs upregulated CXCL12, signaling for trafficking of CXCR4-expressing

BMSCs to specifically migrate to the sites of injured DRG tissues (Chen et al. 2015). Additionally, the intrathecal BMSCs were found to specifically inhibit neuropathic pain by secreting the anti-inflammatory cytokine TGF-β1, which was measured in the cerebral spinal fluid. When TGF-β1 was inhibited by intrathecally injected anti-TGF-β1 neutralizing antibody, the analgesic effect of the BMSCs was transiently reversed. The potency of the analgesic effect of TGF-β1 was indicated by the ability of very low doses of exogenous intrathecal TGF-β1 between 1 and 10 ng to provide pain relief for neuropathic pain models. The ability of BMSCs to survive in DRGs for several months without conversion to other cell types further indicated that these cells served as continuous sources of anti-inflammatory secreted analgesic products for neuropathic pain conditions.

Liu et al. further compared MSC treatment protocols in rat models of neuropathic pain induced by chronic constriction injury of the sciatic nerve and found that both intrathecal and intravenous injections produced powerful and long-lasting analgesic effects and that MSCs from either the bone marrow or adipose tissue produced identical effects (Liu et al. 2017). A one-time injection produced anti-hyperalgesia effects that lasted for the entire experimental course (42 days) without any signs of weaning. Immunohistochemistry studies further confirmed that intrathecally injected MSCs migrated from the injection site to the surface of DRGs affected by the chronic constriction injury of the ipsilateral sciatic nerve.

Recently, a study demonstrated that nasal administration of 1×10^6 MSCs reversed both cisplatin-induced and paclitaxel-induced mechanical allodynia and spontaneous pain (Boukelmoune et al. 2021). Nasally administered MSCs migrate to the meninges of the brain, the brain parenchyma and multiple distal sites which include the meninges of the spinal cord and peripheral lymph nodes where they may directly or indirectly promote the reversal of chemotherapy-induced peripheral neuropathy (CIPN) and restoration of neuronal function (Boukelmoune et al. 2021; Chiu et al. 2018; Galeano et al. 2018). Human MSCs and mouse MSCs were both effective in resolving mechanical pain. Two doses of nasally administered mouse MSC completely restored intraepidermal nerve fiber (IENF) density to levels higher than the controls. Three weeks after the completion of MSC treatment, which was when mechanical allodynia was fully resolved, basal and maximal mitochondrial respiration rates of DRG neurons were normalized compared to the post-chemotherapy baseline.

Opioid tolerance (OT) and opioid-induced hyperalgesia (OIH) are the primary drivers for opioid dose escalation, overdose, and opioid-related deaths. Hua et al. discovered that MSC transplantation effectively prevented the development of OT and OIH and consistently reversed established OT and OIH in rats as well as in mice (Hua et al. 2016; Li et al. 2018). Either intrathecal or intravenous infusion of MSCs prevented the development of OT and OIH that were induced by daily morphine injections. When performed after OT and OIH had fully developed, either intrathecal or intravenous MSC injection effectively reversed it. These studies identified a new therapeutic strategy to overcome the challenges of opioid tolerance and opioid-induced hyperalgesia.

10.3.3 Cancer Pain

Chronic inflammation is a consequence that often develops with the progression of cancer (Spaeth et al. 2008). The role of MSCs with respect to cancer and its progression is not settled in the literature (Prakash et al. 2016), as certain studies suggest that MSCs possess anti-tumor properties (Zhu et al. 2009), while other studies conversely found that MSCs promoted the growth of tumors (Mele et al. 2014). While early-stage cancers in and of themselves may not always cause pain until they undergo metastatic spread to bone or other sites (Chen et al. 2017), the neuropathic pain and visceral pain caused by chemotherapy, surgical interventions, and radiotherapy are experienced by up to half of patients who receive treatment. Researchers found that an intravenous administration of MSCs at the four-week timepoint following radiotherapy was able to reverse visceral allodynia in a rodent model by reducing the interactions between mast cells and PGP9.5+ nerve fibers. MSC treatment also lowered the ulceration of the colon caused by radiotherapy (Durand et al. 2015).

10.4 Different Routes of Administering MSCs/BMSCs

FDA-approved intrathecally administered drugs have a proven safety record, and clinical trials have shown that intrathecally administered MSCs do not lead to adverse side effects in recipients when followed up for 12 months. The intrathecal space is also considered to possess immune privilege due to the isolation of the CSF from systemic circulation due to the blood–brain barrier, and thus MSCs administered intrathecally may avoid host immune responses. Therefore, the intrathecal route of administration has the advantage of addressing major pain pathways involving the spinal cord and DRG, protecting MSCs from the host immune response, which enables their long-term survival, and in turn the MSCs can confer long-term pain relief.

In contrast, systemic administration of MSCs via intravenous injection may require greater dosage numbers of cells due to the accumulation of MSCs in pulmonary capillaries following circulation. On the one side, this route of administration allows cell-to-cell interactions between MSCs and immune cells, which may lead to changes in macrophage immune states, for example, and lead to beneficial immune modulation. On the other side, it has been found that more than 99% of systemically administered MSCs fail to survive in circulation beyond one week (Lee et al. 2009). Within the lungs, MSCs are subject to innate immune responses within a few days of injection, and processes such as macrophage phagocytosis lead to clearance of the MSCs themselves (Karp and Leng Teo 2009). Interestingly, phagocytosis of MSCs induces phenotypical and functional changes in monocytes, which subsequently modulate cells of the adaptive immune system (de Witte et al. 2018). Thus, monocytes play a crucial role in mediating, distributing, and transferring the

immunomodulatory effect of MSC. Interestingly, metabolically inactivated and even fragmented MSCs possess remarkable immunomodulatory capacities (Chang et al. 2012; Gonçalves et al. 2017; Luk et al. 2016). This is consistent with observations that MSCs produce long-term immunomodulatory effects despite having a short half-life after intravenous administration (Eggenhofer et al. 2012; Fischer et al. 2009; Hua et al. 2016; Liu et al. 2017).

Nasally administered MSCs are a promising treatment modality for CIPN as it is safe and has long-lasting efficacy. Intranasally delivered xenogeneic MSCs are effective in resolving CIPN in mice (Boukelmoune et al. 2021; Squillaro et al. 2016). Additionally, nasal administration of MSC does not interfere with the antitumor effects of chemotherapeutic drugs (Chiu et al. 2018; Spanos et al. 2009; Vichaya et al. 2016). However, the effects of MSCs on the fate of tumors that may attract MSCs themselves are unknown. Additionally, although nasal administration of MSC is promising in preclinical studies, the dosage, feasibility, and safety of nasal MSC administration for humans have yet to be determined.

Localized MSC injection is an option to treat localized pathologies. The injections of MSCs directly into injured intervertebral disk, injured spinal cord, or injured ganglia are examples of such applications. Care should be taken to avoid/minimize introducing additional injury to the target tissue.

10.5 MSC Secreted Mediators That Reduce Pain and Neuroinflammation

10.5.1 Transforming Growth Factor Beta 1 (TGF-β1)

TGF-β1 is a widely expressed secreted signaling protein that exists in various tissues that control for a variety of cellular functions including growth, differentiation, and apoptosis (Zhang and An 2007). TGF-β1 is secreted by MSCs and is further upregulated by inflammatory factors such as IFN-γ and TNF-α (Gotherstrom et al. 2005). TGF-β1 is both a powerful immunosuppressive cytokine and a powerful neuromodulator.

Accumulating evidence suggests that TGF-β1 plays a key role in relieving chronic pain through inhibiting nerve injury-induced proliferation and activation of microglia and astrocytes as well as reducing the expression and secretion of proinflammatory cytokines (Chen et al. 2013; Echeverry et al. 2009; Lantero et al. 2012). Recently, TGF-β1 has been shown to rapidly, within 1 minute, modulate synaptic transmission and neuronal excitability in the spinal cord and DRG, respectively, via a noncanonical mechanism (Chen et al. 2015). Identifying the details of TGF-β1 signaling that provide analgesia will be important in understanding how MSCs reduce chronic pain.

10.5.2 Interleukin-10

Interleukin-10 (IL-10) is another powerful anti-inflammatory cytokine that is produced by MSCs, which causes many effects on immunoregulation and inflammation. IL-10 expression can be additionally enhanced by TLR ligands and PEG2 (Plumas et al. 2005). Currently, IL-10 has been shown to inhibit pro-inflammatory factors, antigen-presenting cell maturation, expression of MHC, and costimulatory factors. Relative to the high production of TGF-β by MSCs, the release of IL-10 from MSCs was found to be very low (Chen et al. 2015). The role of IL-10 in MSC-mediated analgesia is controversial. When using MSCs and MSC-conditioned media, some authors reported no significant changes in the level of IL-10 in different ex vivo and in vitro models (Nemeth et al. 2009).

Chen et al. in 2015 demonstrated that IL-10 antibodies did not neutralize the analgesic effects of MSCs, suggesting that IL-10 release does not seem to contribute to pain relief induced by MSCs. However, a recent study showed the opposite to be true; analgesic effects of MSCs were reversed by IL-10 and TGF-β antibody neutralization (Li et al. 2017). Thus, the roles and mechanisms of IL-10 in modulating chronic pain need to be further examined.

10.5.3 Tumor Necrosis Factor-Stimulated Gene-6 (TSG-6)

Tumor necrosis factor-stimulated gene-6 (TSG-6) is an inflammation-associated secreted glycoprotein that is upregulated in many pathological and physiological contexts (Day and Milner 2019; Milner and Day 2003). Aggregated MSCs express TSG-6, which is an important immune-suppressive factor (Sala et al. 2015). TSG-6's relationship with MSCs has been extensively investigated and it seems that MSCs increase levels of TSG-6, which can reduce inflammation (Sala et al. 2015). Intravenously administered MSCs were mostly trapped in the lungs as illustrated by cell tracking studies and the MSCs in the lung had TSG-6 as one of the highest upregulated transcripts (Huh et al. 2017). The silencing of TSG-6 in MSCs resulted in a loss of beneficial effects whereas exogenous TSG-6 administration replicated the therapeutic effects of MSCs (Wang et al. 2012).

10.5.4 Hepatocyte Growth Factor 1 (HGF-1)

Hepatocyte growth factor 1 (HGF-1) was initially isolated as a liver mitogen and is expressed by MSCs (Rasmusson 2006; Son et al. 2006). Overall, HGF is a paracrine cellular growth, motility, and morphogenic factor and its activation is linked with several regenerative processes (Lefebvre et al. 2012). HGF induces IL-10 expression in monocytes and promotes IL-10 signaling in Treg cells. HGF produced by

MSCs also promotes expansion of immune-suppressive myeloid-derived suppressor cells (MDSC), which release soluble factors that are implicated in the regenerative process (Benkhoucha et al. 2010).

In vivo administration of HGF-1 provides a protective effect from autoimmune disease. This occurs by activating regulatory T cells which then produce IL-10 (Okunishi et al. 2007). In the EAE model of multiple sclerosis, the protective effects of MSCs and MSC-conditioned media are no longer observed when blocking HGF-1 using antibodies, demonstrating the key role of HGF-1 in the therapeutic activities of MSCs (Bai et al. 2012). Moreover, HGF-1 is seemingly required for the neuroprotective effects of MSC-conditioned media (Lu et al. 2011). As a result, the production of HGF-1 by MSCs shows a variety of effects including angiogenesis, immune modulation, and protection from apoptosis (Huh et al. 2017).

10.5.5 Tissue Inhibitors of Metalloproteinase (TIMPs)

Metalloproteinases (MMPs) are part of a class of calcium-dependent enzymes that require a zinc ion in their active site for catalytic activity. MMP activity is regulated by endogenous proteins called tissue inhibitors of metalloproteinases (TIMPs) that bind to active and alternative sites of the activated MMP (Malemud 2006). Recently, human umbilical cord plasma was enriched with TIMP-2 and showed that systemic treatments of TIMP-2 and umbilical cord plasma in aged mice increased synaptic plasticity and improved hippocampal-dependent cognition (Castellano et al. 2017).

TIMP proteins were able to suppress neuropathic pain; TIMP-1 alleviated early-phase neuropathic pain and TIMP-2 reduced late-phase neuropathic pain (Kawasaki et al. 2008). These findings, in combination with the fact that TIMP-2 was present in umbilical plasma, suggest that similar adult MSCs may produce TIMP proteins that can inhibit MMP-mediated neuroinflammation and pain (Huh et al. 2017).

10.5.6 Interleukin-1 Receptor Antagonist (IL1Ra)

IL1Ra has been shown to clinically reduce pain following acute anterior cruciate ligament injury (Chevalier et al. 2005; Kraus et al. 2012). Accumulating evidence has demonstrated that IL-1 plays a key role in modulating pain (Honore et al. 2006; Schweizer et al. 1988; Wolf et al. 2003). IL-1 is a pro-inflammatory cytokine; regulating the IL-1 receptor may affect IL-1 function and inflammatory responses. MSCs express IL1Ra and this elevates IL-10 expression and suppresses CD4+ T cell activation, overall causing MSCs to promote M2 macrophage polarization and Treg generation. IL1RA could also suppress B-cell differentiation and antibody production (In 't Anker et al. 2003; Mareschi et al. 2001).

10.5.7 Indoleamine 2,3-Dioxygenase (IDO)

IDO has two isoforms: IDO1 and IDO2. These isoforms deplete the amount of tryptophan by catalyzing the amino acid into different metabolites (Meisel et al. 2004). The depletion of tryptophan results in T cell arrest cells (Bottcher et al. 2016). Tryptophan depletion could induce Treg generation as well (Mauri and Bosma 2012). MSCs express and utilize IDO as an immunosuppressant (Ren et al. 2009). If not expressed in MSCs in the quiescent state, expression can be induced by IFN-γ and enhanced by PGE2 (Dai and Gupta 1990).

10.5.8 Prostaglandin E2 (PGE2)

PGE2 is of particular interest because direct delivery of PGE2 can induce inflammation and the benefits of aspirin-like drugs are at least partially due to their ability to inhibit PGE2 production (McCoy et al. 2002; Stock et al. 2001). MSCs express COX-2 which produces PGE2 (Aggarwal and Pittenger 2005). The production of PGE2 is enhanced by inflammatory stimuli or the combination of IFN-γ and TNF-α treatment demonstrating that MSCs produce high amounts of PGE2 to suppress the immune response (English et al. 2007). Some studies show that PGE2 may have pro-inflammatory effects since it enhances DC maturation and T cell proliferation (Sreeramkumar et al. 2012). PGE2-mediated acute inflammatory response may promote the resolution process.

10.6 Extracellular Vesicles (EVs)

A variety of extracellular vesicles (EVs) are released by MSCs, including exosomes and microvesicles. Exosomes, which range from 40 to 100 nm in size, are formed from the fusion of multi-vesicular bodies (a late endosome containing membrane-bound intraluminal vesicles) within the late endosomal compartment and released from the plasma membrane. This is opposed to microvesicles, which are more typically 100–1000 nm in diameter and are generated via direct budding from the cell membrane (Heijnen et al. 1999). Distinguishing between these two types of extracellular vesicles is challenging given overlapping characteristics and surface markers. While some investigators have ascribed their results specifically to exosomes, exosomes and microvesicles are released concomitantly and commonly co-isolated, and so the findings discussed below are more inclusively and accurately ascribed to MSC extracellular vesicles (Lotvall et al. 2014; Thery et al. 2018). MSC-EVs transport mRNAs that can change the behavior of target cells by affecting different functions including control of transcription, cell proliferation, and immune regulation (Bruno et al. 2009).

MSC-EVs contain a wide variety of biologically active molecules, including cytokines and growth factors, signaling lipids, mRNA, regulatory miRNA, and lncRNA molecules. MSC-EVs can facilitate the transfer of these molecules from the MSCs of origin to distant recipient cells causing a modification in the target cell behavior (Phinney and Pittenger 2017). Mechanisms for EV action on target cells include interaction with cell surface receptors or internalization of biologically active proteins, mRNAs, or miRNAs. EVs secreted from MSCs carry surface markers reflective of their cell of origin, including CD29, CD73, CD44, and CD105 (Camussi et al. 2010). However, MSC-EV content is not simply reflective of the composition of the cells of origin; MSC-EVs are selectively enriched for specific mRNA, miRNA, and proteins (Beninson and Fleshner 2014; Bruno et al. 2009, 2015; Borger et al. 2017; Camussi et al. 2010; Collino et al. 2010; Eirin et al. 2014; Kim et al. 2012; Tsiapalis and O'Driscoll 2020).

Increasingly commonly, MSC-EVs are being found to have a comparable neuroimmune-modulating activity to that of MSCs themselves (Borger et al. 2017; Bruno et al. 2015). MSCs also carry proteins involved in immune tolerance, notably PD-L1, galectin (Gal-1) and TGF-β1 (Mokarizadeh et al. 2012). Diverse studies have demonstrated MSC-EV modulation of microglial activity and attenuation of neuroinflammatory disease (Harrell et al. 2019). In a mouse model of Alzheimer's disease, EVs isolated from human umbilical cord MSCs induced polarization of microglia toward immunosuppressive M2 phenotype. This was associated with significantly increased levels of NEP, IDE, IL-10, and TGF-β and reduced levels of the inflammatory cytokines TNF-α and IL-1β in the brain tissue of treated mice (Ding et al. 2018). In a mouse model of multiple sclerosis, MSC-EVs were shown to attenuate microglia-derived inflammatory cytokines TNF-α, IL-1-β, IL-18, IL-6, and IL-12 as well as T cell-derived IFN-γ and IL-17A (Laso-Garcia et al. 2018). The ability of systemic administration of MSC-EVs to decrease circulating levels of the pro-inflammatory cytokine TNF-α and increase circulating levels of the anti-inflammatory cytokines TGF-β and IL-10 has been reported across animal models as well as in human patients (Nassar et al. 2016). MSC-EV cargo also includes miRNAs, and MSC-EVs are particularly enriched with miR-223, miR-564, and miR-451. These highly expressed miRNAs have been found to affect genes related to multi-organ development, cell survival, and immune regulation (Collino et al. 2010). Furthermore, EVs from MSCs also carry proteins involved in immune tolerance, notably PD-L1, Gal-1, and TGF-β (Mokarizadeh et al. 2012).

The immunologically active contents of MSC-EVs possess the potential to attenuate immune activation (Bruno et al. 2015). Namely, MSC-EV treatment can lead to the downregulation of auto-reactive lymphocyte proliferation, promotion of PGE2 and TGF-β by T cells, and transfer of mRNA and protein such as TGF-β directly to recipient immune cells, all of which can restore an activated immune environment to a physiological state (Zhang et al. 2014a). Therefore, MSC-EVs possess significant potential in treating neuroinflammation and chronic pain.

Regarding the efficacy of MSC-EVs as a treatment for neuropathic pain, a very limited number of studies have investigated MSC-EVs in this context and have been limited to nerve-injury models. In a rat spinal nerve ligation model of neuropathic

pain, Shiue et al. (2019) demonstrated that EVs isolated from cultured human umbilical cord MSCs could attenuate mechanical allodynia and thermal hyperalgesia in a dose-dependent manner when injected intrathecally. This effect was seen as early as 15 minutes after injection. The longevity of a single injection was not elaborated on. Instead, they demonstrated that continuous intrathecal infusion of EVs could both prevent nerve ligation-induced pain, when started immediately following nerve injury, and reverse it, when started four days after nerve ligation. Ligation-induced upregulation of oligodendrocytes, astrocytes, satellite glial cells, and microglia was attenuated within both the spinal cord and DRG following EV administration, as was upregulation of pro-inflammatory cytokines TNF-α and IL-1β in the DRG. In a follow-up study, the same group demonstrated similar effectiveness of a different route of EV administration in the same model of nerve injury-induced pain. A sponge-like alginate scaffold was soaked with MSC-EVs and then wrapped around the spinal nerve ligation site. Major findings paralleled those of intrathecal administration, including attenuation of mechanical allodynia and thermal hyperalgesia, attenuation of local glial activation, attenuation of pro-inflammatory markers, and enhanced expression of anti-inflammatory IL-10 and neurotrophic GDNF. This study also presented limited evidence suggesting that MSC-EV treatment induced myelination (Hsu et al. 2020).

While the investigation into the effectiveness of MSC-EVs to alter neuropathic pain following nerve injury has been limited, a growing body of evidence has demonstrated that MSC-EVs can promote neuronal survival and regeneration. Local delivery of MSC-derived extracellular vesicles has shown promise in promoting neuronal survival and axonal regeneration in several animal models (Li et al. 2020; Rao et al. 2019; Wang et al. 2021). Further investigation should determine whether these outcomes correlate with improvement in neuropathic pain outcomes.

Regarding the efficacy of MSC-EVs as a treatment for degenerative/inflammatory pain, anti-degenerative and immunomodulatory effects have been repeatedly demonstrated, but pain outcomes remain underexplored. In several studies of intervertebral disk degeneration, MSC-EVs derived from varying types of MSCs have been shown to promote extracellular matrix homeostasis and promote cell survival and proliferation (Lu et al. 2021). In studies of osteoarthritis, the evidence suggests that MSC-derived exosomes may protect cartilage and bone from degradation at least in part by regulating the inflammatory response (Mianehsaz et al. 2019). In vivo, MSC-EVs have been reported to reduce the production of the inflammatory mediators TNF-α, IL-6, PGE2, and nitric oxide as well as the production of matrix metallopeptidase 13 from chondrocytes of patients with osteoarthritis, while at the same time enhancing the production of the anti-inflammatory cytokine IL-10 (Tofino-Vian et al. 2018). In vivo, MSC-EVs have been reported to suppress pain and reduce inflammation in a rat model of temporomandibular joint osteoarthritis. Using chondrocyte culture, this separate research group re-demonstrated suppression of nitric oxide and MMP13 production from chondrocytes following treatment with MSC-EVs (Zhang et al. 2019).

While the immunomodulatory effects of MSC-EVs are well-established, the efficacy of MSC-derived extracellular vesicles in addressing chronic pain remains

underexplored. If effective for chronic pain, MSC-EVs possess several advantages as therapeutics as compared to cellular therapy or synthetic drug delivery nanoparticles. These include lack of immunogenicity, the ability to cross major biological barriers such as the blood–brain barrier, increased stability and processing as compared to MSCs, no tumor formation potential, and intrinsic as well as modifiable homing capacity (E. L. Andaloussi et al. 2013; Rashed et al. 2017). Further, MSC-EV composition can be manipulated through the manipulation of MSC culture conditions (Beninson and Fleshner 2014; Tsiapalis and O'Driscoll 2020). Significantly higher concentrations of immunosuppressive cytokines have been reported in EVs from MSCs first primed with inflammatory cytokines (TNF-α and IFN-γ) as compared to MSC-EVs from standard culture conditions (Lai et al. 2019). This suggests that the therapeutic properties of MSC-EVs can be optimized via the manipulation of culture conditions. Culture conditions that replicate inflammation or biological stress may result in an anti-inflammatory and/or pro-survival EV phenotype. With further investigation, MSC-EVs may supplant MSCs in many therapeutic applications.

10.7 Conclusions

MSCs represent a novel therapy for chronic pain due to their immunomodulatory and neuroprotective properties. MSCs have shown the potential to treat chronic pain conditions in preclinical models that have been historically difficult to treat clinically. The analgesic effects conferred by MSCs are mediated by a multitude of secreted mediators that reduce neuroinflammation and pain signaling. Extracellular vesicles secreted from MSCs are increasingly understood to mediate anti-inflammatory, pro-survival, regenerative, and analgesic effects previously attributed to MSCs themselves. These MSC-EVs may hold several advantages as therapeutics as compared to MSC cell therapy, though much remains to be learned. Importantly, there are multiple routes of administration for MSCs that can be catered to clinical needs. Overall, MSCs and their secretome provide a highly promising treatment modality to resolve some of the most prevalent and debilitating chronic pain conditions.

References

Abrams MB, Dominguez C, Pernold K, Reger R, Wiesenfeld-Hallin Z, Olson L, Prockop D. Multipotent mesenchymal stromal cells attenuate chronic inflammation and injury-induced sensitivity to mechanical stimuli in experimental spinal cord injury. Restor Neurol Neurosci. 2009;27(4):307–21.

Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815–22.

Alfaqeh H, Norhamdan MY, Chua KH, Chen HC, Aminuddin BS, Ruszymah BH. Cell based therapy for osteoarthritis in a sheep model: gross and histological assessment. Med J Malaysia. 2008;63(Suppl A):37–8.

Andaloussi SEL, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347–57.

Bai L, Lennon DP, Caplan AI, DeChant A, Hecker J, Kranso J, Zaremba A, Miller RH. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat Neurosci. 2012;15(6):862–70.

Beninson LA, Fleshner M. Exosomes: an emerging factor in stress-induced immunomodulation. Semin Immunol. 2014;26(5):394–401.

Benkhoucha M, Santiago-Raber ML, Schneiter G, Chofflon M, Funakoshi H, Nakamura T, Lalive PH. Hepatocyte growth factor inhibits CNS autoimmunity by inducing tolerogenic dendritic cells and cd25+foxp3+ regulatory t cells. Proc Natl Acad Sci U S A. 2010;107(14):6424–9.

Borger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, Giebel B. Mesenchymal stem/stromal cell-derived extracellular vesicles and their potential as novel immunomodulatory therapeutic agents. Int J Mol Sci. 2017;18(7):1450.

Bottcher M, Hofmann AD, Bruns H, Haibach M, Loschinski R, Saul D, Mackensen A, Le Blanc K, Jitschin R, Mougiakakos D. Mesenchymal stromal cells disrupt mTOR-signaling and aerobic glycolysis during t-cell activation. Stem Cells. 2016;34(2):516–21.

Boukelmoune N, Laumet G, Tang Y, Ma J, Mahant I, Singh SK, Nijboer C, Benders M, Kavelaars A, Heijnen CJ. Nasal administration of mesenchymal stem cells reverses chemotherapy-induced peripheral neuropathy in mice. Brain Behav Immun. 2021;93:43–54.

Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20(5):1053–67.

Bruno S, Deregibus MC, Camussi G. The secretome of mesenchymal stromal cells: role of extracellular vesicles in immunomodulation. Immunol Lett. 2015;168(2):154–8.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood. 2001;98(8):2396–402.

Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int. 2010;78(9):838–48.

Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9(5):641–50.

Caplan AI, Correa D. The msc: an injury drugstore. Cell Stem Cell. 2011;9(1):11–5.

Castellano JM, Mosher KI, Abbey RJ, McBride AA, James ML, Berdnik D, Shen JC, Zou B, Xie XS, Tingle M, et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature. 2017;544(7651):488–92.

Castro-Malaspina H, Gay RE, Resnick G, Kapoor N, Meyers P, Chiarieri D, McKenzie S, Broxmeyer HE, Moore MA. Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. Blood. 1980;56(2):289–301.

Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739–49.

Chang CL, Leu S, Sung HC, Zhen YY, Cho CL, Chen A, Tsai TH, Chung SY, Chai HT, Sun CK, et al. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. J Transl Med. 2012;10:244.

Chen NF, Huang SY, Chen WF, Chen CH, Lu CH, Chen CL, Yang SN, Wang HM, Wen ZH. TGF-beta1 attenuates spinal neuroinflammation and the excitatory amino acid system in rats with neuropathic pain. J Pain. 2013;14(12):1671–85.

Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-beta secretion. J Clin Invest. 2015;125(8):3226–40.

Chen G, Kim YH, Li H, Luo H, Liu DL, Zhang ZJ, Lay M, Chang W, Zhang YQ, Ji RR. PD-L1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via PD-1. Nat Neurosci. 2017;20(7):917–26.

Cheng J. Is it time to redefine neuropathic pain? Malden: Pain Medicine; 2021.

Chevalier X, Giraudeau B, Conrozier T, Marliere J, Kiefer P, Goupille P. Safety study of intraarticular injection of interleukin 1 receptor antagonist in patients with painful knee osteoarthritis: a multicenter study. J Rheumatol. 2005;32(7):1317–23.

Chiu GS, Boukelmoune N, Chiang ACA, Peng B, Rao V, Kingsley C, Liu HL, Kavelaars A, Kesler SR, Heijnen CJ. Nasal administration of mesenchymal stem cells restores cisplatin-induced cognitive impairment and brain damage in mice. Oncotarget. 2018;9(85):35581–97.

Collino F, Deregibus MC, Bruno S, Sterpone L, Aghemo G, Viltono L, Tetta C, Camussi G. Microvesicles derived from adult human bone marrow and tissue specific mesenchymal stem cells shuttle selected pattern of mirnas. PLoS One. 2010;5(7):e11803.

Dai W, Gupta SL. Regulation of indoleamine 2,3-dioxygenase gene expression in human fibroblasts by interferon-gamma. Upstream control region discriminates between interferon-gamma and interferon-alpha. J Biol Chem. 1990;265(32):19871–7.

Day AJ, Milner CM. TSG-6: a multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol. 2019;78–79:60–83.

de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, Shankar AS, O'Flynn L, Elliman SJ, Roy D, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. Stem Cells. 2018;36(4):602–15.

Ding M, Shen Y, Wang P, Xie Z, Xu S, Zhu Z, Wang Y, Lyu Y, Wang D, Xu L, et al. Exosomes isolated from human umbilical cord mesenchymal stem cells alleviate neuroinflammation and reduce amyloid-beta deposition by modulating microglial activation in Alzheimer's disease. Neurochem Res. 2018;43(11):2165–77.

Durand C, Pezet S, Eutamene H, Demarquay C, Mathieu N, Moussa L, Daudin R, Holler V, Sabourin JC, Milliat F, et al. Persistent visceral allodynia in rats exposed to colorectal irradiation is reversed by mesenchymal stromal cell treatment. Pain. 2015;156(8):1465–76.

Echeverry S, Shi XQ, Haw A, Liu H, Zhang ZW, Zhang J. Transforming growth factor-beta1 impairs neuropathic pain through pleiotropic effects. Mol Pain. 2009;5:16.

Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, Baan CC, Dahlke MH, Hoogduijn MJ. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3:297.

Eirin A, Riester SM, Zhu XY, Tang H, Evans JM, O'Brien D, van Wijnen AJ, Lerman LO. MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. Gene. 2014;551(1):55–64.

English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells. Immunol Lett. 2007;110(2):91–100.

Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, Laine GA, Cox CS Jr. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev. 2009;18(5):683–92.

Fischer G, Wang F, Xiang H, Bai X, Yu H, Hogan QH. Inhibition of neuropathic hyperalgesia by intrathecal bone marrow stromal cells is associated with alteration of multiple soluble factors in cerebrospinal fluid. Exp Brain Res. 2017;235(9):2627–38.

Freitag J, Bates D, Boyd R, Shah K, Barnard A, Huguenin L, Tenen A. Mesenchymal stem cell therapy in the treatment of osteoarthritis: reparative pathways, safety and efficacy – a review. BMC Musculoskelet Disord. 2016;17:230.

Galeano C, Qiu Z, Mishra A, Farnsworth SL, Hemmi JJ, Moreira A, Edenhoffer P, Hornsby PJ. The route by which intranasally delivered stem cells enter the central nervous system. Cell Transplant. 2018;27(3):501–14.

Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. J Cell Physiol. 2007;211(1):27–35.
Gonçalves FDC, Luk F, Korevaar SS, Bouzid R, Paz AH, López-Iglesias C, Baan CC, Merino A, Hoogduijn MJ. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. Sci Rep. 2017;7(1):12100.
Gotherstrom C, West A, Liden J, Uzunel M, Lahesmaa R, Le Blanc K. Difference in gene expression between human fetal liver and adult bone marrow mesenchymal stem cells. Haematologica. 2005;90(8):1017–26.
Grigolo B, Lisignoli G, Desando G, Cavallo C, Marconi E, Tschon M, Giavaresi G, Fini M, Giardino R, Facchini A. Osteoarthritis treated with mesenchymal stem cells on hyaluronan-based scaffold in rabbit. Tissue Eng Part C Methods. 2009;15(4):647–58.
Guo W, Wang H, Zou S, Gu M, Watanabe M, Wei F, Dubner R, Huang GT, Ren K. Bone marrow stromal cells produce long-term pain relief in rat models of persistent pain. Stem Cells (Dayton, Ohio). 2011;29(8):1294–303.
Guo W, Chu YX, Imai S, Yang JL, Zou S, Mohammad Z, Wei F, Dubner R, Ren K. Further observations on the behavioral and neural effects of bone marrow stromal cells in rodent pain models. Mol Pain. 2016;12:174480691665804.
Guo W, Imai S, Yang JL, Zou S, Watanabe M, Chu YX, Mohammad Z, Xu H, Moudgil KD, Wei F, et al. In vivo immune interactions of multipotent stromal cells underlie their long-lasting pain-relieving effect. Sci Rep. 2017;7(1):10107.
Harrell CR, Jovicic N, Djonov V, Arsenijevic N, Volarevic V. Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. Cells. 2019;8(12):1605.
Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone. 1992;13(1):69–80.
Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood. 1999;94(11):3791–9.
Holopainen M, Impola U, Lehenkari P, Laitinen S, Kerkela E. Human mesenchymal stromal cell secretome promotes the immunoregulatory phenotype and phagocytosis activity in human macrophages. Cells. 2020;9(9):2142.
Honore P, Wade CL, Zhong C, Harris RR, Wu C, Ghayur T, Iwakura Y, Decker MW, Faltynek C, Sullivan J, et al. Interleukin-1alphabeta gene-deficient mice show reduced nociceptive sensitivity in models of inflammatory and neuropathic pain but not post-operative pain. Behav Brain Res. 2006;167(2):355–64.
Horie M, Choi H, Lee RH, Reger RL, Ylostalo J, Muneta T, Sekiya I, Prockop DJ. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type ii collagen. Osteoarthr Cartil. 2012;20(10):1197–207.
Hsu JM, Shiue SJ, Yang KD, Shiue HS, Hung YW, Pannuru P, Poongodi R, Lin HY, Cheng JK. Locally applied stem cell exosome-scaffold attenuates nerve injury-induced pain in rats. J Pain Res. 2020;13:3257–68.
Hua Z, Liu L, Shen J, Cheng K, Liu A, Yang J, Wang L, Qu T, Yang H, Li Y, et al. Mesenchymal stem cells reversed morphine tolerance and opioid-induced hyperalgesia. Sci Rep. 2016;6:32096.
Huh Y, Ji RR, Chen G. Neuroinflammation, bone marrow stem cells, and chronic pain. Front Immunol. 2017;8:1014.
In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, Willemze R, Fibbe WE, Kanhai HH. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood. 2003;102(4):1548–9.
Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Prolif. 2020;53(1):e12712.
Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood. 2005;105(10):4120–6.

Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell. 2009;4(3):206–16.

Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, Gao YJ, Roy K, Corfas G, Lo EH, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. Nat Med. 2008;14(3):331–6.

Kim HS, Choi DY, Yun SJ, Choi SM, Kang JW, Jung JW, Hwang D, Kim KP, Kim DW. Proteomic analysis of microvesicles derived from human mesenchymal stem cells. J Proteome Res. 2012;11(2):839–49.

Klass M, Gavrikov V, Drury D, Stewart B, Hunter S, Denson DD, Hord A, Csete M. Intravenous mononuclear marrow cells reverse neuropathic pain from experimental mononeuropathy. Anesth Analg. 2007;104(4):944–8.

Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. Stem Cells. 2006;24(2):386–98.

Kraus VB, Birmingham J, Stabler TV, Feng S, Taylor DC, Moorman CT 3rd, Garrett WE, Toth AP. Effects of intraarticular IL1-Ra for acute anterior cruciate ligament knee injury: a randomized controlled pilot trial (nct00332254). Osteoarthr Cartil. 2012;20(4):271–8.

Lai P, Weng J, Guo L, Chen X, Du X. Novel insights into MSC-EVs therapy for immune diseases. Biomark Res. 2019;7:6.

Lantero A, Tramullas M, Diaz A, Hurle MA. Transforming growth factor-beta in normal nociceptive processing and pathological pain models. Mol Neurobiol. 2012;45(1):76–86.

Laso-Garcia F, Ramos-Cejudo J, Carrillo-Salinas FJ, Otero-Ortega L, Feliu A, Gomez-de Frutos M, Mecha M, Diez-Tejedor E, Guaza C, Gutierrez-Fernandez M. Therapeutic potential of extracellular vesicles derived from human mesenchymal stem cells in a model of progressive multiple sclerosis. PLoS One. 2018;13(9):e0202590.

Le Blanc K, Ringden O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2005;11(5):321–34.

Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein tsg-6. Cell Stem Cell. 2009;5(1):54–63.

Lefebvre J, Ancot F, Leroy C, Muharram G, Lemiere A, Tulasne D. Met degradation: more than one stone to shoot a receptor down. FASEB J. 2012;26(4):1387–99.

Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, Katakowski M, Zhang LJ, Lu M, Janakiraman N, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology. 2002;59(4):514–23.

Li J, Deng G, Wang H, Yang M, Yang R, Li X, Zhang X, Yuan H. Interleukin-1beta pre-treated bone marrow stromal cells alleviate neuropathic pain through ccl7-mediated inhibition of microglial activation in the spinal cord. Sci Rep. 2017;7:42260.

Li F, Liu L, Cheng K, Chen Z, Cheng J. The use of stem cell therapy to reverse opioid tolerance. Clin Pharmacol Ther. 2018;103(6):971–4.

Li L, Zhang Y, Mu J, Chen J, Zhang C, Cao H, Gao J. Transplantation of human mesenchymal stem-cell-derived exosomes immobilized in an adhesive hydrogel for effective treatment of spinal cord injury. Nano Lett. 2020;20(6):4298–305.

Liu L, Hua Z, Shen J, Yin Y, Yang J, Cheng K, Liu A, Wang L, Cheng J. Comparative efficacy of multiple variables of mesenchymal stem cell transplantation for the treatment of neuropathic pain in rats. Mil Med. 2017;182(S1):175–84.

Lotvall J, Hill AF, Hochberg F, Buzas EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the international society for extracellular vesicles. J Extracell Vesicles. 2014;3:26913.

Lu S, Lu C, Han Q, Li J, Du Z, Liao L, Zhao RC. Adipose-derived mesenchymal stem cells protect PC12 cells from glutamate excitotoxicity-induced apoptosis by upregulation of XIAP through PI3-K/Akt activation. Toxicology. 2011;279(1–3):189–95.

Lu L, Xu A, Gao F, Tian C, Wang H, Zhang J, Xie Y, Liu P, Liu S, Yang C, et al. Mesenchymal stem cell-derived exosomes as a novel strategy for the treatment of intervertebral disc degeneration. Front Cell Dev Biol. 2021;9:770510.

Luk F, de Witte SF, Korevaar SS, Roemeling-van Rhijn M, Franquesa M, Strini T, van den Engel S, Gargesha M, Roy D, Dor FJ, et al. Inactivated mesenchymal stem cells maintain immunomodulatory capacity. Stem Cells Dev. 2016;25(18):1342–54.

Maccario R, Podesta M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ t-cell subsets expressing a regulatory/suppressive phenotype. Haematologica. 2005;90(4):516–25.

Malemud CJ. Matrix metalloproteinases (MMPs) in health and disease: an overview. Front Biosci. 2006;11:1696–701.

Mareschi K, Biasin E, Piacibello W, Aglietta M, Madon E, Fagioli F. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica. 2001;86(10):1099–100.

Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ, Pelletier JP. Osteoarthritis. Nat Rev Dis Primers. 2016;2:16072.

Mauri C, Bosma A. Immune regulatory function of b cells. Annu Rev Immunol. 2012;30:221–41.

McCoy JM, Wicks JR, Audoly LP. The role of prostaglandin e2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest. 2002;110(5):651–8.

Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic t-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood. 2004;103(12):4619–21.

Mele V, Muraro MG, Calabrese D, Pfaff D, Amatruda N, Amicarella F, Kvinlaug B, Bocelli-Tyndall C, Martin I, Resink TJ, et al. Mesenchymal stromal cells induce epithelial-to-mesenchymal transition in human colorectal cancer cells through the expression of surface-bound TGF-beta. Int J Cancer. 2014;134(11):2583–94.

Mianehsaz E, Mirzaei HR, Mahjoubin-Tehran M, Rezaee A, Sahebnasagh R, Pourhanifeh MH, Mirzaei H, Hamblin MR. Mesenchymal stem cell-derived exosomes: a new therapeutic approach to osteoarthritis? Stem Cell Res Ther. 2019;10(1):340.

Milner CM, Day AJ. TSG-6: a multifunctional protein associated with inflammation. J Cell Sci. 2003;116(Pt 10):1863–73.

Mokarizadeh A, Delirezh N, Morshedi A, Mosayebi G, Farshid AA, Mardani K. Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. Immunol Lett. 2012;147(1–2):47–54.

Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci U S A. 2005;102(50):18171–6.

Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum. 2003;48(12):3464–74.

Musolino PL, Coronel MF, Hokfelt T, Villar MJ. Bone marrow stromal cells induce changes in pain behavior after sciatic nerve constriction. Neurosci Lett. 2007;418(1):97–101.

Naruse K, Sato J, Funakubo M, Hata M, Nakamura N, Kobayashi Y, Kamiya H, Shibata T, Kondo M, Himeno T, et al. Transplantation of bone marrow-derived mononuclear cells improves mechanical hyperalgesia, cold allodynia and nerve function in diabetic neuropathy. PLoS One. 2011;6(11):e27458.

Nassar W, El-Ansary M, Sabry D, Mostafa MA, Fayad T, Kotb E, Temraz M, Saad AN, Essa W, Adel H. Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. Biomater Res. 2016;20:21.

Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin e(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med. 2009;15(1):42–9.

Okunishi K, Dohi M, Fujio K, Nakagome K, Tabata Y, Okasora T, Seki M, Shibuya M, Imamura M, Harada H, et al. Hepatocyte growth factor significantly suppresses collagen-induced arthritis in mice. J Immunol. 2007;179(8):5504–13.

Orozco L, Soler R, Morera C, Alberca M, Sanchez A, Garcia-Sancho J. Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. Transplantation. 2011;92(7):822–8.

Pers YM, Ruiz M, Noel D, Jorgensen C. Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives. Osteoarthr Cartil. 2015;23(11):2027–35.

Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. Stem Cells. 2017;35(4):851–8.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science (New York, NY). 1999;284(5411):143–7.

Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC. Mesenchymal stem cells induce apoptosis of activated t cells. Leukemia. 2005;19(9):1597–604.

Prakash MD, Miller S, Randall-Demllo S, Nurgali K. Mesenchymal stem cell treatment of inflammation-induced cancer. Inflamm Bowel Dis. 2016;22(11):2694–703.

Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science. 1997;276(5309):71–4.

Raffaghello L, Bianchi G, Bertolotto M, Montecucco F, Busca A, Dallegri F, Ottonello L, Pistoia V. Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. Stem Cells. 2008;26(1):151–62.

Rao F, Zhang D, Fang T, Lu C, Wang B, Ding X, Wei S, Zhang Y, Pi W, Xu H, et al. Exosomes from human gingiva-derived mesenchymal stem cells combined with biodegradable chitin conduits promote rat sciatic nerve regeneration. Stem Cells Int. 2019;2019:2546367.

Rashed MH, Bayraktar E, Gouda KH, Abd-Ellah MF, Amero P, Chavez-Reyes A, Rodriguez-Aguayo C. Exosomes: from garbage bins to promising therapeutic targets. Int J Mol Sci. 2017;18(3):538.

Rasmusson I. Immune modulation by mesenchymal stem cells. Exp Cell Res. 2006;312(12):2169–79.

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2(2):141–50.

Ren G, Su J, Zhang L, Zhao X, Ling W, L'Huillie A, Zhang J, Lu Y, Roberts AI, Ji W, et al. Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. Stem Cells. 2009;27(8):1954–62.

Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol. 2014;10(1):44–56.

Sakai D, Andersson GB. Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. Nat Rev Rheumatol. 2015;11(4):243–56.

Sala E, Genua M, Petti L, Anselmo A, Arena V, Cibella J, Zanotti L, D'Alessio S, Scaldaferri F, Luca G, et al. Mesenchymal stem cells reduce colitis in mice via release of tsg6, independently of their localization to the intestine. Gastroenterology. 2015;149(1):163–76. e120

Schnabel LV, Fortier LA, McIlwraith CW, Nobert KM. Therapeutic use of stem cells in horses: which type, how, and when? Vet J. 2013;197(3):570–7.

Schweizer A, Feige U, Fontana A, Muller K, Dinarello CA. Interleukin-1 enhances pain reflexes. Mediation through increased prostaglandin e2 levels. Agents Actions. 1988;25(3–4):246–51.

Shen ZY, Zhang J, Song HL, Zheng WP. Bone-marrow mesenchymal stem cells reduce rat intestinal ischemia-reperfusion injury, ZO-1 downregulation and tight junction disruption via a TNF-alpha-regulated mechanism. World J Gastroenterol. 2013;19(23):3583–95.

Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T, et al. Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. Diabetes. 2008;57(11):3099–107.

Shiue SJ, Rau RH, Shiue HS, Hung YW, Li ZX, Yang KD, Cheng JK. Mesenchymal stem cell exosomes as a cell-free therapy for nerve injury-induced pain in rats. Pain. 2019;160(1):210–23.
Siniscalco D, Giordano C, Galderisi U, Luongo L, Alessio N, Di Bernardo G, de Novellis V, Rossi F, Maione S. Intra-brain microinjection of human mesenchymal stem cells decreases allodynia in neuropathic mice. Cell Mol Life Sci. 2010;67(4):655–69.
Siniscalco D, Giordano C, Galderisi U, Luongo L, de Novellis V, Rossi F, Maione S. Long-lasting effects of human mesenchymal stem cell systemic administration on pain-like behaviors, cellular, and biomolecular modifications in neuropathic mice. Front Integr Neurosci. 2011;5:79.
Son BR, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, Ratajczak MZ, Janowska-Wieczorek A. Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. Stem Cells. 2006;24(5):1254–64.
Spaeth E, Klopp A, Dembinski J, Andreeff M, Marini F. Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Ther. 2008;15(10):730–8.
Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006;107(4):1484–90.
Spanos WC, Nowicki P, Lee DW, Hoover A, Hostager B, Gupta A, Anderson ME, Lee JH. Immune response during therapy with cisplatin or radiation for human papillomavirus-related head and neck cancer. Arch Otolaryngol Head Neck Surg. 2009;135(11):1137–46.
Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. Cell Transplant. 2016;25(5):829–48.
Sreeramkumar V, Fresno M, Cuesta N. Prostaglandin e2 and t cells: friends or foes? Immunol Cell Biol. 2012;90(6):579–86.
Srinath A, Young E, Szigethy E. Pain management in patients with inflammatory bowel disease: translational approaches from bench to bedside. Inflamm Bowel Dis. 2014;20(12):2433–49.
Stock JL, Shinjo K, Burkhardt J, Roach M, Taniguchi K, Ishikawa T, Kim HS, Flannery PJ, Coffman TM, McNeish JD, et al. The prostaglandin e2 ep1 receptor mediates pain perception and regulates blood pressure. J Clin Invest. 2001;107(3):325–31.
Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles. 2018;7(1):1535750.
Tofino-Vian M, Guillen MI, Perez Del Caz MD, Silvestre A, Alcaraz MJ. Microvesicles from human adipose tissue-derived mesenchymal stem cells as a new protective strategy in osteoarthritic chondrocytes. Cell Physiol Biochem. 2018;47(1):11–25.
Tsiapalis D, O'Driscoll L. Mesenchymal stem cell derived extracellular vesicles for tissue engineering and regenerative medicine applications. Cells. 2020;9(4):991.
Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008;8(9):726–36.
Vichaya EG, Molkentine JM, Vermeer DW, Walker AK, Feng R, Holder G, Luu K, Mason RM, Saligan L, Heijnen CJ, et al. Sickness behavior induced by cisplatin chemotherapy and radiotherapy in a murine head and neck cancer model is associated with altered mitochondrial gene expression. Behav Brain Res. 2016;297:241–50.
Wang N, Shao Y, Mei Y, Zhang L, Li Q, Li D, Shi S, Hong Q, Lin H, Chen X. Novel mechanism for mesenchymal stem cells in attenuating peritoneal adhesion: accumulating in the lung and secreting tumor necrosis factor alpha-stimulating gene-6. Stem Cell Res Ther. 2012;3(6):51.
Wang T, Li Y, Guo M, Dong X, Liao M, Du M, Wang X, Yin H, Yan H. Exosome-mediated delivery of the neuroprotective peptide PACAP38 promotes retinal ganglion cell survival and axon regeneration in rats with traumatic optic neuropathy. Front Cell Dev Biol. 2021;9:659783.
Watanabe S, Uchida K, Nakajima H, Matsuo H, Sugita D, Yoshida A, Honjoh K, Johnson WE, Baba H. Early transplantation of mesenchymal stem cells after spinal cord injury relieves pain

hypersensitivity through suppression of pain-related signaling cascades and reduced inflammatory cell recruitment. Stem Cells. 2015;33(6):1902–14.
Wolf G, Yirmiya R, Goshen I, Iverfeldt K, Holmlund L, Takeda K, Shavit Y. Impairment of interleukin-1 (IL-1) signaling reduces basal pain sensitivity in mice: genetic, pharmacological and developmental aspects. Pain. 2003;104(3):471–80.
Wyles CC, Houdek MT, Behfar A, Sierra RJ. Mesenchymal stem cell therapy for osteoarthritis: current perspectives. Stem Cells Cloning. 2015;8:117–24.
Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing t-cell anergy. Blood. 2005;106(5):1755–61.
Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27–37.
Zhang B, Yin Y, Lai RC, Tan SS, Choo AB, Lim SK. Mesenchymal stem cells secrete immunologically active exosomes. Stem Cells Dev. 2014a;23(11):1233–44.
Zhang EJ, Song CH, Ko YK, Lee WH. Intrathecal administration of mesenchymal stem cells reduces the reactive oxygen species and pain behavior in neuropathic rats. Korean J Pain. 2014b;27(3):239–45.
Zhang S, Teo KYW, Chuah SJ, Lai RC, Lim SK, Toh WS. MSC exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis. Biomaterials. 2019;200:35–47.
Zhu Y, Sun Z, Han Q, Liao L, Wang J, Bian C, Li J, Yan X, Liu Y, Shao C, et al. Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. Leukemia. 2009;23(5):925–33.

Chapter 11
Cell-Based Therapies in Clinical Pain Management

Jianguo Cheng

Abstract Development of novel therapies is required to transform care for chronic and refractory pain due to degenerative and neuropathic conditions. Mesenchymal stem cell (MSC)-based therapies are supported by strong biological rationale based upon the remarkable immunomodulatory and analgesic effects of MSCs shown in pre-clinical studies. Such therapies are not only feasible but also are becoming a reality in clinical practice for a wide range of immunologically related diseases. In the field of pain medicine, there is a growing body of literature that reports promising results from randomized clinical trials for joint pain and prospective studies for neuropathic pain. These clinical investigations demonstrate that MSC therapies are not only safe but also efficacious for their respective indications. Further investigations are required to translate research findings to clinical practice. The key to increasing the scientific rigor and success of future clinical trials is to use refined standardized research protocols and cell quality control standards that take into account of factors such as appropriate selection of patients, source of cells, donors of cells, methods of cell processing, route of administration, and number of transplantations (doses). In this chapter, I will focus on these critical aspects of clinical investigation of MSC-based therapies.

Keywords Mesenchymal stem cells (MSCs) · Autologous MSCs · Allogeneic MSCs · Chronic pain · Neuropathic pain · Degenerative joint pain · Discogenic pain · Chronic low back pain · Osteoarthritis · Rheumatoid arthritis · Randomized clinical trials (RCT) · Prospective studies, Systematic reviews

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11.1 Introduction

Managing chronic pain from degenerative and neuropathic conditions is one of the greatest challenges in pain medicine (Cheng 2019a, b; Cheng et al. 2020). Patients with such chronic pain conditions frequently fail to respond to the current treatment continuum (Cheng et al. 2022), ranging from physical, cognitive-behavioral, pharmacological (Xu et al. 2016), interventional (Cheng et al. 2019; Shin and Cheng 2021; Xu et al. 2017, 2021, 2022), to surgical approaches (Cheng et al. 2022; Rogers et al. 2021; Xu et al. 2021) (Fig. 11.1). Thus, novel and more efficacious treatment strategies are urgently needed to relieve the burden of pain, suffering, and disability. Regenerative medicine is a rapidly growing area of research and clinical applications (Buchheit et al. 2020). Recent studies suggest that regenerative therapies may significantly improve symptoms and distinctly modify disease processes of chronic pain through neuroimmune-modulatory and analgesic effects of regenerative agents. In this chapter, we focus on clinical investigations of MSC-based therapies in the management of chronic pain. Preclinical studies of mesenchymal stem cell (MSC) therapies are discussed in Chap. 10. Other regenerative approaches through platelet rich plasma (PRP) and autologous conditioned serum (ACS) are discussed in Chap. 12. Cell-free therapies employing exosomes or gene therapies are active areas of research but are beyond the scope of this chapter.

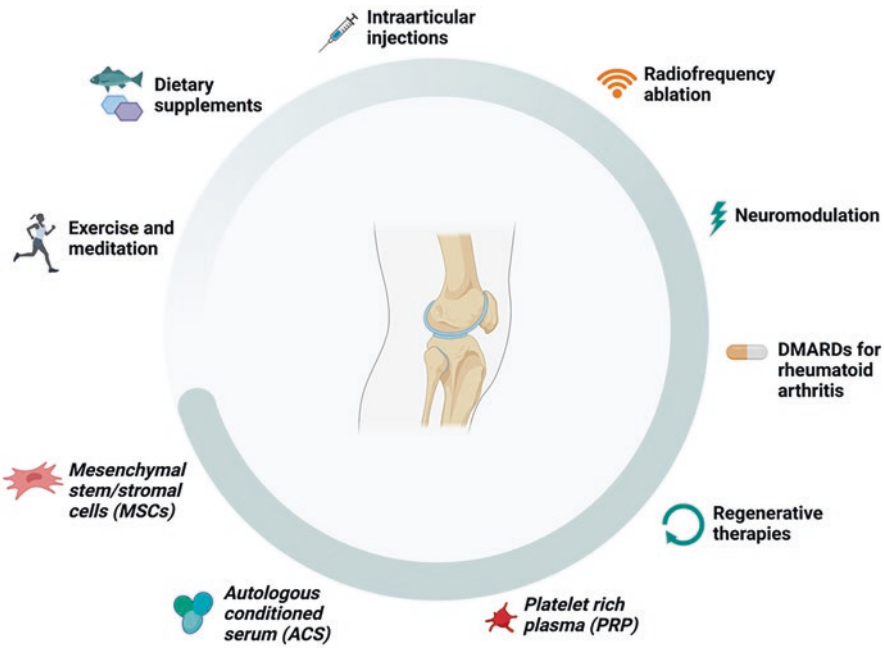

Fig. 11.1 Continuum of joint pain treatment. (Modified from Cheng et al. 2022, Elsevier)

The biological rationale for MSC therapy is multifold and has been discussed extensively in Chap. 10. Briefly, recent studies demonstrate that autoimmune processes and neuroimmune interactions play central roles in the pathogenesis of chronic pain (Birklein et al. 2014; Cuhadar et al. 2019; David Clark et al. 2018; Goebel and Blaes 2013; Helyes et al. 2019; Prasad and Chakravarthy 2021; Tékus et al. 2014; Uçeyler et al. 2007). MSCs, which are present in the perivascular space of nearly all tissues (Lin et al. 2014; Spees et al. 2016), are capable of profoundly modulating neuroimmune functions through multiple mechanisms, including direct cell-to-cell contact, paracrine secretion of cytokines (e.g., TGF-β1, IL-10), chemokines, and growth factors, homing of released exosomes or microvesicles that contain immunoregulatory molecules, and mitochondrial trafficking via tunneling nanotubes (Najar et al. 2016; Song et al. 2020; Spees et al. 2016). Remarkably, it has been demonstrated that apoptotic, metabolically inactivated, or even fragmented MSCs possess immunomodulatory capabilities (Chang et al. 2012; Gonçalves et al. 2017; Luk et al. 2016). As an emerging therapy in pain management (Buchheit et al. 2020), MSC transplantation has shown promise in preclinical studies to treat neuropathic pain (NP) (Chen et al. 2015; Hosseini et al. 2015; Liu et al. 2017), opioid tolerance (Cheng 2018; Hua et al. 2016; Li et al. 2018), and chronic pain due to degenerative musculoskeletal diseases (Centeno et al. 2017; Chakravarthy et al. 2017; Kim et al. 2022; Vega et al. 2015).

Translating MSC-based therapies to clinical practice is not only feasible (Buchheit et al. 2020; Law et al. 2019) but is also a current reality with recent breakthroughs (Levy et al. 2020). The first allogeneic stem cell therapy using a product of adipose-derived hMSCs (Alofisel) has been approved by the European Medicines Agency (EMA) for use in clinical practice in the European Union to treat complex perianal fistulas in Crohn's disease based upon a successful Phase III trial (Panés et al. 2016). In addition to Alofisel, there are 10 globally approved MSC therapies and products to treat a variety of diseases such as graft versus host disease (GvHD) (Canada, New Zealand, and Japan), knee articular cartilage defects (South Korea), spinal cord injury (Japan), critical limb ischemia (India), and acute myocardial infarction (South Korea). Interestingly, in a randomized controlled trial, it is recently demonstrated that infusion of UC-MSCs dramatically improved survival in patients with COVID-19 acute respiratory distress syndrome (Lanzoni et al. 2021). Currently, the use of hMSCs for various diseases is being investigated in nearly 1000 clinical trials (Jayaraman et al. 2021; Kabat et al. 2020), among which about 100 are designed for immune-mediated disorders, such as rheumatoid arthritis (Lopez-Santalla et al. 2020), multiple sclerosis (Zhou et al. 2019), and diabetes (Bhansali et al. 2017; Cai et al. 2016). A significant and consistent finding from published clinical trials is that MSC therapy is not only safe, but also efficacious in improving clinical outcomes in a number of diseases (Saeedi et al. 2019).

11.2 Factors That Determine Clinical Outcomes

11.2.1 Quality Control of MSCs

Quality control of human MSCs is of paramount importance for successful application in clinical investigations and clinical practice. There are established protocols regarding the raw materials, equipment, and processes of generating hMSCs under current good manufacture practice (cGMP). Important considerations include the source of MSCs (bone marrow vs. adipose tissue vs. umbilical cord tissue), autologous vs. allogeneic, age and health status of donors, number of cell passages, and absence of biological and other sources of contamination (Zaim et al. 2012). Early (2nd–3rd) passages of allogeneic hMSCs that meet lot release criteria are typically used to minimize variability and ensure a streamlined and safe supply at low cost (Jayaraman et al. 2021; Pittenger et al. 2019).

Many patients with chronic pain seek experimental therapies after failure of response to available therapies. To fill this void, clinics have emerged to offer "stem cell" therapy using unproven products and methods. This development has led the FDA to issue a Consumer Alert about concerns that patients seeking remedies may be misled by information about products, which place patients at risk (July 22, 2020, FDA).

11.2.2 Transplantation Protocols

Outcomes of clinical investigations of cell-based therapies are determined particular key factors of experimental design, including patient selection, sources of cells, donors of cells, processing methods of cells, route of application, and the number of transplantations (doses). Investigators must take into consideration these critical factors to ensure scientific rigor and the success of clinical investigations. Patient selection through inclusion and exclusion criteria is based upon indications of the therapy and patient characteristics that include biopsychosocial profiling and responsiveness to previous treatments.

Autologous bone marrow aspirate or adipose tissue aspirate containing MSCs are used in many cell-based studies with minimal manipulation of the cells (Durand and Zubair 2022) (Fig. 11.2). Examples of this type of use of autologous MSCs include studies to treat trigeminal nerve neuropathic pain (Vickers et al. 2014), pudendal neuralgia (Venturi et al. 2015), shoulder joint pain (Dwyer et al. 2021), and discogenic pain (Pettine et al. 2015). These types of studies typically lack adequate cell quality control measures and may be unclear about the number, vitality, and purity of cells. Furthermore, the sources of the cells used are cost-prohibitive with limited cell dosages, high donor variability, and potentially biological incompatibility issues. The advantage of using autologous cells, however, is that their use is more permissive under current FDA regulations.

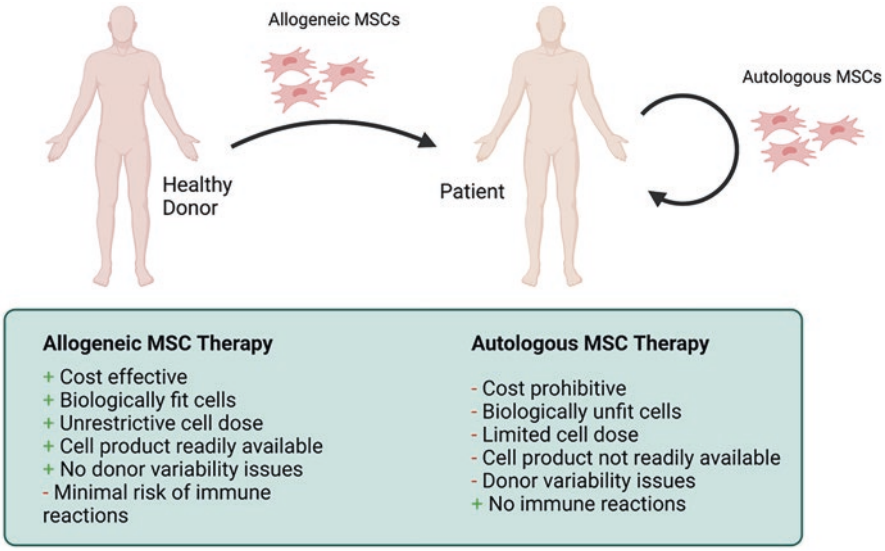

Fig. 11.2 Pros and cons of autologous vs. allogeneic MSCs. (Modified from Durand and Zubair 2022, Elsevier)

Allogeneic MSCs, in contrast, are typically from young and healthy donors who are screened with strict tests and criteria (Durand and Zubair 2022). Cells are processed and multiplied through cell culture under cGMP conditions with comprehensive quality control measures and criteria. Most clinical trials utilize clinical grade BM-MSCs, AD-MSCs, or UC-MSCs that are provided by off-the-shelf and ready-to-use packages. Such cells can be used either directly after thawing or alternatively after subsequent culture so that fresh cells are utilized. The advantages of using allogeneic MSCs include optimal selection of high-quality cells, minimal variability between cell products, cost-effectiveness, and streamlined and safe supply to multiple centers for clinical investigation or application.

A variety of routes of transplantation have been utilized in clinical investigation, including intravenous, intrathecal, intraarticular, intradiscal, subcutaneous, and site of injury applications. The route of application chosen is primarily dictated by the pathophysiology of the clinical indication under investigation. For example, intraarticular injections are used in almost all trials for knee osteoarthritis (OA) as a result of localized degenerative changes in the joint while intravenous injections are most commonly used in trials for knee rheumatoid arthritis (RA) as a result of the systemic autoimmune disorder (Hwang et al. 2021). Either a single injection or repeated multiple dose injections may be studied in clinical trials for sustained therapeutic effects.

11.3 Evidence from Clinical Investigations of MSC Therapy for Degenerative Joint Pain

11.3.1 Knee and Shoulder Osteoarthritis

Osteoarthritis affects more than 46 million Americans and is a major cause of disability. Stem cell therapy for osteoarthritis is an area of intensive research in preclinical and clinical settings (Fig. 11.3) (Cheng 2018). A substantial number of randomized clinical trials (RCTs) on the management of knee OA with stem cells have been reported and 19 meta-analyses of clinical studies were published from January 2020 to July 2021. An independent, systematic review of the literature yielded a total of 183 studies, of which 33 were randomized clinical trials, including a total of 6860 patients with knee OA (Schmitz et al. 2022). The review emphasized that it is important to recognize methodological limitations, interpret the results, and draw conclusions from systematic review and meta-analyses.

Intra-articular injections of MSCs may improve pain and functionality. A systematic review of intra-articular injections of MSCs without adjuvant therapies for knee OA included a total of 19 studies on 440 knees treated (Tan et al. 2021). All

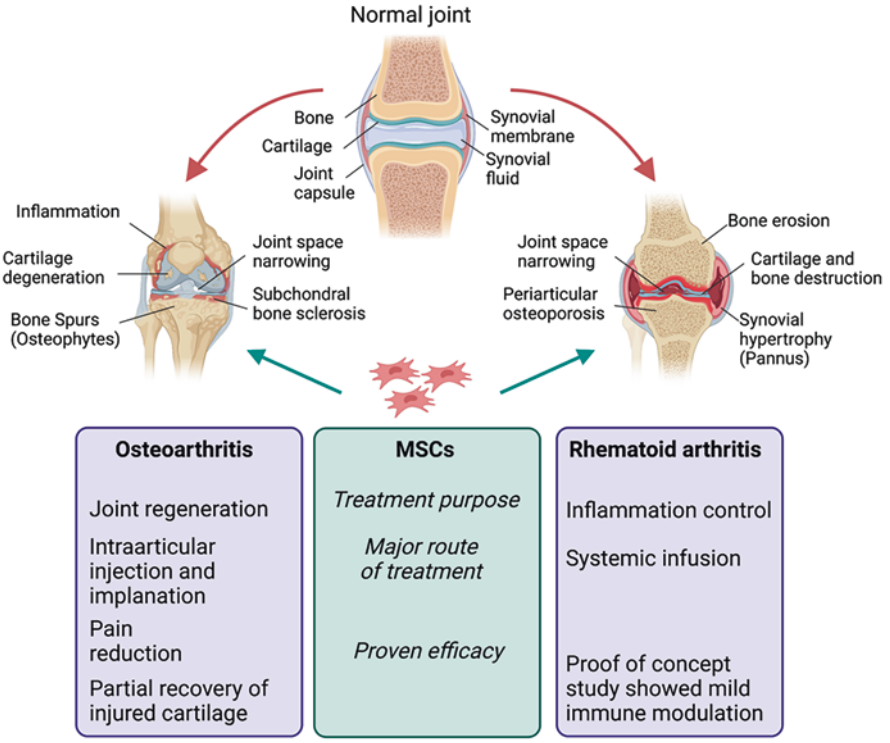

Fig. 11.3 MSCs for OA and RA. (Modified from Hwang et al. 2021)

studies reported an improvement in visual analogue scale (VAS) pain scores and functional outcome measures such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Osteoarthritis Outcome Score following intervention. The review concluded that intra-articular injections of MSCs, without adjuvant therapies, can improve pain and function for osteoarthritis. Significantly better outcomes were obtained with the use of bone marrow-derived MSCs (BM-MSCs) as compared with adipose-derived MSCs (AD-MSCs) and with the use of cultured MSCs as opposed to uncultured MSCs.

There are notable inconsistencies in reported outcomes. A systematic review and meta-analysis of 10 RCTs on MSCs for knee arthritis included studies in 335 patients (Ma et al. 2020). MSC therapy yielded beneficial effects at 6, 12, and 24 months, with significant improvement in VAS, WOMAC and low rates of adverse events. This meta-analysis showed that both BM-MSCs and AD-MSCs had a great application potential in the treatment of knee OA. AD-MSCs tended to be superior to BM-MSCs according to the limited clinical evidence available. However, a similar review of 13 RCTs found that, compared with placebo, there was no significant difference in VAS for pain, WOMAC pain score, WOMAC function score, or WOMAC stiffness score for MSCs (Dai et al. 2021). Therefore, caution must be taken when clinicians interpret the results of meta-analyses of clinical studies on the management of knee OA with stem cells (Schmitz et al. 2022).

The dosage or number of cells administered to a patient, in addition to the source of cells, may impact outcomes. A systematic review and meta-analysis focused on the effects of cell count and included 14 studies involving 564 patients (Muthu et al. 2022). The authors categorized the studies based on the MSC count into four groups, namely less than 1×10^7 cells, between 1–5 $\times 10^7$cells, between 5–10 $\times 10^7$cells, and greater than 10×10^7 cells. They noted incremental decreases in the VAS with increasing dosages of MSCs at 12 months and 24 months and incremental improvement in the WOMAC, KOOS with increasing dosage of MSCs at 12 months respectively. They did not find any significant increase in the adverse events with increased dosage of MSCs in any of the groups compared. It was concluded that treatment with between 5–10 $\times 10^7$ cells showed consistent and significant improvement in pain and functional outcomes compared to the other treatment groups. Hence, a cell count between 5–10 $\times 10^7$ MSCs is recommended for the target site to obtain superior benefits from the procedure.

A recent pilot RCT compared the efficacy of a single, intra-articular, nonconcentrated bone marrow aspirate (BMA) injection in comparison to cortisone for the treatment of glenohumeral joint OA-related shoulder pain (Dwyer et al. 2021). The study included 25 shoulders injections of 22 patients who completed baseline and 12 month patient-reported outcome measures (12 shoulders received cortisone, 13 shoulders received BMA). In the BMA group, a significant improvement was seen in Western Ontario Osteoarthritis of the Shoulder (WOOS) index ($p = 0.002$), the QuickDASH (11 items to measure physical function and symptoms in people with any or multiple musculoskeletal disorders of the upper limb), and the EQ-5D-5L pain dimension between baseline and 12 months. No significant difference was seen for any outcome in the cortisone group between baseline and 12 months. A

significant difference in changes in scores was seen in the QuickDASH and the EQ-5D-5L pain scores and the EQ-5D-5L health scores in favor of BMA. It was concluded that patients with glenohumeral joint OA treated with BMA have superior changes in the QuickDASH and EQ-5D-5L pain and health scores at 12 months post-injection when compared to patients treated with cortisone.

These studies strongly suggest that MSC therapy may provide pain relief and functional improvement in patients with joint pain due to osteoarthritis. Further studies with more rigorous designs should take into account the cell types, cell counts, cell quality, and means of delivery, as well as patient characteristics such as age and stages of disease.

11.3.2 Intervertebral Discogenic Pain

Percutaneously delivered MSC therapy has been proposed as a potential means to holistically ameliorate discogenic low back pain through three mechanisms: mitigation of primary nociceptive disc pain, reduction or reversal of the catabolic metabolism, and restoration of disc tissue.

In an open label pilot study (Pettine et al. 2015), 26 patients (median age 40 years; range 18–61) received autologous bone marrow concentrate (BMC) disc injections (13 one level, 13 two levels). Approximately 1 ml of BMC was analyzed for total nucleated cell (TNC) content, colony-forming unit-fibroblast (CFU-F) frequency, differentiation potential, and phenotype characterization. The average Oswestry disability index (ODI) and VAS scores were reduced to 22.8 and 29.2 at 3 months, 24.4 and 26.3 at 6 months, and 25.0 and 33.2 at 12 months, respectively ($p \leq 0.0001$). Eight of twenty patients had improved disc degenerative severity by one modified Pfirrmann grade on MRI at 1 year. The average BMC contained 121×10^6 TNC/ml with 2713 CFU-F/ml (synonymous with MSCs). Although all subjects presented a substantial reduction in pain, patients receiving greater than 2000 CFU-F/ml experienced a significantly faster and greater reduction in ODI and VAS. Subjects younger than 40 years of age experienced an average pain reduction of 69.5% for ODI ($p = 0.03$) and 70.6% VAS score ($p = 0.01$) at 12 months. This study provides evidence of the safety and feasibility for the nonsurgical treatment of DDD with autologous BMC and indicates an effect of mesenchymal cell concentration on discogenic pain reduction. Follow-up studies further confirmed these findings in the non-surgical treatment of discogenic pain with autologous BMC, with durable pain relief (71% VAS reduction) and ODI improvements (>64%) through two years (Pettine et al. 2016) and three years (Pettine et al. 2017).

A systematic review of 7 studies involving 97 patients found significant improvements in VAS (66.0–20.9 mm, $p < 0.001$) and ODI (44.4–19.1, $p < 0.001$) after the intradiscal BMC injection. It was concluded that intradiscal injection of BMC for lumbar disc degeneration resulted in statistically and clinically significant improvements in VAS and ODI with low re-injection and complication rates (Hirase et al. 2020). More recently, we systematically reviewed the effectiveness of intradiscal

biologic treatments for discogenic low back pain (Schneider et al. 2022). It was found that, for mesenchymal stem cells, the aggregate success rate (≥50% improvement) at six months is 53.5% (95% Confidence Interval: 38.6–68.4%), though using worst-case analysis this rate decreased to 40.7% (95% Confidence Interval: 28.1–53.2%). Also, ≥30% functional improvement was achieved in 74.3% of cases (95% Confidence Interval: 59.8–88.7%) at 6 months but using worst-case analysis, this rate decreased to 44.1% (95% Confidence Interval: 28.1–53.2%). Thus, preliminary observation supports the use of intradiscal biologic agents for the treatment of discogenic low back pain.

11.4 Evidence from Clinical Investigations of MSC Therapy for Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, which affects the lining of the synovial joints causing inflammation, loss of mobility, erosion of joints, and stiffness and pain (Fig. 11.3) (Hwang et al. 2021). A key component for the pathogenesis of RA is abnormal immune responses against the synovium. Progression of RA is characterized by dysfunction of innate and adaptive immunity, including dysregulated cytokine networks and immune complex-mediated complement activation. Current treatments to modulate the altered immune responses include corticosteroids, antirheumatic drugs, and biological agents, which may cause adverse effects to a significant number of RA patients. Additionally, some RA patients are resistant to these therapies. In recent years, MSC-based therapies have been proposed as a novel and promising therapeutic approach in the treatment of RA (Lopez-Santalla et al. 2020). To date, nearly one hundred studies in animal RA models have shown promising results for clinical application. Proof-of-concept clinical studies have demonstrated a satisfactory safety profile for RA treatment with MSC therapy.

Clinical trial registrations in RA patients with MSC therapy have increased linearly since 2011 and reached a plateau in 2018 (Lopez-Santalla et al. 2020). In general, toxicity or adverse effects have not been found in any of the RA clinical trials conducted. Not enough sufficient data on efficacy has been obtained from the completed studies, most likely because these studies were underpowered. Also, the large majority of RA patients enrolled in these studies were refractory to conventional RA treatments with a long history of disease. Given the excellent safety profile of MSC-based therapy, there are eight clinical trials using MSC-based therapy that are registered and active in "clinicaltrials.gov" where MSC therapy at early stages of RA are being explored. For better comparisons of results among clinical trials, an improvement in the standardization of treatment protocols is needed in terms of sources of MSCs, MHC contexts, manufacturing protocols, routes of delivery, cell dosing (cell number), and systematic analysis of the results. Additionally, appropriate selection of patients who are most likely to respond to the therapy will benefit the clinical application of MSC therapy for RA.

11.5 Evidence from Clinical Investigation of MSC Therapy for Neuropathic Pain

Many neuropathic pain conditions are debilitating and difficult to treat. Managing neuropathic pain is one of the most significant challenges in clinical pain. Application of MSC therapy is limited to a few prospective case series, in which autologous adipose or bone marrow aspirates containing "MSCs" were injected locally or intrathecally, and patients reported a significant reduction of neuropathic pain caused by injuries of the trigeminal nerve (Vickers et al. 2014), the pudendal nerve (Venturi et al. 2015), or the spinal cord (Vaquero et al. 2018). While intriguing, these clinical reports are preliminary due to a lack of control groups and small sample sizes.

Trigeminal neuropathic pain is a debilitating condition that affects the face. It is often refractory to pharmacological or procedural treatment. In a case series (Vickers et al. 2014), 10 female patients with symptoms of neuropathic trigeminal pain were prospectively treated with local injections into the pain field with the stromal vascular fraction of lipoaspirate that contained 33 million to 162 million autologous "MSCs" with a cell viability of 62–91%. There were no systemic or local tissue side effects from the stem cell therapy (n = 41 oral and facial injection sites). At 6 months, 5 out of 9 subjects had reductions in both pain intensity scores and use of anti-neuropathic medication. Their mean numeric rating scale (NRS) pain scores were also significantly reduced from 7.5 at the pre-treatment timepoint to 4.3 at 6 months. Thus, this preliminary open-labeled study is promising, showing that administration of autologous stem cells is a safe and well-tolerated intervention for neuropathic trigeminal pain and significantly reduced pain intensity at 6 months.

Pudendal neuralgia is also a difficult-to-treat condition that affects the perineal area. A case series of 15 female patients with pudendal neuralgia were prospectively treated with transperineal injections of autologous adipose tissue with MSCs along the Alcock's canal (Venturi et al. 2015). Twelve patients completed the follow-up protocol. There were no complications. Two patients had no pain improvement and continued to use analgesic drugs. The mean VAS pain score significantly reduced from 8.1 pre-treatment to 3.3 at 12 months while the health quality measure SF36 significantly improve from 85.0 pre-treatment to 75.5 at 12 months. It was concluded that this new treatment is readily administered, carries low risk of complications, and provides significant improvement of symptoms.

Spinal cord injury (SCI)-related neuropathic pain represents a significant cause of decreased quality of life. In a prospective study (Vaquero et al. 2018), 10 patients suffering from chronic SCI received 100 million BM-MSCs into the subarachnoid space by lumbar puncture during the first month of the study. This procedure was repeated at months 4 and 7 of the study, reaching a total dose of 300 million cells. The mean VAS pain score reduced significantly from 5.5 pre-treatment to 1.5 at 10 months post-treatment. This study supports the use of intrathecal administration of autologous MSCs for the treatment of neuropathic pain in patients with SCI.

11.6 Summary

MSC therapy has shown its safety and efficacy in studies ranging from RCTs for joint pain to prospective case series for neuropathic pain. MSC therapy has shown potential for treating both OA and RA with reduced pain, improved joint function, and enhanced overall life satisfaction in patients, although osteoarthritis (OA) patients had more promising results compared to rheumatoid arthritis (RA) patients. Clinical trials on OA and RA demonstrate that MSC therapy is a safe treatment option without serious adverse events. For neuropathic pain, MSC therapy has gained preliminary data that support the safety and efficacy even in patients with the most difficult-to-treat conditions such as trigeminal neuropathic pain, pudendal neuralgia, and SCI-related neuropathic pain. More studies will be required to examine the long-term safety and efficacy of MSC therapies and their respective clinical applications. Future research employing the latest technical advances and experimental protocols hold the key to increasing scientific evidence required to effectively translate MSC therapies to clinical practice in pain management.

References

Bhansali S, Dutta P, Kumar V, Yadav MK, Jain A, Mudaliar S, Bhansali S, Sharma RR, Jha V, Marwaha N, et al. Efficacy of autologous bone marrow-derived mesenchymal stem cell and mononuclear cell transplantation in type 2 diabetes mellitus: a randomized, placebo-controlled comparative study. Stem Cells Dev. 2017;26(7):471–81.

Birklein F, Drummond PD, Li W, Schlereth T, Albrecht N, Finch PM, Dawson LF, Clark JD, Kingery WS. Activation of cutaneous immune responses in complex regional pain syndrome. J Pain. 2014;15(5):485–95.

Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and regenerative pain medicine. J Clin Invest. 2020;130(5):2164–76.

Cai J, Wu Z, Xu X, Liao L, Chen J, Huang L, Wu W, Luo F, Wu C, Pugliese A, et al. Umbilical cord mesenchymal stromal cell with autologous bone marrow cell transplantation in established type 1 diabetes: a pilot randomized controlled open-label clinical study to assess safety and impact on insulin secretion. Diabetes Care. 2016;39(1):149–57.

Centeno C, Markle J, Dodson E, Stemper I, Williams CJ, Hyzy M, Ichim T, Freeman M. Treatment of lumbar degenerative disc disease-associated radicular pain with culture-expanded autologous mesenchymal stem cells: a pilot study on safety and efficacy. J Transl Med. 2017;15(1):197.

Chakravarthy K, Chen Y, He C, Christo PJ. Stem cell therapy for chronic pain management: review of uses, advances, and adverse effects. Pain Physician. 2017;20(4):293–305.

Chang CL, Leu S, Sung HC, Zhen YY, Cho CL, Chen A, Tsai TH, Chung SY, Chai HT, Sun CK, et al. Impact of apoptotic adipose-derived mesenchymal stem cells on attenuating organ damage and reducing mortality in rat sepsis syndrome induced by cecal puncture and ligation. J Transl Med. 2012;10:244.

Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via TGF-beta secretion. J Clin Invest. 2015;125(8):3226–40.

Cheng J. State of the art, challenges, and opportunities for pain medicine. Pain Med. 2018;19(6):1109–11.

Cheng J. Concept and overview of neuropathic pain. In: Cheng J, editor. Neuropathic pain: a case-based approach to practical management. New York: Oxford University Press; 2019a. p. 3–10.
Cheng J. Mechanisms of neuropathic pain. In: Cheng J, editor. Neuropathic pain: a case-based approach to practical management. Oxford: Oxford University Press; 2019b. p. 17–26.
Cheng J, Salmasi V, You J, Grille M, Yang D, Mascha EJ, Cheng OT, Zhao F, Rosenquist RW. Outcomes of sympathetic blocks in the management of complex regional pain syndrome: a retrospective cohort study. Anesthesiology. 2019;131(4):883–93.
Cheng J, Rutherford M, Singh VM. The HHS pain management best practice inter-agency task force report calls for patient-centered and individualized care. Pain Med. 2020;21(1):1–3.
Cheng J, Huh Y, Ji RR. Chapter 16. Mechanism-based treatment and precision medicine. In: Benson HT, editor. Practical management of pain. 6th ed. Philadelphia: Mosby/Elsevier; 2022. p. 183–93.
Cuhadar U, Gentry C, Vastani N, Sensi S, Bevan S, Goebel A, Andersson DA. Autoantibodies produce pain in complex regional pain syndrome by sensitizing nociceptors. Pain. 2019;160(12):2855–65.
Dai W, Leng X, Wang J, Shi Z, Cheng J, Hu X, Ao Y. Intra-articular mesenchymal stromal cell injections are no different from placebo in the treatment of knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. Arthrosc J Arthrosc Relat Surg. 2021;37(1):340–58.
David Clark J, Tawfik VL, Tajerian M, Kingery WS. Autoinflammatory and autoimmune contributions to complex regional pain syndrome. Mol Pain. 2018;14:1744806918799127.
Durand N, Zubair AC. Autologous versus allogeneic mesenchymal stem cell therapy: the pros and cons. Surgery. 2022;171(5):1440–2.
Dwyer T, Hoit G, Lee A, Watkins E, Henry P, Leroux T, Veillette C, Theodoropoulos J, Ogilvie-Harris D, Chahal J. Injection of bone marrow aspirate for glenohumeral joint osteoarthritis: a pilot randomized control trial. Arthrosc Sports Med Rehabil. 2021;3(5):e1431–40.
Goebel A, Blaes F. Complex regional pain syndrome, prototype of a novel kind of autoimmune disease. Autoimmun Rev. 2013;12(6):682–6.
Gonçalves FDC, Luk F, Korevaar SS, Bouzid R, Paz AH, López-Iglesias C, Baan CC, Merino A, Hoogduijn MJ. Membrane particles generated from mesenchymal stromal cells modulate immune responses by selective targeting of pro-inflammatory monocytes. Sci Rep. 2017;7(1):12100.
Helyes Z, Tékus V, Szentes N, Pohóczky K, Botz B, Kiss T, Kemény Á, Környei Z, Tóth K, Lénárt N, et al. Transfer of complex regional pain syndrome to mice via human autoantibodies is mediated by interleukin-1-induced mechanisms. Proc Natl Acad Sci U S A. 2019;116(26):13067–76.
Hirase T, Jack RA 2nd, Sochacki KR, Harris JD, Weiner BK. Systematic review: is intradiscal injection of bone marrow concentrate for lumbar disc degeneration effective? Cureus. 2020;12(7):e9045.
Hosseini M, Yousefifard M, Aziznejad H, Nasirinezhad F. The effect of bone marrow-derived mesenchymal stem cell transplantation on allodynia and hyperalgesia in neuropathic animals: a systematic review with meta-analysis. Biol Blood Marrow Transplant. 2015;21(9):1537–44.
Hua Z, Liu L, Shen J, Cheng K, Liu A, Yang J, Wang L, Qu T, Yang H, Li Y, et al. Mesenchymal stem cells reversed morphine tolerance and opioid-induced hyperalgesia. Sci Rep. 2016;6:32096.
Hwang JJ, Rim YA, Nam Y, Ju JH. Recent developments in clinical applications of mesenchymal stem cells in the treatment of rheumatoid arthritis and osteoarthritis. Front Immunol. 2021;12:631291.
Jayaraman P, Lim R, Ng J, Vemuri MC. Acceleration of translational mesenchymal stromal cell therapy through consistent quality GMP manufacturing. Front Cell Dev Biol. 2021;9:648472.
Kabat M, Bobkov I, Kumar S, Grumet M. Trends in mesenchymal stem cell clinical trials 2004–2018: is efficacy optimal in a narrow dose range? Stem Cells Transl Med. 2020;9(1):17–27.
Kim KI, Kim MS, Kim JH. Intra-articular injection of autologous adipose-derived stem cells or stromal vascular fractions: are they effective for patients with knee osteoarthritis? A systematic review with meta-analysis of randomized controlled trials. Am J Sports Med. 2022;3635465211053893:837.

Lanzoni G, Linetsky E, Correa D, Messinger Cayetano S, Alvarez RA, Kouroupis D, Alvarez Gil A, Poggioli R, Ruiz P, Marttos AC, et al. Umbilical cord mesenchymal stem cells for covid-19 acute respiratory distress syndrome: a double-blind, phase 1/2a, randomized controlled trial. Stem Cells Transl Med. 2021;10(5):660–73.

Law L, Hunt CL, van Wijnen AJ, Nassr A, Larson AN, Eldrige JS, Mauck WD, Pingree MJ, Yang J, Muir CW, et al. Office-based mesenchymal stem cell therapy for the treatment of musculoskeletal disease: a systematic review of recent human studies. Pain Med. 2019;20(8):1570–83.

Levy O, Kuai R, Siren EMJ, Bhere D, Milton Y, Nissar N, De Biasio M, Heinelt M, Reeve B, Abdi R, et al. Shattering barriers toward clinically meaningful MSC therapies. Sci Adv. 6(30):eaba6884. 2020;6:eaba6884.

Li F, Liu L, Cheng K, Chen Z, Cheng J. The use of stem cell therapy to reverse opioid tolerance. Clin Pharmacol Ther. 2018;103(6):971–4.

Lin P, Correa D, Kean TJ, Awadallah A, Dennis JE, Caplan AI. Serial transplantation and long-term engraftment of intra-arterially delivered clonally derived mesenchymal stem cells to injured bone marrow. Mol Ther. 2014;22(1):160–8.

Liu L, Hua Z, Shen J, Yin Y, Yang J, Cheng K, Liu A, Wang L, Cheng J. Comparative efficacy of multiple variables of mesenchymal stem cell transplantation for the treatment of neuropathic pain in rats. Mil Med. 2017;182(S1):175–84.

Lopez-Santalla M, Fernandez-Perez R, Garin MI. Mesenchymal stem/stromal cells for rheumatoid arthritis treatment: an update on clinical applications. Cells. 2020;9(8):1852.

Luk F, de Witte SF, Korevaar SS, Roemeling-van Rhijn M, Franquesa M, Strini T, van den Engel S, Gargesha M, Roy D, Dor FJ, et al. Inactivated mesenchymal stem cells maintain immunomodulatory capacity. Stem Cells Dev. 2016;25(18):1342–54.

Ma W, Liu C, Wang S, Xu H, Sun H, Fan X. Efficacy and safety of intra-articular injection of mesenchymal stem cells in the treatment of knee osteoarthritis: a systematic review and meta-analysis. Medicine. 2020;99(49):e23343.

Muthu S, Mir AA, Kumar R, Yadav V, Jeyaraman M, Khanna M. What is the clinically significant ideal mesenchymal stromal cell count in the management of osteoarthritis of the knee? – meta-analysis of randomized controlled trials. J Clin Orthop Trauma. 2022;25:101744.

Najar M, Raicevic G, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L. Mesenchymal stromal cells and immunomodulation: a gathering of regulatory immune cells. Cytotherapy. 2016;18(2):160–71.

Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. Lancet. 2016;388(10051):1281–90.

Pettine KA, Murphy MB, Suzuki RK, Sand TT. Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. Stem Cells. 2015;33(1):146–56.

Pettine K, Suzuki R, Sand T, Murphy M. Treatment of discogenic back pain with autologous bone marrow concentrate injection with minimum two year follow-up. Int Orthop. 2016;40(1):135–40.

Pettine KA, Suzuki RK, Sand TT, Murphy MB. Autologous bone marrow concentrate intradiscal injection for the treatment of degenerative disc disease with three-year follow-up. Int Orthop. 2017;41(10):2097–103.

Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. NPJ Regen Med. 2019;4:22.

Prasad MA, Chakravarthy MK. Review of complex regional pain syndrome and the role of the neuroimmune axis. Mol Pain. 2021;17:17448069211006617.

Rogers S, El-Hayek T, Cheng J. Spinal cord stimulation and intrathecal pumps. In: Banik R, editor. Anesthesiology in-training exam review: regional anesthesia and chronic pain. Springer-Nature: Cham; 2021.

Saeedi P, Halabian R, Imani Fooladi AA. A revealing review of mesenchymal stem cells therapy, clinical perspectives and modification strategies. Stem Cell Investig. 2019;6:34.
Schmitz C, Alt C, Pearce DA, Furia JP, Maffulli N, Alt EU. Methodological flaws in meta-analyses of clinical studies on the management of knee osteoarthritis with stem cells: a systematic review. Cells. 2022;11(6):965.
Schneider BJ, Hunt C, Conger A, Qu W, Maus TP, Vorobeychik Y, Cheng J, Duszynski B, McCormick ZL. The effectiveness of intradiscal biologic treatments for discogenic low back pain: a systematic review. Spine J. 2022;22(2):226–37.
Shin C, Cheng J. Interventional treatments for CRPS. In: Lawson E, Castellanos J, editors. Complex regional pain syndrome. Cham: Springer Nature; 2021. p. 179–206.
Song N, Scholtemeijer M, Shah K. Mesenchymal stem cell immunomodulation: mechanisms and therapeutic potential. Trends Pharmacol Sci. 2020;41(9):653–64.
Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Res Ther. 2016;7(1):125.
Tan SHS, Kwan YT, Neo WJ, Chong JY, Kuek TYJ, See JZF, Wong KL, Toh WS, Hui JHP. Intra-articular injections of mesenchymal stem cells without adjuvant therapies for knee osteoarthritis: a systematic review and meta-analysis. Am J Sports Med. 2021;49(11):3113–24.
Tékus V, Hajna Z, Borbély É, Markovics A, Bagoly T, Szolcsányi J, Thompson V, Kemény Á, Helyes Z, Goebel A. A CRPS-IGG-transfer-trauma model reproducing inflammatory and positive sensory signs associated with complex regional pain syndrome. Pain. 2014;155(2):299–308.
Uçeyler N, Eberle T, Rolke R, Birklein F, Sommer C. Differential expression patterns of cytokines in complex regional pain syndrome. Pain. 2007;132(1–2):195–205.
Vaquero J, Zurita M, Rico MA, Aguayo C, Fernández C, Gutiérrez R, Rodríguez-Boto G, Saab A, Hassan R, Ortega C. Intrathecal administration of autologous bone marrow stromal cells improves neuropathic pain in patients with spinal cord injury. Neurosci Lett. 2018;670:14–8.
Vega A, Martin-Ferrero MA, Del Canto F, Alberca M, Garcia V, Munar A, Orozco L, Soler R, Fuertes JJ, Huguet M, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. Transplantation. 2015;99(8):1681–90.
Venturi M, Boccasanta P, Lombardi B, Brambilla M, Contessini Avesani E, Vergani C. Pudendal neuralgia: a new option for treatment? Preliminary results on feasibility and efficacy. Pain Med. 2015;16(8):1475–81.
Vickers ER, Karsten E, Flood J, Lilischkis R. A preliminary report on stem cell therapy for neuropathic pain in humans. J Pain Res. 2014;7:255–63.
Xu J, Yang J, Lin P, Rosenquist E, Cheng J. Intravenous therapies for complex regional pain syndrome: a systematic review. Anesth Analg. 2016;122(3):843–56.
Xu J, Liu A, Cheng J. New advancements in spinal cord stimulation for chronic pain management. Curr Opin Anaesthesiol. 2017;30(6):710–7.
Xu J, Sun Z, Wu J, Rana M, Garza J, Zhu AC, Chakravarthy KV, Abd-Elsayed A, Rosenquist E, Basi H, et al. Peripheral nerve stimulation in pain management: a systematic review. Pain Physician. 2021;24(2):E131–e152.
Xu L, Sun Z, Casserly E, Nasr C, Cheng J, Xu J. Advances in interventional therapies for painful diabetic neuropathy: a systematic review. Anesth Analg. 2022;134:1215.
Zaim M, Karaman S, Cetin G, Isik S. Donor age and long-term culture affect differentiation and proliferation of human bone marrow mesenchymal stem cells. Ann Hematol. 2012;91(8):1175–86.
Zhou Y, Zhang X, Xue H, Liu L, Zhu J, Jin T. Autologous mesenchymal stem cell transplantation in multiple sclerosis: a meta-analysis. Stem Cells Int. 2019;2019:8536785.

Chapter 12
Platelet-Rich Plasma and Autologous Conditioned Serum: Non-Cellular Biologic Therapies for Neuroimmune Modulation and the Treatment of Arthritis Pain

Thomas Buchheit

Abstract Osteoarthritis (OA) affects more than 50 million in the United States (Lawrence et al Part II Arthritis Rheum 58(1):26–35, 2008); however, most of these individuals are not considered surgical candidates. Alternative treatment options such as medications, intra-articular corticosteroid or hyaluronic acid injections, or radiofrequency nerve ablations can reduce pain and improve function in some individuals, but many others are left in a state of "orthopedic limbo": conservative therapies are insufficient, and surgery is not an option. Biologically based regenerative pain medicine therapies such as platelet-rich plasma (PRP) and autologous conditioned serum (ACS) offer new options for these patients and are used with increasing frequency in the United States. In this chapter, I will discuss the neuroimmune alterations that drive the development of osteoarthritis, the mechanisms of action of these biologically based, non-stem cell therapies, and clinical outcomes with the use of PRP and ACS.

Keywords Autologous conditioned serum (ACS) · Cytokines · Growth factors · Hyaluronic acid · Osteoarthritis (OA) · Platelet-rich plasma (PRP) · Regenerative therapies

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12.1 Introduction: Current Treatments for Osteoarthritis

The non-surgical management of patients with osteoarthritis (OA) of the knee or other joints follows a common pathway: physical therapy (PT), analgesic medications such as non-steroidal anti-inflammatory drugs (NSAIDs for topical and/or systemic uses) and acetaminophen, or procedures such as intra-articular corticosteroids (IA-C) and intra-articular hyaluronic acid (IA-HA). For patients with refractory pain, radiofrequency lesioning/destruction of the nerves that supply sensation to the joint is also considered. Of these modalities, PT and exercise remain critically important foundational therapies for any individual with degenerative joint disease. Exercise activates cartilage (chondrocytes) and tendon cells (tenocytes), increases collagen synthesis, and builds joint strength and stability (Hinterwimmer et al. 2004; O'Connor et al. 2015). Further, mechanical loading on joints has been shown to inhibit the production of inflammatory cytokines such as IL-1, reducing cartilage breakdown (Torzilli et al. 2010). For these reasons, PT and exercise should be part of all treatment plans.

Other treatments used for arthritis may not be as beneficial, however. For instance, NSAIDS carry significant risks: they have been shown to double the chances of kidney injury in individuals over the age of 65 and increase the risks for gastrointestinal bleeding and cardiovascular disease. Medication alternatives such as acetaminophen may be associated with lower kidney and GI toxicity but is often less effective than NSAIDS and may cause liver damage (Ong et al. 2007).

Injection techniques such as IA-C are performed several million times each year in the United States for the treatment of OA pain; although this procedure provides many patients with rapid analgesia, its benefits are generally short-lived, often lasting only a few weeks (Juni et al. 2015). When performed in a repeated fashion, IA-C also carries the risks of decreased bone density (Al-Shoha et al. 2012), immune system dysfunction (Sytsma et al. 2018), and cellular aging (Poulsen et al. 2014); there is also now clear evidence that repeated IA-C injections will accelerate cartilage loss and worsen joint damage (McAlindon et al. 2017).

IA-HA is another procedure frequently used for patients with OA. Hyaluronic acid, a normal part of synovial fluid, breaks down into smaller, less viscous molecules in arthritis. In efforts to improve joint viscosity, reinjection of HA has been a common procedure since the 1990s for these individuals (Temple-Wong et al. 2016). Hyaluronic acid, manufactured from rooster combs or bacterial sources, has been shown to be effective: a 2006 Cochrane review of 76 studies demonstrated improved pain and function for several months following IA-HA (Bellamy et al. 2006). Subsequent meta-analyses support both the clinical effectiveness for up to 6 months and the superiority to IA-C (Campbell et al. 2015a; He et al. 2017). IA-HA, however, often provides only modest improvements in pain and function for many patients, prompting major societies such as the American Academy of Orthopedic Surgeons and the American College of Rheumatology to recommend against the use of this procedure for the routine treatment of knee OA pain (Jevsevar 2013; Kolasinski et al. 2020). These guidelines, however, do not consider the beneficial

biological functions of IA-HA including the reduction of inflammatory cytokines such as IL-1β, TNFα, and IL-6, and a decrease in joint-damaging enzymes such as matrix metalloproteinases (MMP) (Nicholls et al. 2017). Further, HA increases the synthesis of beneficial cartilage proteoglycan, extracellular matrix proteins, and tissue inhibitors of metalloproteinases (TIMPs) (Campo et al. 2012; Nicholls et al. 2017; Waddell et al. 2007). These biologic activities appear to be magnified with the use of higher molecular weight products (Bowman et al. 2018), and may confer longer-term health benefits to the joint.

The other non-surgical procedure that has gained popularity for the treatment of OA pain (particularly the knee) is the use of geniculate radiofrequency lesioning (RFL) to ablate and reduce the nerve supply to the joint. This procedure has been shown to provide better pain relief than IA-C at 3 months (Chen et al. 2020b) and IA-HA at 6 months (Chen et al. 2020a) but does carry limitations; nerve ablation may decrease joint position sense, one of the important goals of many physical therapy and rehabilitation programs. If effective, RFL may need to be repeated every 10–12 months to maintain analgesia. I generally recommend this procedure for patients with end-stage OA who are not surgical candidates or for those who have persistent pain after joint replacement surgery.

Although these interventions may help, they are insufficient to treat the millions of adults in the United States with functional limitations from OA who are not surgical candidates (Hootman et al. 2016). Many patients remain in "orthopedic limbo": their pain is not significantly improved by traditional non-surgical treatments and their arthritis is not severe enough to require joint replacement surgery. Non-cellular biologic therapies such as PRP and ACS may offer additional analgesic benefits to these individuals through modulation of neuroimmune mechanisms.

12.2 Dissociation of Pain and Degeneration

The diagnosis of OA is based on standardized radiologic criteria such as the Kellgren–Lawrence (K–L) scale that includes four different categories of x-ray findings:

Grade 1, doubtful narrowing of joint space and possible osteophytic lipping
Grade 2, definite osteophytes and possible narrowing of joint space
Grade 3, moderate multiple osteophytes, definite narrowing of joints space, some sclerosis, and possible deformity of bone contours; and
Grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone contours

Although the K–L grading system has been used as the gold standard for OA diagnosis for over 50 years, almost ½ of individuals who meet these criteria for knee arthritis report little or no pain (Hannan et al. 2000). Similarly, while the prevalence of radiographic hip OA in individuals over the age of 50 is nearly 20%, only about 4% experience significant symptoms (Kim et al. 2014). This dramatic disconnect

between anatomy and symptoms challenges the validity of traditional diagnostic criteria and necessitates that we revisit the drivers of pain in OA. Although central sensitization is clearly a factor in regulating the severity of pain with any degenerative condition (Arendt-Nielsen 2017), central modulation is not sufficient to explain the diversity of symptom experiences, particularly in patients with bilateral disease but only unilateral pain (Barreto et al. 2019) or the absence of pain with advanced "bone on bone" OA. In these circumstances, peripheral biochemical alterations clearly play a dominant role in defining the severity of OA symptoms.

12.3 The Link Between Neuroinflammatory Mediators and Pain

Overexpression of inflammatory cytokines such as IL-1β, IL-6, and TNFα plays a prominent role in the initiation of degenerative joint disease (Martel-Pelletier et al. 1992), and the severity of pain in OA correlates with several of these proteins (Cuellar et al. 2009). Although cytokines likely play a diminished role for pain as OA advances (Orita et al. 2011), their early activities induce the production of catabolic enzymes such as matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) causing both joint erosion and neural sensitization (Adams et al. 2015; Goldring and Otero 2011; Nicholls et al. 2017; Pujol et al. 2009; Vandooren et al. 2014). MMP has several subtypes (MMP-1, MMP-3, MMP-13) that are found in higher concentrations in patients with arthritis (Yoshihara et al. 2000); likewise, ADAMTS type 4 and 5 are particularly damaging to joint cartilage (Yang et al. 2017). This catabolic cascade is further magnified by a parallel loss of growth factors such as TGF-β, FGF, IGF, HGF and protective cytokines such as IL-1 receptor antagonist (IL-1Ra) (Arend et al. 1998; Pujol et al. 2009). The composite of these biochemical activities ultimately leads to joint space narrowing, osteophyte overgrowth, and the radiographic diagnosis of OA (Blasioli and Kaplan 2014). The observed cytokine dysregulation in OA has led to several medication trials of disease-modifying antirheumatic drugs (DMARDs) in an attempt to slow or halt disease progression; unfortunately, these drugs have been largely ineffective in improving pain and symptoms (Chevalier et al. 2009; Chevalier et al. 2015). Effective, longer-term treatments for OA must address the complex biochemical alterations that lead to OA progression.

12.4 The Biochemistry of a Healthy Joint

Although no single cytokine appears able to reverse the catabolic cascade of OA, several are capable of improving the inflammatory changes of early OA and reducing pain (Pujol et al. 2009). IL-1Ra binds to the IL-1 receptor but does not induce a intracellular response, thereby inhibiting the damaging effects of this cytokine

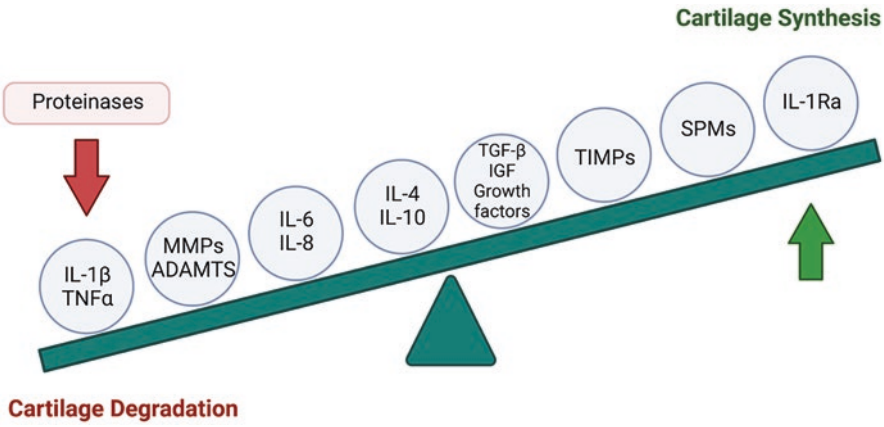

Fig. 12.1 Cytokines, growth factors, and MMP need to be in balance to maintain joint health. Cytokines such as IL-1 and TNF, and enzymes such as MMPs worsen cartilage damage; anabolic factors such as TGF-β and IL-1Ra augment the synthetic capacity of the synovial fluid and joint

(Arend et al. 1998). IL-10 is released by exercise and can both reduce pain and resolve inflammation following injury (da Silva et al. 2015; Grace et al. 2016; Sloane et al. 2009). Growth factors are also a critical component of healthy joints, promoting both collagen and proteoglycan production. In particular, TGF-β plays an important role in additionally reducing neuroinflammation and pain (Chen et al. 2015; Echeverry et al. 2009). The final category of proteins necessary for biochemical balance of the synovial fluid are TIMPS (the inhibitors of MMP and ADAMTS). TIMPS perform a vital role in controlling levels of enzymatic tissue breakdown, thereby offering joint and articular surface protection (Nakamura et al. 2020; Yoshihara et al. 2000). The healthy joint needs these protective cytokines, anabolic growth factors, and enzyme inhibitors to work in concert to maintain (or re-establish) function and reduce pain. This balance is illustrated in Fig. 12.1.

12.5 The Spectrum of Regenerative Therapies

Regenerative pain medicine encompasses a diversity of both "biologic" and "non-biologic" treatments. Common non-biologically based therapies include procedures such as surgical microfracturing, tendon fenestration, and prolotherapy. In surgical microfracturing, multiple lesions are created in the bone surface at the site of cartilage injury; this procedure has been shown to induce both cartilage growth and clinical improvements in the treated joint (Bae et al. 2006). Tendon fenestration employs a parallel process, where multiple needle passes create microinjury and induce the proliferative phase of healing in a chronic tendon injury (Jacobson et al. 2016). Prolotherapy uses a combination of both chemical and mechanical processes to produce controlled inflammation and induction of endogenous healing

mechanisms in the target structure (Topol et al. 2011). In contrast, the biologically based treatments rely on neuroimmune mechanisms rather than physical or chemical methods to induce tissue recovery, and include both cellular (stem cells) and non-cellular (PRP and ACS) interventions. This chapter will present the non-cellular treatments, their mechanisms of action, and their clinical effects in the treatment of osteoarthritis.

12.5.1 Platelet-Rich Plasma (PRP)

PRP has been used clinically since the 1980s when it was found that platelet concentrates stimulated wound healing after surgery (Alves and Grimalt 2018). Platelets contain over 300 growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), fibroblastic growth factor (FGF), hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and others (Blair and Flaumenhaft 2009; Hickey et al. 2003; Xie et al. 2014). In most PRP preparations, white blood cells (WBCs) such as neutrophils and monocytes are also present in varying concentrations. WBC concentrations may increase levels of pro-inflammatory cytokines (Andia and Maffulli 2013), but also play a role in inducing the healing cascade after injection. This progression of cellular activities after tissue injury is shown in Fig. 12.2.

PRP contains additional anabolic factors such as tissue inhibitors of metalloproteases (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) that promote cellular proliferation, matrix formation, and collagen synthesis (Rughetti et al. 2008). The secreted growth factors such as TGF-β, FGF, and IGF are also capable of recruiting endogenous hematopoietic stem cells and progenitor cells to the site, furthering tissue restoration (Baay et al. 2011; Crane and Cao 2014; Le Blanc and Mougiakakos 2012). Several classification systems have been proposed to characterize the PRP products, describing platelet counts, neutrophil, monocyte and growth factor concentrations, and other variables (Lana et al. 2017); ongoing research continues to define the optimal characteristics of PRP for various disorders. Two general methods of preparation include a "buffy coat" system and a "plasma-based" system. The buffy coat system is named after the appearance of plasma following centrifugation: there remains a "whitish" layer (the "buffy coat") on top of the red cells. These systems typically use a single, longer centrifugation process to isolate the platelet layer and often contain a higher concentration of platelets and WBCs.

With a plasma-based process for PRP, two shorter centrifugation steps are often performed; the initial centrifugation (a "soft spin") keeps platelets in plasma suspension; this plasma suspension subsequently undergoes a second spin to isolate the platelets. This method often reduces the WBC concentrations in PRP (and the potential for post-injection inflammatory pain) (Braun et al. 2014; Riboh et al. 2016); however, it also has the potential to reduce platelet counts and growth factor concentrations (Fig. 12.3).

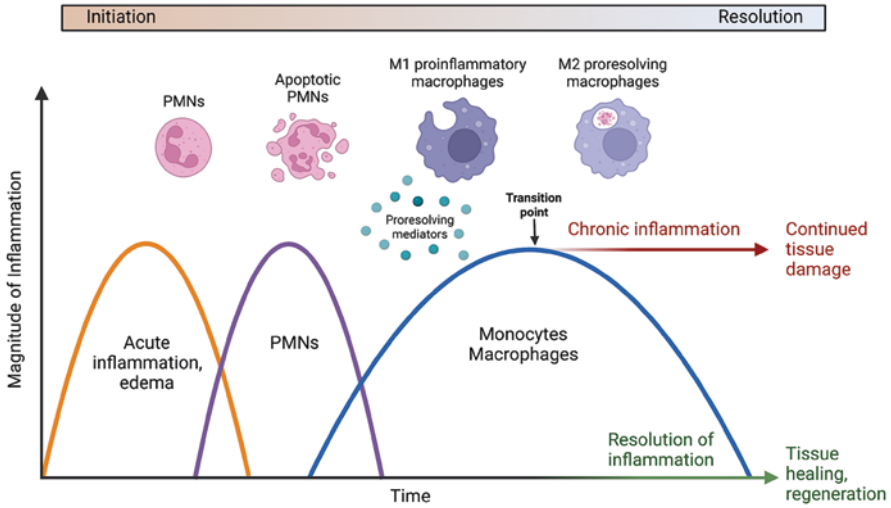

Fig. 12.2 Injury induces platelet activation and the release of growth factors and chemokines on the left. Neutrophils, the most common WBC in circulation initially respond; they are followed by monocytes/macrophages that migrate to the area and begin the transition to an M2 pro-resolving phenotype. This M2 macrophage is capable of releasing growth factors and anabolic cytokines that resolve chronic inflammation and induce tissue healing

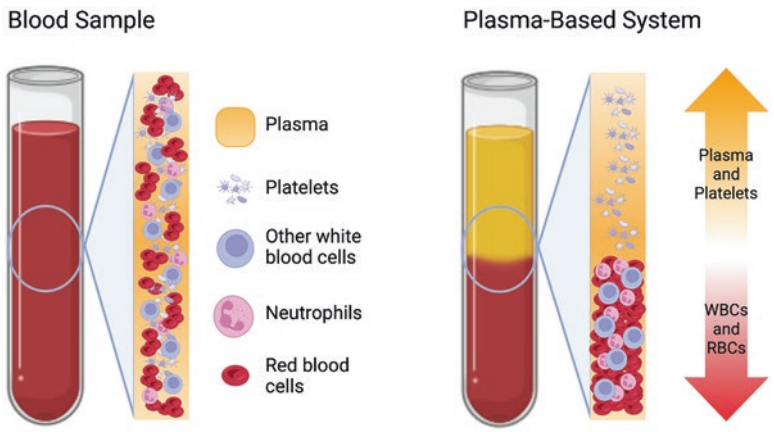

Fig. 12.3 "Plasma" process for PRP. (**a**). Whole blood is collected with anticoagulant and briefly centrifuged with low centrifugal forces (a "softspin"). This process keeps many of the platelets in plasma suspension. (**b**). This plasma suspension then undergoes a second spin to concentrate the platelets. Most of the PPP is then removed, and the platelets re-suspended in the remaining plasma for the final PRP preparation

PRP-based growth factors such as TGF-β, PDGF, IGF, FGF, EGF, VEGF, and HGF have been shown to produce multiple beneficial effects including the reduction of inflammatory cytokines such as IL-1β. The activation of NFκB, a critical

transcription factor for immune and inflammatory processes, is also minimized in osteoarthritic chondrocytes through suppression of CXCR4 in surrounding monocytes/macrophages (Bendinelli et al. 2010; Blair and Flaumenhaft 2009; Hickey et al. 2003; van Buul et al. 2011; Xie et al. 2014). TGF-β and other growth factors additionally promote collagen and proteoglycan production, and chondrocyte proliferation (Akeda et al. 2006; J.-P. Pujol et al. 2009; Sun et al. 2010; van Buul et al. 2011; Wu et al. 2011). PRP-based TIMPs suppress the activity of catabolic enzymes such as MMP3, MMP 13, and ADAMTS, preserving cartilage (Rughetti et al. 2008; Sundman et al. 2014). Collectively, these activities have been shown to enhance cartilage and meniscal cell regeneration in animal models (Ishida et al. 2007; Kwon et al. 2012); however, radiographic restoration of cartilage does not appear to be significant when measured in in human trials (Hart et al. 2013). In addition to direct growth factor and cytokine effects, PRP has been shown to increase endogenous hyaluronic acid secretion in arthritis patients (Anitua et al. 2007), with presumptive improvements in synovial fluid viscosity (Detterline et al. 2008). Overall, PRP appears to improve the health of existing cartilage and tissues by reducing concentrations of damaging cytokines and catabolic enzymes, augmenting beneficial cytokines and growth factors, and promoting endogenous hyaluronic acid production.

The majority of clinical trials of PRP have been in the treatment of knee osteoarthritis where reductions in pain and improvements in function have been demonstrated by both cohort studies (Halpern et al. 2013) and randomized controlled trials (Patel et al. 2013; Sánchez et al. 2012; Vaquerizo et al. 2013). Meta-analyses and systematic reviews additionally support the use of PRP for mild to moderate OA, finding superiority of PRP to IA-HA at 12 months or greater and level I evidence for pain reduction at this period (Belk et al. 2020; Campbell et al. 2015b; Chang et al. 2014; Dai et al. 2017; Johal et al. 2019; Meheux et al. 2016; Sadabad et al. 2016). However, the superiority of PRP over IA-HA has not been noted in all trials (Di Martino et al. 2019; Filardo et al. 2015), leading to an ongoing debate as to the optimal PRP preparation methods and WBC concentrations (Belk et al. 2020). It has been further demonstrated that low platelet PRP products have limited effectiveness in the treatment of knee OA (Bennell et al. 2021). The first randomized comparative trial between PRP and bone marrow stem cells was performed in 2020; the interventions were both found to be effective at 12 months and no difference in outcome was seen between the treatment methods (Anz et al. 2020). The benefits of PRP appear to be greatest in younger patients with earlier stage disease (Chang et al. 2014; Halpern et al. 2013; Patel et al. 2013), likely because of greater autologous growth factor concentrations and fewer senescent chondrocytes. Interestingly, although the impact of hyaluronic acid injection by itself may be modest, there is growing evidence that IA-HA enhances the benefits of PRP by further inhibiting MMP and acting as a matrix for anabolic PRP activities (Chen et al. 2014; Dai et al. 2017; Privata et al. 2019; Zhao et al. 2020).

PRP has also been studied for the treatment of tendinopathy; pre-clinical data reveal that the injection of PRP-based growth factors such as TGFβ and VEGF increases the strength of healing tendons (Docheva et al. 2015; Rodik and

McDermott 2016). Clinically, PRP demonstrates good longer-term outcomes for patients with lateral epicondylopathy (Gosens et al. 2011; Johal et al. 2019; Mishra et al. 2014; Peerbooms et al. 2010) faster recovery of ACL after surgery (Seijas et al. 2013), and evidence of improved tendon healing after injury (Gautam et al. 2015). In a randomized trial, PRP was shown to be superior to dry needling in rotator cuff disease (Rha et al. 2013); however, positive results with the use of PRP in rotator cuff tendinopathy are not universal (Kesikburun et al. 2013; Rha et al. 2013; Schwitzguebel et al. 2019). A 2016 meta-analysis of 18 studies further supports the use of ultrasound-guided PRP in the treatment of chronic tendinopathies of multiple locations (Fitzpatrick et al. 2017), although definitive conclusions and indications for PRP use with various tendinopathies are still being clarified.

12.5.2 Autologous Conditioned Serum (ACS)

Whole blood incubation techniques have also been explored as a method to augment beneficial cytokines and anabolic growth factors. Initial research in this process was performed in Germany and the United States in the 1990s, with the subsequent development of ACS (Evans 2005; Wehling et al. 2007). ACS is now used throughout Europe and in several sites in the United States (Evans et al. 2016). In contrast to PRP, ACS is a filtered serum product without platelets or other cellular components (Evans et al. 2016) (Fig. 12.4).

The ACS incubation process has been shown to significantly increase levels of anti-inflammatory cytokines such as IL-1Ra, IL-4, and IL-10, as well as TGFβ, a critical growth factor for cartilage and tissue health (Evans et al. 2016). When used in animal models, ACS produces thickening of tendons, higher concentrations of type I collagen, and decreases in synovial hyperplasia (Frisbie et al. 2007). In addition to the important induction of cytokines and growth factors in ACS, it also appears that extracellular vesicles such as exosomes may play a vital role in the prolonged analgesia that is observed after injection (Shirokova et al. 2020). The role of exosomes in biologically based therapies remains an active area of investigation.

Initial published use of ACS for the treatment of knee arthritis pain included 1000 patients as part of a prospective, observational trial; WOMAC scores improved by 75% in >70% of patients (Baltzer et al. 2003). A large, blinded, randomized controlled trial (RCT) in 2009 demonstrated superior clinical outcomes of ACS over IA-HA and placebo, and improvements were maintained for at least 2 years (Baltzer et al. 2009). A smaller RCT by Yang et al. was also performed in patients with knee OA; the primary outcome measure of this study did not reach significance; however, the investigators noted that KOOS scores were significantly improved in the ACS group at 12 months in comparison to the saline injections (Yang et al. 2008). The benefits of ACS are further supported by a 2-year observational trial of 118 patients who experienced a 62% decrease in VAS scores and a 56% decrease in WOMAC scores at follow-up (García-Escudero and Trillos 2015). Positive observational

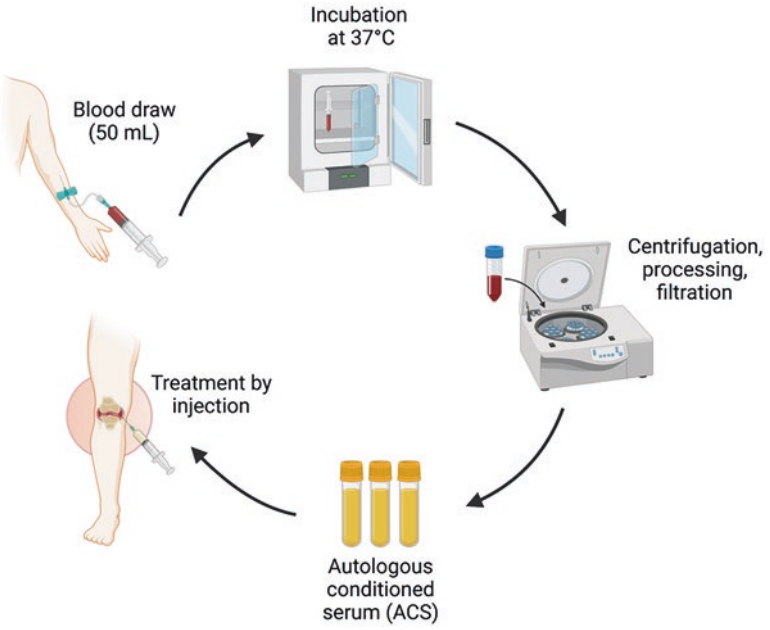

Fig. 12.4 Autologous conditioned serum. Whole blood is incubated under controlled conditions, centrifuged, processed, filtered, and then used for injection

results have also been noted with the use of ACS for hip arthritis (Baltzer et al. 2013), rotator cuff pathology (Damjanov et al. 2018), and Achilles tendinopathy (von Wehren et al. 2019). As a surgical adjuvant, ACS appears to improve outcomes of ACL reconstruction, providing superior WOMAC and IKDC scores compared to the control patients and significant decreases in the synovial fluid levels of IL-1 (Darabos et al. 2011).

12.6 Conclusions

It is increasingly clear that neuroimmune mechanisms drive symptoms in OA and the biochemical imbalance that leads to disease progression is significantly responsive to regenerative pain therapies. Despite the multitude of processing techniques, there are common analgesic mechanisms that these therapies share, including the enhancement of growth factors and anabolic cytokines. PRP and ACS have been used for decades and demonstrate superiority to standard treatments such as steroid or IA-HA injection, not only reducing symptoms, but potentially modifying disease course. As research continues to clarify optimal processing methods and disease-specific indications, the roles for these biologically based interventions will continue to expand in the non-surgical treatment of osteoarthritis.

References

Adams SB, Setton LA, Bell RD, Easley ME, Huebner JL, Stabler T, Kraus VB, Leimer EM, Olson SA, Nettles DL. Inflammatory cytokines and matrix metalloproteinases in the synovial fluid after intra-articular ankle fracture. Foot Ankle Int. 2015;36(11):1264–71.

Akeda K, An HS, Okuma M, Attawia M, Miyamoto K, Thonar EJMA, Lenz ME, Sah RL, Masuda K. Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthr Cartil. 2006;14(12):1272–80.

Al-Shoha A, Rao DS, Schilling J, Peterson E, Mandel S. Effect of epidural steroid injection on bone mineral density and markers of bone turnover in postmenopausal women. Spine (Phila Pa 1976). 2012;37(25):E1567–71.

Alves R, Grimalt R. A review of platelet-rich plasma: history, biology, mechanism of action, and classification. Skin Append Disord. 2018;4(1):18–24.

Andia I, Maffulli N. Platelet-rich plasma for managing pain and inflammation in osteoarthritis. Nat Rev Rheumatol. 2013;9(12):721–30.

Anitua E, Sanchez M, Nurden AT, Zalduendo MM, de la Fuente M, Azofra J, Andia I. Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. Rheumatology (Oxford). 2007;46(12):1769–72.

Anz AW, Hubbard R, Rendos NK, Everts PA, Andrews JR, Hackel JG. Bone marrow aspirate concentrate is equivalent to platelet-rich plasma for the treatment of knee osteoarthritis at 1 year: a prospective, randomized trial. Orthop J Sports Med. 2020;8(2):2325967119900958.

Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. Annu Rev Immunol. 1998;16:27–55.

Arendt-Nielsen L. Joint pain: more to it than just structural damage? Pain. 2017;158(Suppl 1):S66–73.

Baay M, Brouwer A, Pauwels P, Peeters M, Lardon F. Tumor cells and tumor-associated macrophages: secreted proteins as potential targets for therapy. Clin Dev Immunol. 2011;2011:565187.

Bae DK, Yoon KH, Song SJ. Cartilage healing after microfracture in osteoarthritic knees. Arthroscopy. 2006;22(4):367–74.

Baltzer ADR, Granrath M, et al. Intra-articular treatment of osteoarthritis using autologous interleukin-1 receptor antagonist (il1-ra) conditioned serum. Dtsch Z Sportmed. 2003;54:209–11.

Baltzer AWA, Moser C, Jansen SA, Krauspe R. Autologous conditioned serum (orthokine) is an effective treatment for knee osteoarthritis. Osteoarthr Cartil. 2009;17(2):152–60.

Baltzer AWA, Ostapczuk MS, Stosch D, Seidel F, Granrath M. A new treatment for hip osteoarthritis: clinical evidence for the efficacy of autologous conditioned serum. Orthop Rev (Pavia). 2013;5(2):e13.

Barreto RPG, Braman JP, Ludewig PM, Ribeiro LP, Camargo PR. Bilateral magnetic resonance imaging findings in individuals with unilateral shoulder pain. J Shoulder Elb Surg. 2019;28(9):1699–706.

Belk JW, Kraeutler MJ, Houck DA, Goodrich JA, Dragoo JL, McCarty EC. Platelet-rich plasma versus hyaluronic acid for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. Am J Sports Med.363546520909397. 2020.

Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev. (2):CD005321. 2006.

Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, Desiderio MA. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: mechanisms of nf-kappab inhibition via hgf. J Cell Physiol. 2010;225(3):757–66.

Bennell K, Paterson B, Metcalf V, Duong J, Eyles J, Kasza Y, Wang F, Cicuttini R, Buchbinder A, Forbes A, Harris S, Yu D, Connell J, Linklater B, Wang W, Oo M, Hunter D. Effect of intra-articular platelet-rich plasma vs placebo injection on pain and medial Tibial cartilage volume in patients with knee osteoarthritis: the RESTORE randomized clinical trial. JAMA. 2021;326(20):2021–30.

Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev. 2009;23(4):177–89.

Blasioli DJ, Kaplan DL. The roles of catabolic factors in the development of osteoarthritis. Tissue Eng Part B Rev. 2014;20(4):355–63.

Bowman S, Awad ME, Hamrick MW, Hunter M, Fulzele S. Recent advances in hyaluronic acid based therapy for osteoarthritis. Clin Transl Med. 2018;7(1):6.

Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes. Am J Sports Med. 2014;42(5):1204–10.

Campbell KA, Erickson BJ, Saltzman BM, Mascarenhas R, Bach BR Jr, Cole BJ, Verma NN. Is local viscosupplementation injection clinically superior to other therapies in the treatment of osteoarthritis of the knee: a systematic review of overlapping meta-analyses. Arthroscopy. 2015a;31(10):2036–45.

Campbell KA, Saltzman BM, Mascarenhas R, Khair MM, Verma NN, Bach BR Jr, Cole BJ. Does intra-articular platelet-rich plasma injection provide clinically superior outcomes compared with other therapies in the treatment of knee osteoarthritis? A systematic review of overlapping meta-analyses. Arthroscopy. 2015b;31(11):2213–21.

Campo GM, Avenoso A, D'Ascola A, Prestipino V, Scuruchi M, Nastasi G, Calatroni A, Campo S. Hyaluronan differently modulates tlr-4 and the inflammatory response in mouse chondrocytes. Biofactors. 2012;38(1):69–76.

Chang KV, Hung CY, Aliwarga F, Wang TG, Han DS, Chen WS. Comparative effectiveness of platelet-rich plasma injections for treating knee joint cartilage degenerative pathology: a systematic review and meta-analysis. Arch Phys Med Rehabil. 2014;95(3):562–75.

Chen WH, Lo WC, Hsu WC, Wei HJ, Liu HY, Lee CH, Tina Chen SY, Shieh YH, Williams DF, Deng WP. Synergistic anabolic actions of hyaluronic acid and platelet-rich plasma on cartilage regeneration in osteoarthritis therapy. Biomaterials. 2014;35(36):9599–607.

Chen G, Park CK, Xie RG, Ji RR. Intrathecal bone marrow stromal cells inhibit neuropathic pain via tgf-beta secretion. J Clin Invest. 2015;125(8):3226–40.

Chen AF, Khalouf F, Zora K, DePalma M, Kohan L, Guirguis M, Beall D, Loudermilk E, Pingree M, Badiola I, et al. Cooled radiofrequency ablation compared with a single injection of hyaluronic acid for chronic knee pain: a multicenter, randomized clinical trial demonstrating greater efficacy and equivalent safety for cooled radiofrequency ablation. J Bone Joint Surg Am. 2020a;102(17):1501–10.

Chen AF, Mullen K, Casambre F, Visvabharathy V, Brown GA. Thermal nerve radiofrequency ablation for the nonsurgical treatment of knee osteoarthritis: a systematic literature review. J Am Acad Orthop Surg. 2020b.

Chevalier X, Goupille P, Beaulieu AD, Burch FX, Bensen WG, Conrozier T, Loeuille D, Kivitz AJ, Silver D, Appleton BE. Intraarticular injection of anakinra in osteoarthritis of the knee: a multicenter, randomized, double-blind, placebo-controlled study. Arthritis Rheum. 2009;61(3):344–52.

Chevalier X, Ravaud P, Maheu E, Baron G, Rialland A, Vergnaud P, Roux C, Maugars Y, Mulleman D, Lukas C, et al. Adalimumab in patients with hand osteoarthritis refractory to analgesics and nsaids: a randomised, multicentre, double-blind, placebo-controlled trial. Ann Rheum Dis. 2015;74(9):1697–705.

Crane JL, Cao X. 2014. Bone marrow mesenchymal stem cells and tgf-β signaling in bone remodeling. J Clin invest. 124(2):466-472-472.

Cuellar JM, Scuderi GJ, Cuellar VG, Golish SR, Yeomans DC. 2009. Diagnostic utility of cytokine biomarkers in the evaluation of acute knee pain. The journal of Bone & Joint Surgery. 91(10):2313-2320-2320.

da Silva MD, Bobinski F, Sato KL, Kolker SJ, Sluka KA, Santos AR. Il-10 cytokine released from m2 macrophages is crucial for analgesic and anti-inflammatory effects of acupuncture in a model of inflammatory muscle pain. Mol Neurobiol. 2015;51(1):19–31.

Dai WL, Zhou AG, Zhang H, Zhang J. Efficacy of platelet-rich plasma in the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. Arthroscopy. 2017;33(3):659–70.

Damjanov N, Barac B, Colic J, Stevanovic V, Zekovic A, Tulic G. The efficacy and safety of autologous conditioned serum (acs) injections compared with betamethasone and placebo injections in the treatment of chronic shoulder joint pain due to supraspinatus tendinopathy: a prospective, randomized, double-blind, controlled study. Med Ultrason. 2018;20(3):335–41.

Darabos N, Haspl M, Moser C, Darabos A, Bartolek D, Groenemeyer D. Intraarticular application of autologous conditioned serum (acs) reduces bone tunnel widening after acl reconstructive surgery in a randomized controlled trial. Knee Surg Sports Traumatol Arthrosc. 2011;19(Suppl 1):S36–46.

Detterline AJ, Goldstein JL, Rue JP, Bach BR Jr. Evaluation and treatment of osteochondritis dissecans lesions of the knee. J Knee Surg. 2008;21(2):106–15.

Di Martino A, Di Matteo B, Papio T, Tentoni F, Selleri F, Cenacchi A, Kon E, Filardo G. Platelet-rich plasma versus hyaluronic acid injections for the treatment of knee osteoarthritis: results at 5 years of a double-blind, randomized controlled trial. Am J Sports Med. 2019;47(2):347–54.

Docheva D, Muller SA, Majewski M, Evans CH. Biologics for tendon repair. Adv Drug Deliv Rev. 2015;84:222–39.

Echeverry S, Shi XQ, Haw A, Liu H, Zhang ZW, Zhang J. Transforming growth factor-beta1 impairs neuropathic pain through pleiotropic effects. Mol Pain. 2009;5:16.

Evans CH. Novel biological approaches to the intra-articular treatment of osteoarthritis. Biodrugs. 2005;19(6):355–62.

Evans CH, Chevalier X, Wehling P. Autologous conditioned serum. Phys Med Rehabil Clin N Am. 2016;27(4):893–908.

Filardo G, Di Matteo B, Di Martino A, Merli ML, Cenacchi A, Fornasari P, Marcacci M, Kon E. Platelet-rich plasma intra-articular knee injections show no superiority versus viscosupplementation: a randomized controlled trial. Am J Sports Med. 2015;43(7):1575–82.

Fitzpatrick J, Bulsara M, Zheng MH. The effectiveness of platelet-rich plasma in the treatment of tendinopathy: a meta-analysis of randomized controlled clinical trials. Am J Sports Med. 2017;45(1):226–33.

Frisbie DD, Kawcak CE, Werpy NM, Park RD, McIlwraith CW. Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis. Am J Vet Res. 2007;68(3):290–6.

García-Escudero JB, Trillos PMH. Treatment of osteoarthritis of the knee with a combination of autologous conditioned serum and physiotherapy: a two-year observational study. PLoS One. 2015;10(12):e0145551.

Gautam VK, Verma S, Batra S, Bhatnagar N, Arora S. Platelet-rich plasma versus corticosteroid injection for recalcitrant lateral epicondylitis: clinical and ultrasonographic evaluation. J Orthop Surg (Hong Kong). 2015;23(1):1–5.

Goldring MB, Otero M. Inflammation in osteoarthritis. Curr Opin Rheumatol. 2011;23(5):471–8.

Gosens T, Peerbooms JC, Laar WV, Oudsten BLD. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis. The American Journal of Sports Medicine. 2011;39(6):1200–8.

Grace PM, Fabisiak TJ, Green-Fulgham SM, Anderson ND, Strand KA, Kwilasz AJ, Galer EL, Walker FR, Greenwood BN, Maier SF, et al. Prior voluntary wheel running attenuates neuropathic pain. Pain. 2016;157(9):2012–23.

Halpern B, Chaudhury S, Rodeo SA, Hayter C, Bogner E, Potter HG, Nguyen J. Clinical and mri outcomes after platelet-rich plasma treatment for knee osteoarthritis. Clin J Sport Med. 2013;23(3):238–9.

Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol. 2000;27(6):1513–7.

Hart R, Safi A, Komzak M, Jajtner P, Puskeiler M, Hartova P. Platelet-rich plasma in patients with tibiofemoral cartilage degeneration. Arch Orthop Trauma Surg. 2013;133(9):1295–301.

He WW, Kuang MJ, Zhao J, Sun L, Lu B, Wang Y, Ma JX, Ma XL. Efficacy and safety of intraarticular hyaluronic acid and corticosteroid for knee osteoarthritis: a meta-analysis. Int J Surg. 2017;39:95–103.

Hickey DG, Frenkel SR, Di Cesare PE. Clinical applications of growth factors for articular cartilage repair. Am J Orthop (Belle Mead NJ). 2003;32(2):70–6.
Hinterwimmer S, Krammer M, Krötz M, Glaser C, Baumgart R, Reiser M, Eckstein F. Cartilage atrophy in the knees of patients after seven weeks of partial load bearing. Arthritis Rheum. 2004;50(8):2516–20.
Hootman JM, Helmick CG, Barbour KE, Theis KA, Boring MA. Updated projected prevalence of self-reported doctor-diagnosed arthritis and arthritis-attributable activity limitation among us adults, 2015-2040. Arthritis Rheumatol. 2016;68(7):1582–7.
Ishida K, Kuroda R, Miwa M, Tabata Y, Hokugo A, Kawamoto T, Sasaki K, Doita M, Kurosaka M. The regenerative effects of platelet-rich plasma on meniscal cells in vitro and its in vivo application with biodegradable gelatin hydrogel. Tissue Eng. 2007;13(5):1103–12.
Jacobson JA, Yablon CM, Henning PT, Kazmers IS, Urquhart A, Hallstrom B, Bedi A, Parameswaran A. Greater trochanteric pain syndrome: percutaneous tendon fenestration versus platelet-rich plasma injection for treatment of gluteal tendinosis. J Ultrasound Med. 2016;35(11):2413–20.
Jevsevar DS. Treatment of osteoarthritis of the knee: evidence-based guideline, 2nd edition. J Am Acad Orthop Surg. 2013;21(9):571–6.
Johal H, Khan M, Yung SP, Dhillon MS, Fu FH, Bedi A, Bhandari M. Impact of platelet-rich plasma use on pain in orthopaedic surgery: a systematic review and meta-analysis. Sports Health. 2019;11(4):355–66.
Juni P, Hari R, Rutjes AW, Fischer R, Silletta MG, Reichenbach S, da Costa BR. Intra-articular corticosteroid for knee osteoarthritis. Cochrane Database Syst Rev. 2015;10:CD005328.
Kesikburun S, Tan AK, Yılmaz B, Yaşar E, Yazıcıoğlu K. 2013. Platelet-rich plasma injections in the treatment of chronic rotator cuff tendinopathy. The American journal of sports medicine. 41(11):2609-2616-2616.
Kim C, Linsenmeyer KD, Vlad SC, Guermazi A, Clancy MM, Niu J, Felson DT. Prevalence of radiographic and symptomatic hip osteoarthritis in an urban United States community: the Framingham osteoarthritis study. Arthritis Rheumatol. 2014;66(11):3013–7.
Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, Callahan L, Copenhaver C, Dodge C, Felson D, et al. 2019 American College of Rheumatology/Arthritis Foundation guideline for the management of osteoarthritis of the hand, hip, and knee. Arthritis Rheumatol. 2020;72(2):220–33.
Kwon DR, Park GY, Lee S-u. The effects of intra-articular platelet-rich plasma injection according to the severity of collagenase-induced knee osteoarthritis in a rabbit model. Ann Rehabil Med. 2012;36(4):458–65.
Lana JFSD, Purita J, Paulus C, Huber SC, Rodrigues BL, Rodrigues AA, Santana MH, Luzo ÂCM, Belangero WD, et al. Contributions for classification of platelet rich plasma – proposal of a new classification: Marspill. Regen Med. 2017;12:565.
Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, Gabriel S, Hirsch R, Hochberg MC, Hunder GG, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II Arthritis Rheum. 2008;58(1):26–35.
Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol. 2012;12(5):383–96.
Martel-Pelletier J, McCollum R, DiBattista J, Faure MP, Chin JA, Fournier S, Sarfati M, Pelletier JP. The interleukin-1 receptor in normal and osteoarthritic human articular chondrocytes. Identification as the type i receptor and analysis of binding kinetics and biologic function. Arthritis Rheum. 1992;35(5):530–40.
McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, Ward RJ. Effect of intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: a randomized clinical trial. JAMA. 2017;317(19):1967–75.
Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. 2016. Efficacy of intra-articular platelet-rich plasma injections in knee osteoarthritis: a systematic review. Arthroscopy. 32(3):495-505-505.

Mishra AK, Skrepnik NV, Edwards SG, Jones GL, Sampson S, Vermillion DA, Ramsey ML, Karli DC, Rettig AC. Efficacy of platelet-rich plasma for chronic tennis elbow: a double-blind, prospective, multicenter, randomized controlled trial of 230 patients. Am J Sports Med. 2014;42(2):463–71.

Nakamura H, Vo P, Kanakis I, Liu K, Bou-Gharios G. Aggrecanase-selective tissue inhibitor of metalloproteinase-3 (timp3) protects articular cartilage in a surgical mouse model of osteoarthritis. Sci Rep. 2020;10(1):9288.

Nicholls MA, Fierlinger A, Niazi F, Bhandari M. The disease-modifying effects of hyaluronan in the osteoarthritic disease state. Clin Med Insights Arthritis Musculoskelet Disord. 2017;10:1179544117723611.

O'Connor SR, Tully MA, Ryan B, Bleakley CM, Baxter GD, Bradley JM, McDonough SM. Walking exercise for chronic musculoskeletal pain: systematic review and meta-analysis. Arch Phys Med Rehabil. 2015;96(4):724–34. e723

Ong CK, Lirk P, Tan CH, Seymour RA. An evidence-based update on nonsteroidal anti-inflammatory drugs. Clin Med Res. 2007;5(1):19–34.

Orita S, Koshi T, Mitsuka T, Miyagi M, Inoue G, Arai G, Ishikawa T, Hanaoka E, Yamashita K, Yamashita M, et al. Associations between proinflammatory cytokines in the synovial fluid and radiographic grading and pain-related scores in 47 consecutive patients with osteoarthritis of the knee. BMC Musculoskelet Disord. 2011;12:144.

Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. Am J Sports Med. 2013;41(2):356–64.

Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. Am J Sports Med. 2010;38(2):255–62.

Poulsen RC, Watts AC, Murphy RJ, Snelling SJ, Carr AJ, Hulley PA. Glucocorticoids induce senescence in primary human tenocytes by inhibition of sirtuin 1 and activation of the p53/p21 pathway: in vivo and in vitro evidence. Ann Rheum Dis. 2014;73(7):1405–13.

Pujol J-P, Chadjichristos C, Legendre F, Baugé C, Beauchef G, Andriamanalijaona R, Galéra P, Boumediene K. Interleukin-1 and transforming growth factor-ß 1 as crucial factors in osteoarthritic cartilage metabolism. Connect Tissue Res. 2009;49(3-4):293–7.

Rha DW, Park GY, Kim YK, Kim MT, Lee SC. Comparison of the therapeutic effects of ultrasound-guided platelet-rich plasma injection and dry needling in rotator cuff disease: a randomized controlled trial. Clin Rehabil. 2013;27(2):113–22.

Riboh JC, Saltzman BM, Yanke AB, Fortier L, Cole BJ. Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. Am J Sports Med. 2016;44(3):792–800.

Rodik T, McDermott B. Platelet-rich plasma compared with other common injection therapies in the treatment of chronic lateral epicondylitis. J Sport Rehabil. 2016;25(1):77–82.

Rughetti A, Giusti I, D'Ascenzo S, Leocata P, Carta G, Pavan A, Dell'Orso L, Dolo V. Platelet gel-released supernatant modulates the angiogenic capability of human endothelial cells. Blood Transfus. 2008;6(1):12–7.

Sadabad HN, Behzadifar M, Arasteh F, Behzadifar M, Dehghan HR. Efficacy of platelet-rich plasma versus hyaluronic acid for treatment of knee osteoarthritis: a systematic review and meta-analysis. Electron Physician. 2016;8(3):2115–22.

Sánchez M, Fiz N, Azofra J, Usabiaga J, Recalde EA, Gutierrez AG, Albillos J, Gárate R, Aguirre JJ, Padilla S, et al. A randomized clinical trial evaluating plasma rich in growth factors (prgf-endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. Arthroscopy. J Arthroscop Relat Surg. 2012;28(8):1070–8.

Schwitzguebel AJ, Kolo FC, Tirefort J, Kourhani A, Nowak A, Gremeaux V, Saffarini M, Ladermann A. Efficacy of platelet-rich plasma for the treatment of interstitial supraspinatus tears: a double-blinded, randomized controlled trial. Am J Sports Med. 363546519851097. 2019.

Seijas R, Ares O, Catala J, Alvarez-Diaz P, Cusco X, Cugat R. Magnetic resonance imaging evaluation of patellar tendon graft remodelling after anterior cruciate ligament reconstruction with or without platelet-rich plasma. J Orthopaed Surg. 2013;21(1):10–4.

Shirokova L, Noskov S, Gorokhova V, Reinecke J, Shirokova K. Intra-articular injections of a whole blood clot secretome, autologous conditioned serum, have superior clinical and biochemical efficacy over platelet-rich plasma and induce rejuvenation-associated changes of joint metabolism: a prospective, controlled open-label clinical study in chronic knee osteoarthritis. Rejuvenation Res. 2020;23(5):401–10.

Sloane EM, Soderquist RG, Maier SF, Mahoney MJ, Watkins LR, Milligan ED. Long-term control of neuropathic pain in a non-viral gene therapy paradigm. Gene Ther. 2009;16(4):470–5.

Sun Y, Feng Y, Zhang CQ, Chen SB, Cheng XG. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. Int Orthop. 2010;34(4):589–97.

Sundman EA, Cole BJ, Karas V, Della Valle C, Tetreault MW, Mohammed HO, Fortier LA. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med. 2014;42(1):35–41.

Sytsma TT, Greenlund LK, Greenlund LS. Joint corticosteroid injection associated with increased influenza risk. Mayo Clin Proc Innov Qual Outcomes. 2018;2(2):194–8.

Temple-Wong MM, Ren S, Quach P, Hansen BC, Chen AC, Hasegawa A, D'Lima DD, Koziol J, Masuda K, Lotz MK, et al. Hyaluronan concentration and size distribution in human knee synovial fluid: variations with age and cartilage degeneration. Arthritis Res Ther. 2016;18:18.

Topol GA, Podesta LA, Reeves KD, Raya MF, Fullerton BD, Yeh HW. Hyperosmolar dextrose injection for recalcitrant osgood-schlatter disease. Pediatrics. 2011;128(5):e1121–8.

Torzilli PA, Bhargava M, Park S, Chen CT. Mechanical load inhibits il-1 induced matrix degradation in articular cartilage. Osteoarthr Cartil. 2010;18(1):97–105.

van Buul GM, Koevoet WL, Kops N, Bos PK, Verhaar JA, Weinans H, Bernsen MR, van Osch GJ. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. Am J Sports Med. 2011;39(11):2362–70.

Vandooren J, Van Damme J, Opdenakker G. On the structure and functions of gelatinase b/matrix metalloproteinase-9 in neuroinflammation. Prog Brain Res. 2014;214:193–206.

Vaquerizo V, Plasencia MÁ, Arribas I, Seijas R, Padilla S, Orive G, Anitua E. Comparison of intra-articular injections of plasma rich in growth factors (prgf-endoret) versus durolane hyaluronic acid in the treatment of patients with symptomatic osteoarthritis: a randomized controlled trial. Arthroscop J Arthroscop Relat Surg. 2013;29(10):1635–43.

von Wehren L, Pokorny K, Blanke F, Sailer J, Majewski M. Injection with autologous conditioned serum has better clinical results than eccentric training for chronic achilles tendinopathy. Knee Surg Sports Traumatol Arthrosc. 2019.

Waddell DD, Kolomytkin OV, Dunn S, Marino AA. Hyaluronan suppresses il-1beta-induced metalloproteinase activity from synovial tissue. Clin Orthop Relat Res. 2007;465:241–8.

Wehling P, Moser C, Frisbie D, McIlwraith CW, Kawcak CE, Krauspe R, Reinecke JA. Autologous conditioned serum in the treatment of orthopedic diseases. Biodrugs. 2007;21(5):323–33.

Wu C-C, Chen W-H, Zao B, Lai P-L, Lin T-C, Lo H-Y, Shieh Y-H, Wu C-H, Deng W-P. Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokine-inhibited chondrogenesis. Biomaterials. 2011;32(25):5847–54.

Xie X, Zhang C, Tuan RS. Biology of platelet-rich plasma and its clinical application in cartilage repair. Arthritis Res Ther. 2014;16(1):204.

Xix Congresso Nazionale S.I.C.O.O.P. Societa' Italiana Chirurghi Ortopedici Dell'Ospedalita' Privata A, Papalia R, Zampogna B, Russo F, Torre G, De Salvatore S, Nobile C, Tirindelli MC, Grasso A, Vadala G, et al. The combined use of platelet rich plasma and hyaluronic acid: Prospective results for the treatment of knee osteoarthritis. J Biol Regul Homeost Agents. 2019;33(2 Suppl. 1):21–8.

Yang KGA, Raijmakers NJH, ERAV A, Caron JJ, Rijk PC, Willems WJ, JAC Z, Verbout AJ, WJA D, DBF S. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. Osteoarthritis Cartilage. 2008;16(4):498–505.

Yang CY, Chanalaris A, Troeberg L. Adamts and Adam metalloproteinases in osteoarthritis - looking beyond the 'usual suspects'. Osteoarthr Cartil. 2017;25(7):1000–9.

Yoshihara Y, Nakamura H, Obata K, Yamada H, Hayakawa T, Fujikawa K, Okada Y. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. Ann Rheum Dis. 2000;59(6):455–61.

Zhao J, Huang H, Liang G, Zeng LF, Yang W, Liu J. Effects and safety of the combination of platelet-rich plasma (prp) and hyaluronic acid (ha) in the treatment of knee osteoarthritis: a systematic review and meta-analysis. BMC Musculoskelet Disord. 2020;21(1):224.

Chapter 13
Exercise and Diet in the Control of Inflammation and Pain

Jasmine Ji, Aidan McGinnis, and Ru-Rong Ji

Abstract Lifestyle choices, such as exercise and diet, can play significant roles in mediating inflammation and consequently, pain. Functional medicine is an emerging medical specialty that focuses on lifestyle influences, genetics, and the environment to determine what is causing disease or chronic conditions such as chronic pain. The foundation of functional medicine is the use of food as a first-line therapy. The right nutrition, combined with the right lifestyle and behavioral interventions, will help individuals take charge of their health. Healthy diets are enriched with omega-3 unsaturated fatty acids, such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). These are precursors of specialized proresolving mediators (SPMs) which are known to potently inhibit pain in various animal models of inflammation. Exercise can profoundly change immune cell phenotypes and promote the resolution of inflammation and pain. In particular, a combination of exercise and a healthy diet can facilitate the biosynthesis of SPMs from DHA and EPA, generating synergistic health benefits.

Keywords Inflammation · Chronic pain · COVID-19 · Diet · Exercise · Exerkines · Functional medicine · Myokines · Omega-3 unsaturated fatty acids · Specialized proresolving mediators (SPMs) · Resolution of inflammation

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13.1 Introduction

Behavior change cannot be picked up at the pharmacy and taken twice daily. As a result, exercise and diet are often overlooked in the management of pain in favor of pharmacological, interventional, or surgical treatments. Nonetheless, exercise and diet can have consequential roles in helping to reduce inflammation and attenuate pain. Functional medicine is an emerging specialty that focuses on lifestyle influences, genetics, and the environment to determine what is causing disease or chronic conditions such as chronic pain. The foundation of functional medicine is that lifestyle and behavioral interventions can help individuals take charge of their health. In this chapter, we examine the evidence backing various types of lifestyle modifications in the context of inflammation and pain and propose possible mechanisms for these effects.

The role of diet in pain stands to reason; supplying the body with building blocks for pro-resolution molecules such as specialized proresolving mediators (SPMs) may be of benefit. Certain diets may also contribute by reducing or promoting inflammation. Dietary interventions typically fall into three categories: restricting or eliminating particular types of food (e.g., low-fat diets, gluten-free diets, ketogenic diets, and vegetarian and vegan diets), altering total calorie intake, and supplementation via the addition of non-drug products (such as fish oil).

The benefit of exercise, however, may initially seem counterintuitive. It is widely accepted in the research field that pain is aversive, and that pain relief is rewarding (King et al. 2009; Navratilova et al. 2012). So why do so many people seek out strenuous exercise, and how can causing acute stress aid resolution? One well-known phenomenon of exercise is the "runner's high"; runners and many other athletes often report experiencing a brief period of euphoria after intense exercise. Endorphins, cannabinoids, and dopamine may be responsible for this phenomenon, and each of these has been separately tied to pain modulation. Still, the runner's high is just one of the many ways in which exercise interacts with the pain system. A wealth of studies suggest that exercise may contribute to the resolution of inflammation and the attenuation of pain by interacting with the immune system.

Despite the difficulties in implementing behavior change as medicine, the role of diet and exercise in chronic pain remains an important field of study. The mechanisms underlying these effects should be elucidated both to highlight the importance of healthy habits and to improve the field's understanding of protective and pro-resolution mechanisms.

13.2 Exercise, Inflammation, and Pain

Exercise can reduce inflammation and pain (Gleeson et al. 2011; Runhaar et al. 2019). Exercise has long been used in rehabilitation programs, especially for patients with chronic musculoskeletal conditions and chronic pain, and clinical

trials support this practice for patients with conditions such as low back pain or musculoskeletal pain (Tan et al. 2022; Van Middelkoop et al. 2010). In rodents, swim therapy has been found to alleviate the chronic pain caused by nerve injury. It was found in two studies that regular swim therapy sessions significantly reduced the mechanical allodynia and thermal hyperalgesia in both rats with chronic constriction injury (CCI) and mice with partial sciatic nerve ligation (PSNL) compared to animals receiving a control intervention (Shen et al. 2013; Kami et al. 2018). Rats with a spinal cord injury saw substantial improvements in mechanical allodynia after commencing either of swimming and treadmill training as compared to stand training or no intervention (Hutchinson et al. 2004). However, mice with chronic inflammatory and neuropathic pain exhibit deficits in voluntary wheel running as well as other movement-related assays (e.g., open field test, see Chap. 3). This presents a challenge: those who need exercise most may find their condition inhibitive.

Exercise is not just beneficial in resolving pain: it's also a powerful preventative measure. Correlational epidemiology repeatedly finds that those who are more active are less likely to develop a chronic pain condition (Law and Sluka 2017). This is consistent with the notion that long periods of sitting, as many experience in their day-to-day lives, can increase the excitability of pain pathways while decreasing the inhibitory effects of the central nervous system (CNS), making people who sit for long periods of time more susceptible to developing chronic pain. Conversely, regular exercise can decrease pain pathway excitability while increasing inhibitory effects in both the central nervous system and the immune system, powerfully reducing the risk of developing chronic pain. These ideas have led some experts to declare chronic pain to be, in part, a "disease of inactivity" (Sluka et al. 2018). This is also supported by animal research. Strikingly, six weeks of voluntary wheel running prior to nerve injury has been found to reduce the severity of pain and to hasten recovery in rats (Grace et al. 2016). A similar trend was found in mice allowed seven days of wheel running prior to establishment of chemotherapy-induced peripheral neuropathy (Slivicki et al. 2019).

The use of exercise, however, is very much a balancing act: the act of exercising can trigger pain in patients, which makes it difficult to continue exercise regimens during rehabilitation programs. Studies using animal models of exercise-induced pain suggest that activation of NMDA (N-methyl-D-aspartate) receptors in pain-modulating areas may be the cause of this pain; in other words, the induction of this pain follows a central mechanism (Sluka et al. 2018). A related concern is that some animal work has relied upon forced, not voluntary, exercise. Still, exposure to voluntary exercise has proven effective, even when animals exercised relatively little (Pitcher et al. 2017). In other words, even a little exercise may go a long way in helping relieve persistent pain. Extrapolating these data, it seems that encouraging chronic pain patients to partake in any degree of physical activity may be beneficial, even if their pain prevents them from engaging in strenuous exercise.

13.2.1 Release of Neurochemicals and Exerkines

The question remains: how does exercise exert these striking effects? One explanation points to the release of neurochemicals—some of which may be at the root of the "runner's high." Endorphins and endocannabinoids are two neuropeptides released during exercise. Dopamine may also be stimulated by voluntary exercise. Notably, these exercise-produced signaling molecules can serve as neuromodulators and inhibit pain in certain contexts (Pilozzi et al. 2020). They are best known for contributing to the phenomenon of the "runner's high," a brief feel-good period after intensive exercise (Boecker et al. 2008; Fuss et al. 2015). Endorphins, or "endogenous morphine" are named as such because they imitate the effects of morphine, by binding to morphine receptors. Twenty different kinds of endorphins exist; the one most tied to exercise is known as a beta-endorphin. Beta-endorphin is also released during acupuncture and meditation (Han 2003). Endorphins tend to be too large to cross the blood–brain barrier but may be endogenously produced in the CNS and travel via cerebrospinal fluid (Veening et al. 2012).

Like endorphins, endocannabinoids are endogenously produced and can create pleasurable feelings during exercise, in this case by acting on the endocannabinoid system. Unlike endorphins, endocannabinoids are small enough that they can cross the blood–brain barrier to reach the brain. In particular, endocannabinoids produce effects similar to tetrahydrocannabinol (THC), the main psychoactive component found in marijuana. Endocannabinoids are known to interact with sensory neurons and microglia through both the cannabinoid 1 receptors (CB1) and cannabinoid 2 receptors (CB2), respectively. Notably, CB2 is expressed in spinal cord microglia and activation of CB2 can induce an anti-inflammatory phenotype of microglia and inhibit neuropathic pain in rodents (Romero-Sandoval et al. 2009).

A third hypothesis is that exercise may release dopamine. Ventral tegmental area dopaminergic neurons are a critical part of the mesolimbic system. The exercised-induced hypoalgesia provided by voluntary wheel running has been tied to increased activation of the mesolimbic reward system (Kami et al. 2018). Recent studies using a range of exercise protocols suggest that central inhibitory mechanisms may play a role in the analgesic effect of exercise. Opioid, serotonin, and NMDA mechanisms in the rostral ventromedial medulla all increase exercise-induced analgesia (Lima et al. 2017).

The realization that skeletal muscle is an endocrine organ capable of secreting myokines (e.g., interleukin-6 [IL-6]), which participate in tissue and immune crosstalk, has provided a critical link between exercise and the health benefits it induces (Whitham and Febbraio 2016). These myokines are a subset of the exerkines released following exercise; there are cardiokines from heart, hepatokines from liver, adipokines from white adipose tissue, baptokines from brown adipose tissue, and neurokines from neurons. Based on these findings, exerkines are defined as a broad category of signaling moieties released in response to acute and chronic exercise, which exert their beneficial effects, including pain relief, through endocrine, paracrine and/or autocrine pathways and neuroimmune interactions (Chow et al. 2022).

These exerckines could interact with pain in numerous ways. Exercise triggers multiple endogenous protective and repair processes by altering gene expression and releasing a range of factors that prepare our body for the next challenge. These factors involve anti-oxidation, energy metabolism, and anti-inflammation. Oxidative stress can be triggered following an acute injury and cause secondary damage leading to chronic pain onset. Safakhah et al. examined whether exercise can reduce these secondary mechanisms after CCI in animals. They found that post-injury exercise not only reduced allodynia and hyperalgesia, but also increased ferric reducing ability of plasma (FRAP) and reduced TNF-alpha levels (Safakhah et al. 2017). Other ways in which exerkines may affect pain processing by inducing neuroplasticity and stimulating the secretion of BDNF (brain-derived neurotrophic factor) (Chow et al. 2022). Chronic pain is commonly thought of as maladaptive long-term potentiation (Ji et al. 2003); successful amelioration of SCI-induced allodynia via treadmill exercise in rats has been tied to increased BDNF transcription (Hutchinson et al. 2004). Still other exerkines may work to maintain brain homeostasis and confer protection against pathological insults by regulating glial activation and neuroinflammation. Activated microglia and multiple pro-inflammatory cytokines play active roles in the pathogenesis of pain as well as other neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). It is safe to say that pain is not the only neurological condition improved by exercise. In the following section, we will explore how exerkines affect pain via crosstalk with the immune system.

13.2.2 Immune Crosstalk in Exercise–Induced Pain Modulation

In addition to its neuromodulatory effects, the anti-inflammatory effects of exercise are also well recognized and documented (Gleeson et al. 2011; Runhaar et al. 2019). Long-term exercise at low-to-moderate intensity beneficially regulates the inflammatory response (Mee-Inta et al. 2019). Nerve injury results in immune system activation leading to an increase in proinflammatory cytokines at both the location of injury and the spinal dorsal horn. Exercise has been found to stimulate macrophages to make a phenotypic switch, from a pro-inflammatory phenotype (M1) to an anti-inflammatory phenotype (M2) in uninjured muscle and, after a nerve injury and to promote the production of anti-inflammatory cytokines, contributing to an analgesic effect. Much of this effect is thought to be regulated via exerkine secretion.

The cytokine IL-6 was the first identified myokine, as skeletal muscle releases large amounts of IL-6 (Chow et al. 2022; Steensberg et al. 2000). Extensive research has shown that IL-6 can be both proinflammatory and anti-inflammatory in a context-dependent manner (Kistner et al. 2022). It was proposed that distinct signaling pathways (classic-signaling and trans-signaling) may mediate different effects of IL-6. The pro-inflammatory effects of IL-6 use trans-signaling by which IL-6 binds to a soluble form of IL-6 receptor. In contrast, the anti-inflammatory effects

of IL-6 are mediated by classic-signaling in which IL-6 acts on the membrane-bound non-signaling α-receptor. Notably, the levels of IL-6 may be reduced in several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease.

Other myokines may also exhibit different anti-inflammatory properties. IL-6 upregulates the expressions of anti-inflammatory cytokine IL-10 and IL-1 receptor antagonist (IL-1Ra). IL-1Ra has a higher affinity for the IL-1R than IL-1α or IL-1β. Blocking the binding of IL-1 to its receptor interrupts the pro-inflammatory IL-1 signaling cascade and related microglial activity. It was found that long-term exercise can increase the production and secretion of IL-10 in the skeletal muscles and drives resident macrophages to take on an anti-inflammatory "M2" phenotype. This phenotypic shifting has also been observed in spinal cord microglia following exercise in a mouse model of neuropathic pain (Gong et al. 2017). Furthermore, inhibiting IL-10 blocked the benefit of wheel running in mice with musculoskeletal pain (Leung et al. 2016). These anti-inflammatory myokines can also be transported into the CNS from the peripheral circulation. Thus, exercise can alleviate pain by upregulation of the anti-inflammatory cytokines and inhibition of microglial activation in the spinal cord (Lesnak and Sluka 2020).

Recent evidence also points to the cytokine IL-4 as being an exerkine of critical importance in exercise-induced pain modulation. In a mouse model of peripheral nerve injury, two weeks of low-intensity exercise inhibited peripheral and central neuroinflammatory responses via upregulation of IL-4 (Bobinski et al. 2018). Moreover, treadmill exercise resulted in an increase in M2 macrophages and decrease in M1 macrophages, which secrete anti-inflammatory and inflammatory macrophages, respectively, in comparison to sedentary mice. Importantly, these results were not seen in IL-4 knockout mice. Furthermore, IL-4 has been found to contribute to pain amelioration by stimulating the secretion of endorphins from macrophages at the site of nerve injury (Celik et al. 2020), perhaps suggesting a link between exerkine and neuropeptide mechanisms.

13.2.3 Respiration and Circulation

Inflammation is notable for causing various breathing problems, perhaps most commonly seen in asthma, which affects over 20 million Americans. However, increased breathing rate and thus circulation can contribute to reducing inflammation following exercise. In general, decreased circulation reduces the body's ability to repair damaged tissues and protect tissues against further damage, as the body requires the flow of blood to deliver nutrients and carry away toxins, dead cells, and other debris. Thus, not only does increased inflammation impair circulation, poor circulation can also cause chronic inflammation. (Interestingly, as will be discussed later about the diet, nutrients found in nuts, seeds, olive oil, and oily fish can help improve circulatory health while inhibiting inflammation). One group, examining the role of improving heart circulation in mice as a method of treating coronary heart disease,

found that activating blood circulation could help inhibit inflammation (Ma et al. 2014).

Breathing during exercise can also activate the sympathetic nervous system. It is well established that the sympathetic and parasympathetic nervous systems control the heart rate. While the sympathetic nervous system releases catecholamines (epinephrine and norepinephrine) to accelerate the heart rate, the parasympathetic nervous system releases the hormone acetylcholine to slow the heart rate. Exercising for any duration increases heart rate, which will stay elevated for as long as the exercise is continued. At the beginning of exercise, the body removes the parasympathetic stimulation, which allows the heart rate to gradually increase. As exercise becomes more intensive, the sympathetic system acts to accelerate the heart rate even more. By contrast, regular cardiovascular exercise over an extended period of time is known to decrease your resting heart rate by increasing heart size, contractile strength, and length of time the heart fills with blood. This results in an increase in activity of the parasympathetic nervous system and decrease in sympathetic nervous system. In short, an accelerated heart rate, as seen in exercise, requires the use of the parasympathetic system to slow down.

The sympathetic nervous system has been linked to inflammation. The sympathetic system is well known for the "fight or flight" response. In addition, it is part of constant regulatory machinery that keeps body functions in a steady-state equilibrium. The sympathetic nervous system operates closely with the hypothalamic-pituitary axis (HPA) and the sensory nervous system and vagal nervous system (VNS), to accomplish its functions. When a pathogen enters the body, local activation of immune cells releases proinflammatory mediators, which are able to excite or sensitize (by lowering thresholds) of nociceptive afferent and vagal afferent nerve fibers. The inflammatory signals reach to the brain and result in activation of the two major stress axes, the HPA axis and the sympathetic nervous system. The proinflammatory cytokines IL-1β and TNF are crucial in this communication from the immune system to the central nervous system. In turn, central sympathetic activity also talks to the immune system, exhibiting direct effects on inflammatory cytokines. In a study with hypertensive patients, central inhibition of the sympathetic nervous system decreased peripheral TNF serum levels. Similarly, stress responses that modulate sympathetic nervous system activity have a great impact on inflammation. However, there might be a disruption of this communication between the brain and the immune system in the course of protracted inflammation, as shown in an arthritis model in rats. Thus, the activation of the sympathetic nervous system during exercise may also influence inflammation through the increased sympathetic nervous system activity (Wei et al. 2020).

Exercise has also been shown to benefit people with knee osteoarthritis. Subchondral bone degeneration and synovitis are the main characteristics of knee osteoarthritis, which can be exacerbated with mechanical overload, inflammation, factors involving metabolism or hormones and aging. Although surgery for knee osteoarthritis is available, it tends to be for patients with end-stage knee osteoarthritis only. As such, exercise becomes an important part of treatment for those with knee osteoarthritis. Exercise can not only inhibit inflammation and further

degeneration of cartilage, it can also stop the loss of subchondral bone and metaphyseal bone trabeculae. According to recent studies, pain, stiffness, joint dysfunction, and muscle weakness are also improved with exercise in patients with knee osteoarthritis. Varied forms of exercise, including aerobic exercise, strength training, neuromuscular exercise, balance training, proprioception training, aquatic exercise, as well as traditional exercise can all provide benefits, such as reduced inflammation, delayed cartilage and bone degeneration, and improved tendon and muscle structure (Zeng et al. 2021).

The mechanisms by which exercise helps prevent the exacerbation of chronic inflammatory diseases requires further elucidation. One study has shown that exercise can promote resolution of acute inflammation by increasing levels of resolvin D1 and macrophage phagocytic activity. The study found that mice given a four-week treadmill exercise regimen had higher RvD1 and macrophage phagocytic activity levels. They also found that neutrophil clearance occurred earlier after acute inflammation. The authors further determined that exercise may achieve these effects through the release of epinephrine, which is known to have immunomodulatory effects. Macrophages treated with epinephrine show higher levels of RvD1 and 15-lipoxygenase-1 abundance. These changes were prevented by incubation with the α1 adrenergic receptor (α1-AR) antagonist prazosin. It was found that stimulation of α1-AR with phenylephrine also enhanced RvD1 production and macrophage phagocytosis. During acute inflammation, prazosin abrogated exercise-enhanced neutrophil clearance, macrophage phagocytosis, and RvD1 biosynthesis. These results point to the possibility that exercise-stimulated epinephrine enhances resolution of acute inflammation in an α1-AR-dependent manner (Zheng et al. 2019).

Of particular interest is a study from Harvard Medical School reported that stretching can impact inflammation resolution. Tissue stretching was shown to activate local pro-resolving mechanisms in the acute phase of inflammation. In a rat model of inflammation induced by carrageenan, stretching was shown to reduce edema and neutrophil count and increase RvD1 concentrations within inflamed tissue. Interestingly, subcutaneous resolvin administration could recapitulate the action of stretching. The *in vivo* effects can be reproduced in ex vivo conditions, which demonstrated that stretching of connective tissue was sufficient to inhibit the migration of neutrophils and enhance tissue RvD1 concentration (Lisbeth Berrueta et al. 2016).

Another study characterized the inflammatory lipid mediator response in blood to unaccustomed resistance exercise in humans. It was found that acute proinflammatory signaling is mechanistically linked to the induction of an active resolution program, regulated by proresolving lipid mediators during post-exercise period of recovery. Postexercise recovery was characterized by elevated levels of cyclooxygenase (COX)-derived prostanoids, lipoxygenase-derived leukotrienes, as well as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)-derived resolvins (RvD1 and RvE1), and protectins. Notably, Ibuprofen, an anti-inflammatory treatment, not only blocked the exercise-induced prostanoids but also suppressed the

SPM response (Markworth et al. 2013). Thus, exercise-induced acute inflammation may have beneficial effects by promoting SPM production and signaling.

13.3 Diet in Inflammation and Pain

Diet also plays a significant role in modulating inflammation. Microbiota and its metabolites have been shown to alter CNS function. Probiotics restore the eubiosis in the gut while a gluten-free diet, by modulation of microbiota profile and intestinal permeability, can alter the activity of microbiota-gut-brain axis, which was associated with the pathogenesis of depression. Of note is that microbiota being able to digest gluten may play a role in the formation of peptides with different immunogenic capacities. It was found that the combination of a gluten-free diet and probiotic supplementation may inhibit the immune-inflammatory cascade in major depression disorder, improving both psychiatric and gut barrier-associated features (Karakula-Juchnowicz et al. 2019).

Gluten-related disorders include Celiac disease (CD) and non-celiac gluten/wheat sensitivity (NCG/WS). Both are triggered and worsened by ingestion of gluten proteins. Other components, such as amylase/trypsin inhibitors and fermentable oligosaccharides, disaccharides, monosaccharides and polyols, may also play a role in the development of NCG/WS onset. The only effective treatment to date is a lifelong adherence to a strictly gluten-free diet. Emerging evidence shows the involvement of intestinal barrier which regulates the delicate crosstalk between metabolic, neuroendocrine, and immunological functions. Especially, the microbiota plays a crucial role in regulating the gut integrity and inflammation process, which is associated with the outbreak of CD and NCG/WS (Caio et al. 2020).

In NOD/ShiLtJ (NOD) mice, in which leukocytes infiltrate pancreatic islets to produce insulitis, a lifelong gluten-free diet was found to decrease infiltration of monocytes/macrophages and T cells in salivary glands, leading to reduced inflammation in pancreatic islets. Thus, autoimmune diseases, such as type 1 diabetes (T1D) and Sjogren's syndrome (SS), may be alleviated by a gluten-free diet (Haupt-Jorgensen et al. 2022).

Omega-3 polyunsaturated fatty acids (PUFAs) are enriched in healthy diet and fish oil and demonstrate a wide range of benefits in human health and animal disease models. PUFAs include eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). It is generally believed that many of the biological functions of PUFAs are mediated via bioactive metabolites, produced by the actions of fatty acid oxygenases, including cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases (Ishihara et al. 2019).

In a clinical trial with EPA intervention, icosapent ethyl (the ethyl ester form of EPA) demonstrates a significant improvement of cardiovascular events. Importantly, EPA is a precursor for the formation of E series of resolvins, which belong to a

subfamily of specialized proresolving mediators (SPMs). Resolvin E1 (RvE1) is the best investigated member of E series of resolvins. It stimulates the resolution of inflammation and reduces atherosclerosis through its specific receptor ChemR23 (also named ERV1). Furthermore, ω-3 fatty acids produce additional benefits by decreasing the levels of proinflammatory and proatherosclerotic leukotrienes. Interestingly, the ratio of resolvins and leukotrienes is an emerging marker of resolving vs. non-resolving vascular inflammation. SPMs was shown to alleviate atherosclerosis independently of changing cholesterol and triglyceride levels. The findings of the recent clinical trials of ω-3 fatty acid supplementation have demonstrated the importance of the type and dose of ω-3 supplementation. They also highlight the need for risk stratification in terms of patient selection for ω-3 supplementation for prevention of primary and secondary cardiovascular diseases (Back et al. 2019).

Diet can have a profound impact on both inflammation and pain. One diet that has been popularized as a method of reducing inflammation is the omega-3 diet. Omega-3 fatty acids are also known as "essential fats" because they cannot be produced in the human body and thus must be obtained through food. These fats can be divided into three groups: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA can be found in various nuts, such as walnuts, and many plants. EPA and DHA can both be found in cold-water fish, including salmon and tuna. This section explores the effects of omega-3 fatty acids and diets including these fats on inflammation and pain.

PUFAs have been found to be analgesic (Bjørklund et al. 2019). EPA and DHA are essential precursors for the biosynthesis of SPMs, which includes resolvins, protectins, and maresins (Ji 2023; Serhan 2014). These mediators all act as potent analgesics in various animal models of pain, including inflammatory, post-operative, and neuropathic pain models. Resolvins can powerfully suppress inflammatory pain. They are 1000 times more effective than EPA and DHA precursors found in food items and 100 times more effective than morphine. These mediators not only resolve inflammation, but also suppress neural plasticity, glial function, and TRP (transient receptor potential) channel function (Ji 2023). Previous studies have found fish oil diets to be ineffective for treating migraines and rheumatoid arthritis. In these studies, however, it is ambiguous whether or not the PUFAs from the diets were transferred to the patients in any significant way. In another study, in which PUFAs were directly administered to patients suffering from migraines, patients saw significant reductions in pain, supporting the effectiveness of PUFAs in suppressing pain (Van De Ven and Ji 2013).

Another diet known to reduce inflammation is the ketogenic diet, which consists of high-fat, low carbohydrate food that causes cells to rely on ketone rather than glucose for energy. The ketogenic diet has been shown to be effective in treating pediatric epilepsy and type II diabetes, and may potentially even reduce brain injury. The cellular mechanisms enabled by a dependence on ketone suggest that the ketogenic diet may be able to reduce pain and inflammation. One study found a significant reduction in inflammation in rats given a ketogenic diet, based on hindpaw swelling and plasma extraversion measurements. In addition, the diet resulted in

thermal hypoalgesia. As such, the ketogenic diet may be another option for reducing inflammation via diet (Ruskin et al. 2009).

Another study examined the effects of a meat-, gluten-, and lactose-free diet in rheumatoid arthritis patients. They found that the diet reduced the number of circulating leukocytes and neutrophils, as well as the level of hs-C-Reactive Protein after three months, suggesting that such a diet may help further control inflammation in rheumatoid arthritis patients who already have proper drug treatments in place (Guagnano et al. 2021).

Conversely, some diets can also contribute to inflammation. The stereotypical "western diet," full of processed oils and fats, and known for promoting obesity, has been identified as a diet that can promote inflammation through omega-6 PUFAs. Omega-6 PUFAs can be found in many vegetable oils, such as sunflower, corn, soybean, and cottonseed oils. These PUFAs can build up in membrane phospholipids, then oxidize into pronociceptive oxylipins to induce pain. In mice, omega-6 PUFAs have been found to induce peripheral neuropathy, perhaps explaining the neuropathy found in many obese but non-diabetic people (McGinnis and Ji 2021). In particular, mice administered an ω-6 PUFA-enriched diet develop lasting hypersensitivity, develop persistent spontaneously active and hyper-responsive glabrous afferent fibers, and histologic markers of peripheral nerve damage characteristic of peripheral neuropathy. Moreover, omega-6 PUFAs, such as linoleic and arachidonic acids, build up in lumbar dorsal root ganglia and can be allowed to move more easily by phospholipase PLA2 activity. Omega-6-induced peripheral neuropathy is reversible, however, by either inhibiting platelet-activating factor acetylhydrolase (PLA2G7) or with a diet rich in omega-3 PUFAs. These treatments can reduce nociceptive behaviors, neurophysiologic abnormalities, and afferent histopathology induced by high omega-6 consumption in mice. Omega-6 PUFA accumulation also worsens allodynia found in preclinical inflammatory and neuropathic pain models, which is highly correlated with many pain indices of clinical diabetic neuropathy (Boyd et al. 2021).

13.4 Conclusions and Future Directions

This chapter examined the benefits of exercise and diet in terms of reducing inflammation and pain. During exercise, the release of neurochemicals (i.e., endorphins and endocannabinoids) and increased respiration and circulation can help reduce inflammation. Over time, exercise can also contribute to altering fat composition, increasing brown fat while decreasing white fat, which is associated with inflammation. Moreover, exercise results in release of epinephrine and IL-6, which can promote SPM levels and phagocytic activity by macrophages to resolve inflammation. Exercise is particularly helpful to those with knee osteoarthritis, which otherwise impairs mobility and decreases quality of life.

Diet can also play an important role in reducing inflammation, with omega-3 PUFAs, found in fish oil, containing precursors for a variety of SPMs, all of which help resolve pain and inflammation. High-fat, low-carbohydrate ketogenic diets and meat-, gluten-, and lactose-free diets were also shown to decrease inflammation. On the other hand, omega-6 PUFAs, often found in vegetable oils used in high-fat "western" diets which promote obesity, increase inflammation, leading to painful peripheral neuropathy. The effects of omega-6 PUFAs, however, can be reversed via the administration of omega-3 PUFAs.

The anti-inflammatory effects of both exercise and omega-3 diets may depend on SPMs. SPMs consist of cell signaling molecules that are produced via the metabolism of PUFAs by one or several of the enzymes, including lipoxygenase, cyclooxygenase, and cytochrome P450 monooxygenase. SPMs have potent anti-inflammatory and neuroprotective capabilities. As mentioned before, SPMs consist of resolvins, protectins, and maresins and possess potent analgesic actions in various animal models (Ji 2023). Exercise and diets can effectively change immune cell phenotypes and contribute to the resolution of inflammation and pain. We highlight that a combination of exercise and a healthy diet can facilitate the biosynthesis of SPMs from omega-3 PUFAs, leading to synergistic health benefits (Figs. 13.1 and 13.2).

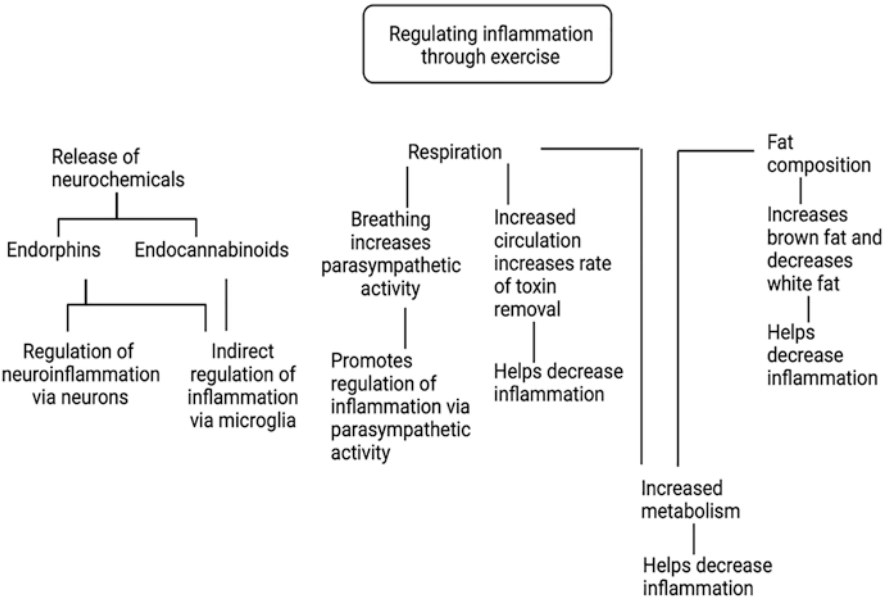

Fig. 13.1 Regulation of inflammation through exercise. Exercise can help regulate inflammation through a variety of processes, including the release of neurochemicals, respiration, and changing fat composition. The latter two processes contribute to increased metabolism. In such a way, exercise is able to promote the regulation of inflammation in various ways, leading to pain relief

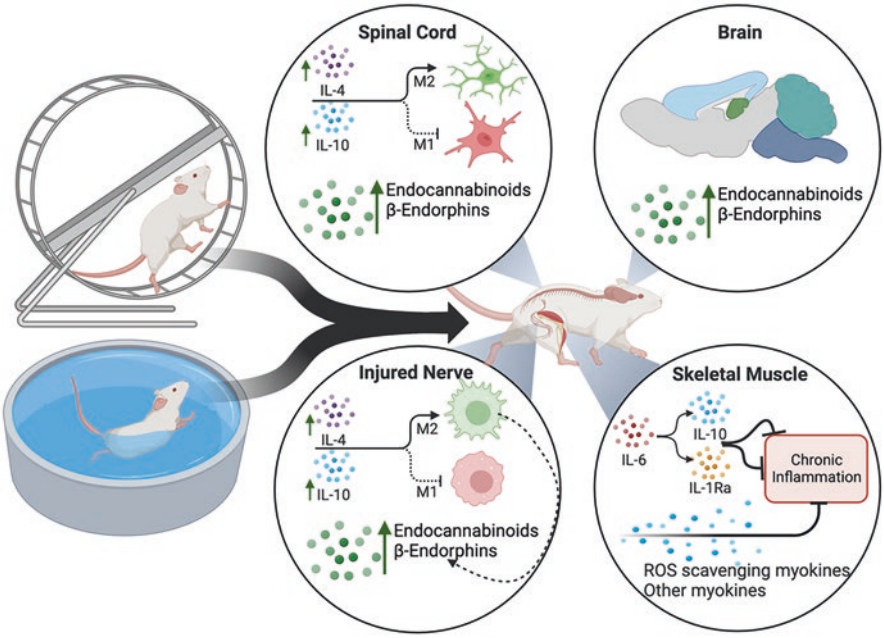

Fig. 13.2 Effects of exercise on a nerve-injured rodent. Exercise drives pain relief at multiple levels. In all nervous tissues (nerve, spinal cord, and brain), endocannabinoids and β-endorphins modulate neuronal activity. IL-4 and IL-10 drive microglia toward an anti-inflammatory phenotype in the spinal cord and macrophages toward a similar phenotype near the injured nerve. In skeletal muscle, IL-6 drives secretion of IL-10 and IL-1Ra, contributing along with other myokines toward reduction of chronic inflammation and chronic pain

References

Berrueta L, Muskaj I, Olenich S, Butler T, Badger GJ, Colas RA, Spite M, Serhan CN, Langevin HM. Stretching impacts inflammation resolution in connective tissue. J Cell Physiol. 2016;231(7):1621–7. https://doi.org/10.1002/jcp.25263.

Bjørklund G, Aaseth J, Doşa MD, Pivina L, Dadar M, Pen JJ, Chirumbolo S. Does diet play a role in reducing nociception related to inflammation and chronic pain? Nutrition (Burbank, Los Angeles County, Calif.). 2019;66:153–65. https://doi.org/10.1016/j.nut.2019.04.007.

Bobinski F, Teixeira JM, Sluka KA, Santos A. Interleukin-4 mediates the analgesia produced by low-intensity exercise in mice with neuropathic pain. Pain. 2018;159(3):437–50. https://doi.org/10.1097/j.pain.0000000000001109.

Boecker H, Sprenger T, Spilker ME, Henriksen G, Koppenhoefer M, Wagner KJ, Valet M, Berthele A, Tolle TR. The runner's high: Opioidergic mechanisms in the human brain. Cereb Cortex. 2008;18(11):2523–31.

Boyd JT, LoCoco PM, Furr AR, Bendele MR, Tram M, Li Q, Chang FM, Colley ME, Samenuk GM, Arris DA, Locke EE, Bach S, Tobon A, Ruparel SB, Hargreaves KM. Elevated dietary ω-6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. Nat Metab. 2021;3(6):762–73. https://doi.org/10.1038/s42255-021-00410-x.

Caio G, Lungaro L, Segata N, Guarino M, Zoli G, Volta U, De Giorgio R. Effect of gluten-free diet on gut microbiota composition in patients with celiac disease and non-celiac gluten/wheat sensitivity. Nutrients. 2020;12(6):1832.
Celik MÖ, Labuz D, Keye J, Glauben R, Machelska H. Il-4 induces m2 macrophages to produce sustained analgesia via opioids. JCI Insight. 2020;5(4)
Chow LS, Gerszten RE, Taylor JM, Pedersen BK, van Praag H, Trappe S, Febbraio MA, Galis ZS, Gao Y, Haus JM. Exerkines in health, resilience and disease. Nat Rev Endocrinol. 2022;18(5):273–89.
Fuss J, Steinle J, Bindila L, Auer MK, Kirchherr H, Lutz B, Gass P. A runner's high depends on cannabinoid receptors in mice. Proc Natl Acad Sci. 2015;112(42):13105–8.
Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol. 2011;11(9):607–15.
Gong X, Chen Y, Fu B, Jiang J, Zhang M. Infant nerve injury induces delayed microglial polarization to the m1 phenotype, and exercise reduces delayed neuropathic pain by modulating microglial activity. Neuroscience. 2017;349:76–86.
Grace PM, Fabisiak TJ, Green-Fulgham SM, Anderson ND, Strand KA, Kwilasz AJ, Galer EL, Walker FR, Greenwood BN, Maier SF. Prior voluntary wheel running attenuates neuropathic pain. Pain. 2016;157(9):2012.
Guagnano MT, D'Angelo C, Caniglia D, Di Giovanni P, Celletti E, Sabatini E, Speranza L, Bucci M, Cipollone F, Paganelli R. Improvement of inflammation and pain after three Months' exclusion diet in rheumatoid arthritis patients. Nutrients. 2021;13(10):3535.
Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci. 2003;26(1):17–22.
Haupt-Jorgensen M, Groule V, Reibel J, Buschard K, Pedersen A. Gluten-free diet modulates inflammation in salivary glands and pancreatic islets. Oral Dis. 2022;28(3):639–47.
Hutchinson KJ, Gómez-Pinilla F, Crowe MJ, Ying Z, Basso DM. Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. Brain. 2004;127(6):1403–14.
Ishihara T, Yoshida M, Arita M. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. Int Immunol. 2019;31(9):559–67.
Ji RR. Specialized pro-resolving mediators as resolution pharmacology for the control of pain and itch. Annu Rev Pharmacol Toxicol. 2023;63:273–293. https://doi.org/10.1146/annurev-pharmtox-051921-084047.
Ji R-R, Kohno T, Moore KA, Woolf CJ. Central sensitization and ltp: do pain and memory share similar mechanisms? Trends Neurosci. 2003;26(12):696–705.
Kami K, Tajima F, Senba E. Activation of mesolimbic reward system via laterodorsal tegmental nucleus and hypothalamus in exercise-induced hypoalgesia. Sci Rep. 2018;8(1):1–14.
Karakula-Juchnowicz H, Rog J, Juchnowicz D, Łoniewski I, Skonieczna-Żydecka K, et al. The study evaluating the effect of probiotic supplementation on the mental status, inflammation, and intestinal barrier in major depressive disorder patients using gluten-free or gluten-containing diet (SANGUT study): a 12-week, randomized, double-blind, and placebo-controlled clinical study protocol. Nutr J. 2019;18(1):50. https://doi.org/10.1186/s12937-019-0475-x.
King T, Vera-Portocarrero L, Gutierrez T, Vanderah TW, Dussor G, Lai J, Fields HL, Porreca F. Unmasking the tonic-aversive state in neuropathic pain. Nat Neurosci. 2009;12(11):1364–6.
Kistner TM, Pedersen BK, Lieberman DE. Interleukin 6 as an energy allocator in muscle tissue. Nat Metab. 2022;4(2):170–9.
Law LF, Sluka KA. How does physical activity modulate pain? Pain. 2017;158(3):369.
Lesnak JB, Sluka KA. Mechanism of exercise-induced analgesia: what we can learn from physically active animals. Pain Reports. 2020;5(5):e850.
Leung A, Gregory NS, Allen L-AH, Sluka KA. Regular physical activity prevents chronic pain by altering resident muscle macrophage phenotype and increasing il-10 in mice. Pain. 2016;157(1):70.

Lima LV, Abner T, Sluka KA. Does exercise increase or decrease pain? Central mechanisms underlying these two phenomena. J Physiol. 2017;595(13):4141–50.

Markworth JF, Vella L, Lingard BS, Tull DL, Rupasinghe TW, Sinclair AJ, Maddipati KR, Cameron-Smith D. Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. Am J Physiol Regul Integr Comp Physiol. 2013;305(11):R1281–96. https://doi.org/10.1152/ajpregu.00128.2013.

Ma XJ, Guo CY, Yin HJ, Liu Y, Shi DZ. [Effect of activating blood circulation or activating blood circulation and detoxication on platelet activation, inflammation, and coagulation status in acute myocardial infarction rats]. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2014;34(11):1329–34.

McGinnis A, Ji RR. Can a Western high-fat diet lead to painful neuropathy? Nat Metab. 2021;3(6):735–6. https://doi.org/10.1038/s42255-021-00411-w.

Navratilova E, Xie JY, Okun A, Qu C, Eyde N, Ci S, Ossipov MH, King T, Fields HL, Porreca F. Pain relief produces negative reinforcement through activation of mesolimbic reward-valuation circuitry. Proc Natl Acad Sci U S A. 2012;109(50):20709–13.

Pilozzi A, Carro C, Huang X. Roles of β-endorphin in stress, behavior, Neuroinflammation, and brain energy metabolism. Int J Mol Sci. 2020;22(1):338. https://doi.org/10.3390/ijms22010338. PMID: 33396962; PMCID: PMC7796446.

Pitcher MH, Tarum F, Rauf IZ, Low LA, Bushnell C. Modest amounts of voluntary exercise reduce pain- and stress-related outcomes in a rat model of persistent hind limb inflammation. J Pain. 2017;18(6):687–701. https://doi.org/10.1016/j.jpain.2017.01.006.

Romero-Sandoval EA, Horvath R, Landry RP, DeLeo JA. Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via mkp induction and erk dephosphorylation. Mol Pain. 2009;5:25.

Runhaar J, Beavers DP, Miller GD, Nicklas BJ, Loeser RF, Bierma-Zeinstra S, Messier SP. Inflammatory cytokines mediate the effects of diet and exercise on pain and function in knee osteoarthritis independent of BMI. Osteoarthr Cartil. 2019;27(8):1118–23. https://doi.org/10.1016/j.joca.2019.04.009. Epub 2019 Apr 20. PMID: 31009749.

Ruskin DN, Kawamura M, Masino SA. Reduced pain and inflammation in juvenile and adult rats fed a ketogenic diet. PLoS One. 2009;4(12):e8349. https://doi.org/10.1371/journal.pone.0008349.

Safakhah HA, Moradi Kor N, Bazargani A, Bandegi AR, Gholami Pourbadie H, Khoshkholgh-Sima B, Ghanbari A. Forced exercise attenuates neuropathic pain in chronic constriction injury of male rat: an investigation of oxidative stress and inflammation. J Pain Res. 2017;10:1457–66.

Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510:92–101.

Shen J, Fox LE, Cheng J. Swim therapy reduces mechanical allodynia and thermal hyperalgesia induced by chronic constriction nerve injury in rats. Pain Med. 2013;14:516–25.

Slivicki RA, Mali SS, Hohmann AG. Voluntary exercise reduces both chemotherapy-induced neuropathic nociception and deficits in hippocampal cellular proliferation in a mouse model of paclitaxel-induced peripheral neuropathy. Neurobiol Pain. 2019;6:100035.

Sluka KA, Frey-Law L, Hoeger Bement M. Exercise-induced pain and analgesia? Underlying mechanisms and clinical translation. Pain. 2018;159 Suppl 1(Suppl 1):S91–7.

Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Pedersen BK. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6, vol. 529. Wiley Online Library; 2000. p. 237.

Tan L, Cicuttini FM, Fairley J, Romero L, Estee M, Hussain SM, Urquhart DM. Does aerobic exercise effect pain sensitisation in individuals with musculoskeletal pain? Systemat Rev BMC Musculoskelet Disord. 2022;23(1):1–21.

Van De Ven T, Ji RR. Dietary control of chronic headache: involvement of pro-resolution lipid mediators. Pain. 2013;154(11):2247–8.

Van Middelkoop M, Rubinstein SM, Verhagen AP, Ostelo RW, Koes BW, van Tulder MW. Exercise therapy for chronic nonspecific low-back pain. Best Pract Res Clin Rheumatol. 2010;24(2):193–204.

Veening JG, Gerrits PO, Barendregt HP. Volume transmission of beta-endorphin via the cerebrospinal fluid; a review. Fluids Barriers CNS. 2012;9(1):1–16.

Whitham M, Febbraio MA. The ever-expanding myokinome: discovery challenges and therapeutic implications. Nat Rev Drug Discov. 2016;15(10):719–29.

Wei Y, Liang Y, Lin H, Dai Y, Yao S. Autonomic nervous system and inflammation interaction in endometriosis-associated pain. J Neuroinflammation. 20207;17(1):80. doi: https://doi.org/10.1186/s12974-020-01752-1.

Zeng CY, Zhang ZR, Tang ZM, Hua FZ. Benefits and mechanisms of exercise training for knee osteoarthritis. Front Physiol. 2021;12:794062. https://doi.org/10.3389/fphys.2021.794062.

Zheng JJ, Pena Calderin E, Hill BG, Bhatnagar A, Hellmann J. Exercise promotes resolution of acute inflammation by catecholamine-mediated stimulation of Resolvin D1 biosynthesis. J Immunol (Baltimore, Md.: 1950). 2019;203(11):3013–22. https://doi.org/10.4049/jimmunol.1900144.

Chapter 14
Mechanisms-Based Pain Therapies

Raissa Quezado da Nobrega, Ru-Rong Ji, and Jianguo Cheng

Abstract Chronic pain is a prevalent disease with high impact on public health and individual's quality of life. Understanding the complex mechanisms and causes of pain is crucial for precise diagnosis, adequate management, and better patient outcomes. As we deepen our knowledge, new therapeutic targets and strategies are expanding and becoming more mechanism-based. Current mechanism-based therapies include approaches to modulating the transduction, conduction, transmission, perception, and adaptation of pain through pharmacological, interventional, surgical, physical/psychological behavioral treatments. Increasing evidence suggests that some of these treatments can not only alleviate pain symptoms but also control disease progression by modulation of inflammation and neuroinflammation.

Keywords Acupuncture · Cognitive-behavioral therapy · Electrotherapeutics · Integrative therapy · Interventional therapy · Multimodal therapy · Nerve blocks · Nerve ablation · Peripheral nerve stimulation · Pharmacotherapy · Physiotherapy · Spinal cord stimulation · Transcutaneous electrical nerve stimulation (TENS)

14.1 Introduction

Chronic pain affects approximately 50 million Americans and contributes to an estimate $560 billion per year in health care costs (Cheng et al. 2020; Dahlhamer et al. 2018). Despite the scientific advances and better understanding of the

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pathophysiology of chronic pain over the past decades, the management of chronic pain remains challenging. Chronic pain can be a result of the dysfunction of multiple disease processes involving complex neural components such as peripheral nerves, the spinal cord, and higher brain centers (Vardeh et al. 2016). For a given pain condition, different pain mechanisms can be present simultaneously (e.g., nociceptive, nociplastic, neuropathic). The condition can also be affected by the patient's comorbidities, gender, genetics and epigenetic factors, and psychosocial factors (Mills et al. 2019). Thus, identifying the exact mechanism involved can be challenging or even impossible due to limitations in our understanding of the pain conditions. Consequently, the treatment is frequently guided by the diagnosis and symptoms' characteristics (Cohen et al. 2021). Furthermore, therapies may lack specificity, have different mechanisms, and act on several targets causing unwanted side effects. Hence, it is fundamental to thoroughly assess the patient and accurately define the diagnosis and differential diagnosis, taking into consideration of the anatomy, cellular and molecular mechanisms, genetic implications, and the influences of biopsychosocial factors as anxiety, depression, social economic status, and pain catastrophizing (Cheng 2018; Cheng et al. 2020). Whenever possible, mechanism-based therapy for pain should be the guiding principle for pain management and pain research. Currently, a patient-centered, multimodal, and integrated approach is the best practice for optimal clinical outcomes (Cheng 2018; Cheng et al. 2020). In this chapter, we concisely review the commonly used and mechanism-based therapies that include pharmacological treatments, interventional procedures, surgeries, and physical and cognitive behavioral therapies (PT and CBT).

14.2 Pharmacological Therapies

Understanding the underlying pain mechanism is important in order to guide selection of medications for optimal pain relief. For example, neuropathic pain occurs when there is a lesion within the somatosensory nervous system leading to ectopic activity by voltage-gated sodium channels and transient receptor channels causing pain. Thus, medications that target and modulate those channels such as carbamazepine and lidocaine have a role on the treatment. Nociceptive pain is associated with nociceptor activation by an inflammatory process and can be treated by using non-steroidal anti-inflammatory drugs (NSAIDs) and tumor necrosis factor inhibitors (Scholz and Woolf 2002). Central mechanisms of pain are therapeutic target as well. When the peripheral noxious input reaches the spinal cord dorsal horn, there is release of substance P and glutamate into the synaptic cleft. Glutamate receptors such as N-methyl-D-aspartate (NMDA) and a-amino-3-hydroxy-5-methyl-4 isoxazolepropironic acid (AMPA) receptors play a role in the transmission of pain signals to supraspinal centers and in central sensitization of pain at the spinal cord level. Compromise of spinal inhibitory effects by GABAergic or glycinergic interneurons in the spinal cord is another mechanism of central sensitization. Furthermore, activation of immune cells (microglia) and glial cells (astrocytes) also contributes to

central sensitization through cytokine/chemokines release. Moreover, reduced descending inhibitory control from supraspinal centers may also contribute to central sensitization. Inhibiting the reuptake of neurotransmitters necessary for this path by antidepressants or NMDA receptors antagonists can lead to pain relief (Rekatsina et al. 2020). Multiple mechanisms may be present simultaneously in different pain conditions so that anti-inflammatory drugs, commonly prescribed for nociceptive pain, might sometimes work for a neuropathic condition and a membrane stabilizer, such as antiepileptic drugs, might improve postsurgical pain (Scholz and Woolf 2002).

The commonly used medications include local anesthetics, NSAIDs, anticonvulsants, antidepressants, muscle relaxants, opioids, and ketamine, among other drugs. The therapeutic targets are summarized in Table 14.1, while the guiding principle for selection of drugs are shown in Table 14.2. The mechanism of action of the local anesthetics is through a reversible blockade of voltage-gated sodium channels, inhibiting action potential propagation (Hermanns et al. 2019). NSAIDs reduce inflammation by inhibiting the COX enzyme, responsible for catalyzing the conversion of arachidonic acid into prostaglandins. The NSAIDs are divided into selective (COX-1) or non-selective (COX-2), with celecoxib representing a unique COX-2 selective medication that has decreased gastrointestinal symptoms (Bovill 1997). Anticonvulsants medications are membrane stabilizers that block calcium or sodium channels. Those channels play an important role in peripheral and central hyperexcitability (Sills and Rogawski 2020). Most muscle relaxants act centrally, activating alpha-2 adrenergic receptor and presumably increasing presynaptic inhibition of motor neurons (tizanidine) or inhibiting 5-HT2 receptor (cyclobenzaprine), improving muscle spasms (Coward 1994; Kobayashi et al. 1996). Baclofen may cause muscle relaxation via activation of GABAB receptors. Opioids activates mu, kappa, or delta opioid receptors to produce analgesia. Those receptors are coupled with G1 proteins, and the resulting effect is mainly inhibitory with a resulting hyperpolarization and reduction in neuronal excitability (Bovill 1997). There are multiple classes of antidepressants used in pain management including serotonin-norepinephrine reuptake inhibitors (e.g., duloxetine and tricyclic antidepressants (TCAs)), selective serotonin reuptake inhibitors (e.g., fluoxetine) and selective norepinephrine reuptake inhibitors (e.g., tapentadol). These medications have inhibitory influence on nociceptive transmission through enhancing the descending bulbospinal inhibitory pathway (Fishbain et al. 2000). Ketamine is an anesthetic used to manage refractory chronic pain through its action inhibiting the NMDA receptors (Pickering et al. 2020). Many of the medications have a complex mechanism, frequently acting in multiple sites. For example, tramadol is a mu opioid receptor agonist but also inhibits serotonin and norepinephrine reuptake; methadone is a Mu opioid receptor agonist and a NMDA receptor antagonist; and gabapentin blocks voltage-gated calcium channels and modulates other targets such as transient receptor potential channels and NMDA-receptors.

The search for new pharmacotherapies continues due to limitations of the current treatments, such as lack of efficacy or undesirable adverse effects. Recently, oliceridine, a biased opioid medication, was Food and Drug Administration

Table 14.1 Therapeutic agents in clinical practice

Therapeutic target/ mechanisms	Representative drugs
μ opioid receptor agonists	Opioids: Morphine, Hydrocodone, Hydromorphone, Methadone, Fentanyl, Tramadol, Tapentadol
Cyclooxygenase (Cox-1 and Cox-2) non-selective inhibitors	Meloxicam, Ibuprofen, Naproxen
Cox-2 selective inhibitor	Celecoxib (NSAIDs)
Voltage-gated sodium channels: non-selective blockers	Antiepileptic drugs: Carbamazepine, Oxycarbazapine; Local anesthetics: Lidocaine, Bupivacaine, Ropivacaine
Voltage-gated calcium channels: $Ca_V2.2$ blocker	Ziconotide (used intrathecally for cancer and chronic non-cancer pain)
Ca^{2+} channel $\alpha 2\delta 1$ subunit blocker	Gabapentinoid: gabapentin, pregabalin (Antiepileptic drugs)
TRPV1 agonists	Capsaicin, Resiniferatoxin (RTX)
NMDA Receptor antagonist	Ketamine, a dissociative anesthetic
5HT 1B/D agonists	Triptans for Migraine
5HT/NE transporter, serotonin-norepinephrine reuptake inhibitors (SNRIs)	Duloxetine (Antidepressants); Tramadol, Tricyclic antidepressants: Amitriptyline, Nortriptyline, Desipramine, etc.
5HT transporter, serotonin selective reuptake inhibitors (SSRIs)	Fluoxetine (alleviation of nociceptive pain, and attenuation of opioid tolerance and dependence)
Norepinephrine transporter, Norepinephrine reuptake inhibitors (NRI)	Tapentadol
Adrenergic α2 receptor agonist	Tizanidine (muscle relaxant)
5HT2 receptor antagonist	Cyclobenzaprine (muscle relaxant)
Synaptosome-associated protein (SNAP-25)	Botulinum toxin A
Vesicle-associated membrane protein (VAMP)	Botulinum toxin B
CGRP receptor monoclonal antibody	Erenumab for migraine headache prevention
CGRP monoclonal antibodies	Fremanezumab, Galcanezumab, and Eptinezumab for migraine headache treatment and prevention
TNF-α inhibitors	Infliximab, Adalimumab, Etanercept, Golimumab, and Eertolizumab for rheumatoid arthritis (RA), juvenile arthritis, psoriatic arthritis, plaque psoriasis, ankylosing spondylitis, ulcerative colitis (UC), and Crohn's disease

(FDA)-approved for the management of acute pain in controlled settings for its selectivity for the mu-receptor and reduced side effects due to low potency for beta-arrestin recruitment (Markham 2020; Tan and Habib 2021). Also, biologic therapy through the use of monoclonal antibodies (mABs) takes advantage of its high

Table 14.2 Selection of therapeutic agents in clinical practice

Types of pain	Nociceptive	Neuropathic	Nociplastic
Mechanisms	Nociceptor activation by inflammatory process	Lesion of the somatosensory nervous system leading to ectopic discharge	Immune and inflammatory processes leading to peripheral sensitisation and central sensitisation
Examples	*Somatic*: bone fracture, metastases, muscle spasm, osteoartrhitis, postoperative pain, burns	*Central*: spinal cord injury, stroke, Parkinson's disease, multiple sclerosis	Fibromyalgia, irritable bowel syndrome, complex regional pain syndrome type 1, temporomandibular (TMJ) disorder
	Visceral: cholecistitis, nephrolithiasis, angina, mesenteric ischemia, cancer	*Peripheral*: Diabetic neuropathy, postherpetic neuralgia; trigeminal neuralgia, CRPS-2, chemotherapy-induced neuropathy	
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Y	?	Y
Analgesics (e.g. acetaminophen)	Y	Y	Y
Anticonvulsants	?	Y	Y
Antidepressants		Y	Y
Opioids	Y	?	?
Image guided injections	Y	Y	Y for CRPS-1 and TMJ pain
Neuromodulation		Y	Y for CRPS-1
Exercise	Y	Y	Y
Behavioural interventions	Y	Y	Y

affinity and specificity for predetermined ligands or targets in pain transmission and neurogenic inflammation with reduced adverse effects. Calcitonin gene-related peptide (CGRP) binds to calcitonin-like receptors and activates the cascade involved with nociceptive transmission. Several mABs against CGRP or CRRP receptors have been successfully utilized in clinical practice for prophylaxis and treatment of headaches based on strong evidence provided by high-impact clinical trials. Another therapy frequently used in the management of migraine headaches is botulinum toxin, which involves multiple and complex mechanisms in the peripheral and central nervous systems including reducing expression of critical pain-related the channels and receptors and inhibiting secretion of neurotransmitters that are related to peripheral and central sensitization (Matak et al. 2019; Sim 2011).

Emerging new molecular targets are under investigation or in clinical trials. For example, anti-nerve growth factor (anti-NGF) antibodies are a promising therapy for osteoarthritis with studies showing improved pain control and function; however, meta-analyses of clinical trials identified adverse effects in patients treated

with tanezumab (Sánchez-Robles et al. 2021). The transient receptor potential ankyrin 1 (TRPA1) has a major role in pain and several studies have showed its role in the inflammatory and immune response. Despite exciting preclinical findings, clinical trials had disappointing pharmacokinetics and pharmacodynamic features (Souza Monteiro de Araujo et al. 2020). Other analgesic target is the voltage-gated sodium channel Nav1.7, but human data so far has been disappointing in chronic pain conditions (Nguyen and Yarov-Yarovoy 2022).

14.3 Non-pharmacological Therapies

Acupuncture is part of the traditional Chinese medicine and has been used for more than 3000 years. It provides pain relief by activating a variety of bioactive chemicals through peripheral, spinal, and supraspinal mechanisms, include endogenous opioids, serotonin, and norepinephrine. Endogenous opioids desensitize peripheral nociceptors and reduce proinflammatory cytokines peripherally and in the spinal cord. Serotonin and norepinephrine may decrease spinal NMDA receptor subunit GluN1 phosphorylation (Zhang et al. 2014). Activation of the descending inhibitory pathways may also contribute to acupuncture analgesic effects. A placebo effect may also account for some of the analgesic effects. Functional magnetic resonance imaging (MRI) studies have identified signal changes in the brain regions associated with nociceptive processing and pain perception (e.g., insula, thalamus, median prefrontal cortex); however, the available data shows no difference between verum and sham acupuncture group. Nevertheless, it has been shown that the procedure provided pain relief in many painful conditions. Based on favorable benefit/risks ratio and low cost, the procedure is among the treatments recommended for low back pain by the American College of physicians (Coutaux 2017; Ezzo et al. 2000).

The transcutaneous electrical nerve stimulation (TENS) is a non-invasive, inexpensive, self-administered technique to relieve pain. TENS techniques include conventional TENS, acupuncture-like TENS and intense TENS. Clinical experience and systematic reviews suggest that TENS is beneficial for several types of chronic pain and possibly for acute pain as an adjunct to pharmacotherapy. The mechanisms of TENS are to selectively activate large diameter non-noxious afferents (A-beta) to reduce nociceptor cell activity and sensitization at a segmental level in the central nervous system. Pain relief with conventional TENS is rapid in onset and offset and is maximal when the patient experiences a strong but non-painful paresthesia beneath the electrodes (Johnson 2007).

14.4 Interventional Procedures

Interventional techniques for pain management have increased significantly in the past 20 years. These procedures can be diagnostic (e.g., facet medial branch block) and/or therapeutic (e.g., radio frequency ablation [RFA]) and randomized studies showed that when these techniques are performed under a multimodal approach, the outcome is better compared to the injections alone (Cohen et al. 2014). Intraarticular injections of corticosteroids, hyaluronic acid, or blood-derived products aim to target the inflammation at several levels of the cascade, restore lubricant and shock-absorbing effect and reduce synovial inflammation, and deliver a broad spectrum of growth factors and other specific molecules to the injury site, respectively (Ayhan et al. 2014). Guidelines recommend intraarticular steroid injection for inflammatory disease such as osteoarthritis that affects large and medium joints; however, frequent injections may cause decrease in cartilage volume (Cohen et al. 2021). Nerve blocks with local anesthetic with or without steroid aim to reversibly block the nociceptive afferent fibers and decrease the inflammation process. Interestingly, the clinical response for the blocks often far outlasts the pharmacological effect of the medications and a central or systemic effect might also be present (Caracas et al. 2009; Gracely et al. 1992). Epidural steroid injection (ESI), for example, is routinely performed for conditions such as radiculopathy. A recent meta-analysis showed that the use of ESI for lumbosacral radicular pain is more effective compared to conservative measures (Yang et al. 2020) and only low-quality data supports the procedure for non-radicular pain(Cohen et al. 2013). For cervical conditions as disc herniation or stenosis, level II evidence supports ESI for long-term pain improvement (Manchikanti et al. 2015). Nerve ablation is a modality that irreversibly blocks the nociceptive afferent signal and possibly provides a longer-term relief of joint pain or peripheral neuropathic pain conditions through radio frequency ablation (RFA), cryoneurolysis, chemoneurolysis (phenol or alcohol), and balloon compression. RFA is the most frequently performed procedure in this category and targets sensory nerves without a significant motor component (alpha fibers). It is most commonly used to provide longer-term pain relief from facet, sacroiliac, knee, and other joints depending on the technique applied and patient characteristics (Cohen et al. 2021). The duration of pain relief is believed to be limited by re-innervation of the target region through regeneration of the ablated nerve fibers and/or sprouting of adjacent nerve fibers.

14.5 Surgical Approach

There are multiple surgical procedures designed for chronic pain conditions, particularly involving large joints and the spine. Large joint procedures such as total knee or hip replacement not only can reduce pain but also improve functionality. Studies showed that up to 38% of the patients continued to have post-surgical pain

after the arthroplasty (Fletcher et al. 2015), most likely due to neuropathic, nociplastic, or a central component of the pain. Spine surgeries, such as laminectomy, foraminotomy and discectomy, aim to decompress specific nerves, decrease the inflammatory process, and improve pain and other symptoms. The outcomes are variable depending on many factors. For example, in a systematic review, low-quality evidence supported surgical decompression for lumbar disc herniation compared with non-surgical management for pain improvement at 6-month and physical functions at 1-year follow-up (Chen et al. 2018). For lumbar discogenic pain, no significant differences in disability scores were identified between patients who had lumbar fusion versus the nonoperative group (Chen et al. 2018), suggesting that lumbar fusion is not indicated unless there is a significant spinal instability. A systematic review showed that patients with degenerative cervical myelopathy that underwent surgical management had similar functional outcomes compared to the non-surgical group, although those managed conservatively had higher rates of hospital admission and treatment for spinal cord injury (Rhee et al. 2017).

14.6 Neuromodulation

Neurostimulation is a growing field in pain management and is discussed in detail in Chap. 14. This modality of treatment provides pain relief by electrical modulation of the nervous system through spinal cord stimulator (SCS), dorsal root ganglion stimulator (DRG) and peripheral nerve stimulator (PNS), among others. Here, we briefly highlight the major advances of neuromodulation in recent years.

SCS is most frequently used to treat failed back surgery syndrome (FBSS), non-surgical refractory back pain (NSRBP), painful diabetic neuropathy (PDN), and complex regional pain syndrome (CRPS). Electrode(s) are implanted percutaneously or surgically in the posterior epidural space in the thoracic or cervical spine to target the dorsal column of the spinal cord. The mechanisms of action are complex and not fully understood, although it is initially based upon the gate control theory. More recent studies indicate that both neuronal and non-neuronal mechanisms may contribute the therapeutic effects. For example, RNA sequence analysis studies reveal that SCS-induced differentially-expressed genes (DEGs) are concentrated around signaling pathways in the immune functions mediated by microglia, and synaptic signaling/cell-cell signaling/trans-synaptic signaling between neuronal cells (Stephens et al. 2018).

In recent years, several high-impact randomized controlled trials (RCT) provide strong evidence supporting the efficacy of SCS in several refractory pain conditions. In failed back surgery syndrome, two RCTs observed significant pain improved when SCS was compared to conservative medical treatment alone (Kumar et al. 2007, 2008). However, in these studies SCS was less effective to manage low back pain compared to leg pain. More recently, new modalities have been used to address low back pain through multicolumn leads and new stimulation modalities such as high-frequency (10 k Hertz) stimulation (HF-10), burst stimulation, and close-loop

stimulation with better outcomes (Moisset et al. 2020). A RCT demonstrated superiority of high-frequency (HF-10) SCS over traditional SCS with higher success rates (~80%) in reducing both the back and leg pain by at least 50% in patients with FBSS (Kapural et al. 2015). This effect was sustained in 24-month follow-up (Kapural et al. 2016). HF-10 SCS was further studied for non-surgical refractory back pain in a RCT that demonstrated similar efficacy in follow-up over 12 months, providing evidence for durability of the therapy to improve pain, physical function, quality of life, and opioid use (Kapural et al. 2022). Notably, in a recent RCT, HF-10 SCS has been demonstrated to achieve 85% success rate in reducing pain by at least 50% in patients with painful diabetic neuropathy (PDN), a common and debilitating complication of diabetes, in contrast to conventional medical management (CMM), which has a success rate of 5%.

Closed-loop SCS takes advantage of recording and utilizing evoked compound action potentials (ECAP) from spinal cord stimulation to automatically control the stimulus intensity of SCS through a closed-loop feedback mechanism, thereby to deliver stimulation at therapeutic intensity continuously. This modality has recently FDA-approved for clinical application for patients with failed back surgery syndrome or non-surgical refractory back pain, based on a recently published RCT (Mekhail et al. 2020, 2022).

DRG stimulation has recently been studied in patients with CRPS in the lower extremity in a RCT. Specifically designed electrodes are placed in close proximity to the DRGs concordant to the involved regions of the CRPS in the leg, such as L4, L5, and S1 DRGs. The RCT demonstrated that DRG stimulation is safe, efficacious, and superior compared to SCS in managing CRPS in the lower extremities (Deer et al. 2017). PNS may be considered when the pain is restricted to one or two specific nerve distribution. More detailed information is provided in a systematic review we recently published (Xu et al. 2021).

14.7 Physical and Cognitive Behavioral Therapies

Decreasing pain and restoring function are the primary goal of physical therapy (PT). The treatment plan is delineated by the physical therapist and might include manual therapy (e.g., massage and joint manipulation), exercise, education, TENS, heat/ice, among others. Although mechanisms of PT are complex, studies have shown that clinical practice might be guided by mechanistic understanding of the particular pain condition at hand. For example, if a nociceptive-driven pain is suspected, a region-specific exercise might have a better outcome compared to a pain with a central sensitization component that may benefit more from a generalized strengthening or aerobic exercises with a focus on modulating central inhibition and excitation (Chimenti et al. 2018).

Cognitive behavioral therapy (CBT) is the most common psychological approach for patients with chronic pain and consists of evaluating and managing maladaptive thoughts and behaviors with the goal to improve emotions and coping techniques.

CBT has been studied in different pain conditions as a stand-alone therapy or combined therapy. A systematic review showed that CBT provided small benefit for reducing pain, disability, and distress in the short-term compared with treatment as usual/waiting list (Eccleston et al. 2009). Mindfulness therapy is a subtype of CBT that promotes nonreactive self-regulated awareness. It can be performed by different meditation routines and does not require a specific training (Jinich-Diamant et al. 2020). Neuro-imaging studies utilizing functional MRI demonstrated potential mechanisms supporting short and long-term pain attenuation (Zeidan et al. 2019). A systematic review found sufficient evidence across a large body of literature (59 studies, over 5000 participants) that CBT has small or very small beneficial effects for reducing pain, disability, and distress in chronic pain (Williams et al. 2020).

14.8 Multimodal Approach

Pain is determined by biological, psychological, and social factors and treatment plan should address each of these components by a multidisciplinary team. Guidelines recommend incorporating a biopsychosocial approach into individualized plans of care. Although this approach can be costly and time-consuming, the long-term benefits in terms of quality of life and function likely outweigh these issues (Steglitz et al. 2012). In addition to pharmacological and non-pharmacological measures to manage chronic pain, a comprehensive approach should also include self-care, sleep hygiene, smoking cessation, weight loss if indicated, and a healthy lifestyle. Pain-related disability is lower in patients who have an active role in their care (Steglitz et al. 2012). Exercise is the most frequently used self-management that improves deconditioning and helps weight loss, sleep, and general well-being, as discussed in Chap. 15. Exercise is more beneficial for function than pain relief and for musculoskeletal and diffuse pain compared to neuropathic pain, although it is beneficial across different pain conditions (Geneen et al. 2017). Several RCTs showed that exercising two to three times per week for 20–30 min is beneficial for pain relief and functionality (Chimenti et al. 2018).

Pain can also be the cause and/or consequence of sleep deficiency. Poor sleep quality and duration are risk factor for chronic pain. Deficient sleep can lead to hyperalgesia and exacerbation of the pain symptoms (Haack et al. 2020). Hence, focusing on improving sleep is an important part of pain management. Healthy and balanced diets might reduce inflammation and sensitization and improve chronic pain, as discussed in Chap. 15. However, there is no data favoring a specific type of diet as superior (Field et al. 2022). Educating and supporting the patient to have realistic expectation on the management of the pain condition are also important. In conclusion, given the complexity of chronic pain, successful management must be patient-centered, multimodal, and integrated through self-care combined with a multidisciplinary team approach.

It is important to point out that some of the above-mentioned treatments can not only alleviate pain symptoms but may also control diseases progression, by

modulation of inflammation and neuroinflammation. These inflammation-modulating treatments are not restricted to anti-inflammatory treatments, such as NSAIDs. They also include neuromodulation (SCS, vagal nerve stimulation (VNS)) and non-pharmacological/complementary treatments (acupuncture, TENS) (da Silva et al. 2015; Sato et al. 2014; Ji et al. 2018; Tao et al. 2020, 2021).

References

Ayhan E, Kesmezacar H, Akgun I. Intraarticular injections (corticosteroid, hyaluronic acid, platelet rich plasma) for the knee osteoarthritis. World J Orthop. 2014;5(3):351–61. https://doi.org/10.5312/wjo.v5.i3.351.

Bovill JG. Mechanisms of actions of opioids and non-steroidal anti-inflammatory drugs. Eur J Anaesthesiol Suppl. 1997;15:9–15. https://doi.org/10.1097/00003643-199705001-00003.

Caracas HC, Maciel JV, Martins PM, de Souza MM, Maia LC. The use of lidocaine as an anti-inflammatory substance: a systematic review. J Dent. 2009;37(2):93–7. https://doi.org/10.1016/j.jdent.2008.10.005.

Chen BL, Guo JB, Zhang HW, Zhang YJ, Zhu Y, Zhang J, Wang XQ. Surgical versus non-operative treatment for lumbar disc herniation: a systematic review and meta-analysis. Clin Rehabil. 2018;32(2):146–60. https://doi.org/10.1177/0269215517719952.

Cheng J. My take on the biopsychosocial model of patient-centered care. Pain Med. 2018;19(11):2101–3. https://doi.org/10.1093/pm/pny187.

Cheng J, Rutherford M, Singh VM. The HHS pain management best practice inter-agency task force report calls for patient-centered and individualized care. Pain Med. 2020;21(1):1–3. https://doi.org/10.1093/pm/pnz303.

Chimenti RL, Frey-Law LA, Sluka KA. A mechanism-based approach to physical therapist management of pain. Phys Ther. 2018;98(5):302–14. https://doi.org/10.1093/ptj/pzy030.

Cohen SP, Bicket MC, Jamison D, Wilkinson I, Rathmell JP. Epidural steroids: a comprehensive, evidence-based review. Reg Anesth Pain Med. 2013;38(3):175–200. https://doi.org/10.1097/AAP.0b013e31828ea086.

Cohen SP, Hayek S, Semenov Y, Pasquina PF, White RL, Veizi E, Vorobeychik Y. Epidural steroid injections, conservative treatment, or combination treatment for cervical radicular pain: a multicenter, randomized, comparative-effectiveness study. Anesthesiology. 2014;121(5):1045–55. https://doi.org/10.1097/aln.0000000000000409.

Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. Lancet. 2021;397(10289):2082–97. https://doi.org/10.1016/s0140-6736(21)00393-7.

Coutaux A. Non-pharmacological treatments for pain relief: TENS and acupuncture. Joint Bone Spine. 2017;84(6):657–61. https://doi.org/10.1016/j.jbspin.2017.02.005.

Coward DM. Tizanidine: neuropharmacology and mechanism of action. Neurology. 1994;44(11 Suppl 9):S6–10. discussion S10-11

da Silva MD, Bobinski F, Sato KL, Kolker SJ, Sluka KA, Santos AR. Il-10 cytokine released from m2 macrophages is crucial for analgesic and anti-inflammatory effects of acupuncture in a model of inflammatory muscle pain. Mol Neurobiol. 2015;51(1):19–31.

Dahlhamer J, Lucas J, Zelaya C, Nahin R, Mackey S, DeBar L, Helmick C. Prevalence of chronic pain and high-impact chronic pain among adults – United States, 2016. Morb Mortal Wkly Rep. 2018;67(36):1001–6. https://doi.org/10.15585/mmwr.mm6736a2.

Deer TR, Levy RM, Kramer J, Poree L, Amirdelfan K, Grigsby E, Mekhail N. Dorsal root ganglion stimulation yielded higher treatment success rate for complex regional pain syndrome and causalgia at 3 and 12 months: a randomized comparative trial. Pain. 2017;158(4):669–81. https://doi.org/10.1097/j.pain.0000000000000814.

Eccleston C, Williams AC, Morley S. Psychological therapies for the management of chronic pain (excluding headache) in adults. Cochrane Database Syst Rev. 2009;2:Cd007407. https://doi.org/10.1002/14651858.CD007407.pub2.

Ezzo J, Berman B, Hadhazy VA, Jadad AR, Lao L, Singh BB. Is acupuncture effective for the treatment of chronic pain? A systematic review. Pain. 2000;86(3):217–25. https://doi.org/10.1016/s0304-3959(99)00304-8.

Field R, Pourkazemi F, Rooney K. Effects of a low-carbohydrate ketogenic diet on reported pain, blood biomarkers and quality of life in patients with chronic pain: a pilot randomized clinical trial. Pain Med. 2022;23(2):326–38. https://doi.org/10.1093/pm/pnab278.

Fishbain DA, Cutler R, Rosomoff HL, Rosomoff RS. Evidence-based data from animal and human experimental studies on pain relief with antidepressants: a structured review. Pain Med. 2000;1(4):310–6. https://doi.org/10.1046/j.1526-4637.2000.00042.x.

Fletcher D, Stamer UM, Pogatzki-Zahn E, Zaslansky R, Tanase NV, Perruchoud C, Meissner W. Chronic postsurgical pain in Europe: an observational study. Eur J Anaesthesiol. 2015;32(10):725–34. https://doi.org/10.1097/eja.0000000000000319.

Geneen LJ, Moore RA, Clarke C, Martin D, Colvin LA, Smith BH. Physical activity and exercise for chronic pain in adults: an overview of cochrane reviews. Cochrane Database Syst Rev. 2017;4(4):Cd011279. https://doi.org/10.1002/14651858.CD011279.pub3.

Gracely RH, Lynch SA, Bennett GJ. Painful neuropathy: altered central processing maintained dynamically by peripheral input. Pain. 1992;51(2):175–94. https://doi.org/10.1016/0304-3959(92)90259-e.

Haack M, Simpson N, Sethna N, Kaur S, Mullington J. Sleep deficiency and chronic pain: potential underlying mechanisms and clinical implications. Neuropsychopharmacology. 2020;45(1):205–16. https://doi.org/10.1038/s41386-019-0439-z.

Hermanns H, Hollmann MW, Stevens MF, Lirk P, Brandenburger T, Piegeler T, Werdehausen R. Molecular mechanisms of action of systemic lidocaine in acute and chronic pain: a narrative review. Br J Anaesth. 2019;123(3):335–49. https://doi.org/10.1016/j.bja.2019.06.014.

Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and central sensitization in chronic and widespread pain. Anesthesiology. 2018;129:343.

Jinich-Diamant A, Garland E, Baumgartner J, Gonzalez N, Riegner G, Birenbaum J, Zeidan F. Neurophysiological mechanisms supporting mindfulness meditation-based pain relief: an updated review. Curr Pain Headache Rep. 2020;24(10):56. https://doi.org/10.1007/s11916-020-00890-8.

Johnson M. Transcutaneous electrical nerve stimulation: mechanisms, clinical application and evidence. Rev Pain. 2007;1(1):7–11. https://doi.org/10.1177/204946370700100103.

Kapural L, Yu C, Doust MW, Gliner BE, Vallejo R, Sitzman BT, Burgher AH. Novel 10-kHz high-frequency therapy (HF10 therapy) is superior to traditional low-frequency spinal cord stimulation for the treatment of chronic back and leg pain: the SENZA-RCT randomized controlled trial. Anesthesiology. 2015;123(4):851–60. https://doi.org/10.1097/aln.0000000000000774.

Kapural L, Yu C, Doust MW, Gliner BE, Vallejo R, Sitzman BT, Burgher AH. Comparison of 10-kHz high-frequency and traditional low-frequency spinal cord stimulation for the treatment of chronic back and leg pain: 24-month results from a multicenter, randomized, controlled pivotal trial. Neurosurgery. 2016;79(5):667–77. https://doi.org/10.1227/neu.0000000000001418.

Kapural L, Jameson J, Johnson C, Kloster D, Calodney A, Kosek P, Patel NP. Treatment of nonsurgical refractory back pain with high-frequency spinal cord stimulation at 10 kHz: 12-month results of a pragmatic, multicenter, randomized controlled trial. J Neurosurg Spine. 2022;37:1–12. https://doi.org/10.3171/2021.12.Spine211301.

Kobayashi H, Hasegawa Y, Ono H. Cyclobenzaprine, a centrally acting muscle relaxant, acts on descending serotonergic systems. Eur J Pharmacol. 1996;311(1):29–35. https://doi.org/10.1016/0014-2999(96)00402-5.

Kumar K, Taylor RS, Jacques L, Eldabe S, Meglio M, Molet J, North RB. Spinal cord stimulation versus conventional medical management for neuropathic pain: a multicentre randomised

controlled trial in patients with failed back surgery syndrome. Pain. 2007;132(1–2):179–88. https://doi.org/10.1016/j.pain.2007.07.028.

Kumar K, Taylor RS, Jacques L, Eldabe S, Meglio M, Molet J, North RB. The effects of spinal cord stimulation in neuropathic pain are sustained: a 24-month follow-up of the prospective randomized controlled multicenter trial of the effectiveness of spinal cord stimulation. Neurosurgery. 2008;63(4):762–70.; discussion 770. https://doi.org/10.1227/01.Neu.0000325731.46702.D9.

Manchikanti L, Nampiaparampil DE, Candido KD, Bakshi S, Grider JS, Falco FJ, Hirsch JA. Do cervical epidural injections provide long-term relief in neck and upper extremity pain? A systematic review. Pain Physician. 2015;18(1):39–60.

Markham A. Oliceridine: first approval. Drugs. 2020;80(16):1739–44. https://doi.org/10.1007/s40265-020-01414-9.

Matak I, Bölcskei K, Bach-Rojecky L, Helyes Z. Mechanisms of botulinum toxin type a action on pain. Toxins (Basel). 2019;11(8) https://doi.org/10.3390/toxins11080459.

Mekhail N, Levy RM, Deer TR, Kapural L, Li S, Amirdelfan K, Poree L. Long-term safety and efficacy of closed-loop spinal cord stimulation to treat chronic back and leg pain (evoke): a double-blind, randomised, controlled trial. Lancet Neurol. 2020;19(2):123–34. https://doi.org/10.1016/s1474-4422(19)30414-4.

Mekhail N, Levy RM, Deer TR, Kapural L, Li S, Amirdelfan K, Soliday N. Durability of clinical and quality-of-life outcomes of closed-loop spinal cord stimulation for chronic back and leg pain: a secondary analysis of the evoke randomized clinical trial. JAMA Neurol. 2022;79(3):251–60. https://doi.org/10.1001/jamaneurol.2021.4998.

Mills SEE, Nicolson KP, Smith BH. Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth. 2019;123(2):e273–83. https://doi.org/10.1016/j.bja.2019.03.023.

Moisset X, Lanteri-Minet M, Fontaine D. Neurostimulation methods in the treatment of chronic pain. J Neural Transm (Vienna). 2020;127(4):673–86. https://doi.org/10.1007/s00702-019-02092-y.

Nguyen PT, Yarov-Yarovoy V. Towards structure-guided development of pain therapeutics targeting voltage-gated sodium channels. Front Pharmacol. 2022;13:842032. https://doi.org/10.3389/fphar.2022.842032.

Pickering G, Pereira B, Morel V, Corriger A, Giron F, Marcaillou F, Delage N. Ketamine and magnesium for refractory neuropathic pain: a randomized, double-blind. Crossover Trial Anesthesiol. 2020;133(1):154–64. https://doi.org/10.1097/aln.0000000000003345.

Rekatsina M, Paladini A, Piroli A, Zis P, Pergolizzi JV, Varrassi G. Pathophysiologic approach to pain therapy for complex pain entities: a narrative review. Pain Ther. 2020;9(1):7–21. https://doi.org/10.1007/s40122-019-00147-2.

Rhee J, Tetreault LA, Chapman JR, Wilson JR, Smith JS, Martin AR, Fehlings MG. Nonoperative versus operative management for the treatment degenerative cervical myelopathy: an updated systematic review. Global Spine J. 2017;7(3 Suppl):35s–41s. https://doi.org/10.1177/2192568217703083.

Sánchez-Robles EM, Girón R, Paniagua N, Rodríguez-Rivera C, Pascual D, Goicoechea C. Monoclonal antibodies for chronic pain treatment: present and future. Int J Mol Sci. 2021;22(19) https://doi.org/10.3390/ijms221910325.

Sato KL, Johanek LM, Sanada LS, Sluka KA. Spinal cord stimulation reduces mechanical hyperalgesia and glial cell activation in animals with neuropathic pain. Anesth Analg. 2014;118(2):464–72.

Scholz J, Woolf CJ. Can we conquer pain? Nat Neurosci. 2002;5(Suppl):1062–7. https://doi.org/10.1038/nn942.

Sills GJ, Rogawski MA. Mechanisms of action of currently used antiseizure drugs. Neuropharmacology. 2020;168:107966. https://doi.org/10.1016/j.neuropharm.2020.107966.

Sim WS. Application of botulinum toxin in pain management. Korean J Pain. 2011;24(1):1–6. https://doi.org/10.3344/kjp.2011.24.1.1.

Souza Monteiro de Araujo D, Nassini R, Geppetti P, De Logu F. TRPA1 as a therapeutic target for nociceptive pain. Expert Opin Ther Targets. 2020;24(10):997–1008. https://doi.org/10.1080/14728222.2020.1815191.

Steglitz J, Buscemi J, Ferguson MJ. The future of pain research, education, and treatment: a summary of the IOM report "relieving pain in America: a blueprint for transforming prevention, care, education, and research". Transl Behav Med. 2012;2(1):6–8. https://doi.org/10.1007/s13142-012-0110-2.

Stephens KE, Chen Z, Sivanesan E, Raja SN, Linderoth B, Taverna SD, Guan Y. RNA-seq of spinal cord from nerve-injured rats after spinal cord stimulation. Mol Pain. 2018;14:1744806918817429. https://doi.org/10.1177/1744806918817429.

Tan HS, Habib AS. Safety evaluation of oliceridine for the management of postoperative moderate-to-severe acute pain. Expert Opin Drug Saf. 2021;20(11):1291–8. https://doi.org/10.1080/14740338.2021.1965989.

Tao X, Lee MS, Donnelly CR, Ji RR. Neuromodulation, specialized pro-resolving mediators, and resolution of pain Neurotherapeutics. Neurotherapeutics. 2020;17:886–99.

Tao X, Luo X, Zhang T, Hershey B, Esteller R, Ji RR. Spinal cord stimulation attenuates mechanical allodynia and increases central resolvin D1 levels in rats with spared nerve injury. Front Physiol. 2021;12:687046.

Vardeh D, Mannion RJ, Woolf CJ. Toward a mechanism-based approach to pain diagnosis. J Pain. 2016;17(9 Suppl):T50–69. https://doi.org/10.1016/j.jpain.2016.03.001.

Williams ACC, Fisher E, Hearn L, Eccleston C. Psychological therapies for the management of chronic pain (excluding headache) in adults. Cochrane Database Syst Rev. 2020;8(8):Cd007407. https://doi.org/10.1002/14651858.CD007407.pub4.

Xu J, Sun Z, Wu J, Rana M, Garza J, Zhu AC, Chakravarthy KV, Abd-Elsayed A, Rosenquist E, Basi H, Christo P, Cheng J. Peripheral nerve stimulation in pain management: a systematic review. Pain Physician. 2021;24(2):E131–e152.

Yang S, Kim W, Kong HH, Do KH, Choi KH. Epidural steroid injection versus conservative treatment for patients with lumbosacral radicular pain: a meta-analysis of randomized controlled trials. Medicine (Baltimore). 2020;99(30):e21283. https://doi.org/10.1097/md.0000000000021283.

Zeidan F, Baumgartner JN, Coghill RC. The neural mechanisms of mindfulness-based pain relief: a functional magnetic resonance imaging-based review and primer. Pain Rep. 2019;4(4):e759. https://doi.org/10.1097/pr9.0000000000000759.

Zhang R, Lao L, Ren K, Berman BM. Mechanisms of acupuncture-electroacupuncture on persistent pain. Anesthesiology. 2014;120(2):482–503. https://doi.org/10.1097/aln.0000000000000101.

Chapter 15
Neuromodulation in Pain Management

Paul Guillod and Jianguo Cheng

Abstract Neuromodulation employs electrical stimulation along nervous tissue to reduce the perception of pain. This can be achieved distally along peripheral nerves, centrally in the spinal column or brain, or at autonomic targets such as the vagus nerve. The field is rapidly advancing in its technology and clinical application as well as in our understanding of its wide-ranging biological effects. Beyond its putative role of suppressing transmission of pain signals, neuromodulation has been demonstrated to influence and even reverse neuroimmune mechanisms involved in the generation and maintenance of chronic pain states. Novel waveforms and closed-loop systems offer clinicians highly configurable systems to optimize patient experience and maximize therapeutic benefit for an expanding range of chronic pain conditions.

15.1 Introduction

15.1.1 Basics/History

Neuromodulation refers to external influence on neuronal circuity outside of its intrinsic physiologic mechanisms. There are multiple techniques to achieve this objective. In the context of this chapter, neuromodulation refers to the use of electrical stimulation of targeted neural structures for pain relief. The discovery of using electricity for treating pain dates as far back as the early AD period with the use of the electric ray fish for gout pain. It was not until the eighteenth century with the discovery of methods for generating electric fields that heralded the advent of the first patient electrical therapy device in 1902, the first spinal cord stimulation in the 1960s, and further progression to multiple advanced neuromodulation modalities today (Mekhail et al. 2010, 2018). Neuromodulation is among the most active areas

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of research and new clinical applications in the field of pain management, particularly for some of the most common, debilitating, and refractory pain conditions in daily practice.

The goal with neuromodulation, as with any therapy, is to be targeted, specific, and least-invasive as possible. Pain information travels through the electrochemical signaling pathways in the nervous system, which begins with transduction in the periphery, conduction along peripheral nerves, transmission/modulation in the spinal cord and brain, and perception in the cerebral cortex. As the nervous system relies on action potentials for information transmission, stimulation at any point along this path can be utilized for neuromodulation therapy. In addition, non-neuronal cells, such as glial cells and immune cells, have been strongly implicated in the mechanisms of neuromodulation for pain relief.

15.1.2 Common Modalities of Neuromodulation

Neuromodulation modalities include transcutaneous electrical nerve stimulation (TENS) across the skin, peripheral nerve stimulation (PNS) along nerve tracts, dorsal root ganglion (DRG) stimulation at the cell bodies of the primary sensory neurons, spinal cord stimulation (SCS) along the dorsal column, deep brain stimulation (DBS) in select areas of the cerebrum, and motor cortical stimulation (MCS). Modulation of pain additionally occurs through the autonomic nervous system which confers additional targets such as with vagal nerve stimulation (VNS). In each case, an array of electrodes (or leads) are selectively placed at intended site and are connected to a pulse generator that is worn or implanted. Stimulation therapy has historically been most efficacious in neuropathic pain states—pain generated by lesions or dysfunction in the somatosensory nervous system through a maladaptive process. Recent studies have shown promise in reducing nociceptive and inflammatory mediated pain states as well.

Neuromodulation is in the process of a rapid evolution in clinical application supported by research evidence for safety and efficacy with novel targets, waveforms, frequency, lead stability, and other technological advances (Mekhail et al. 2018). When assessing for success, most studies define ≥50% improvement in the visual analog scale (VAS) or numerical rating score (NRS) as a therapy responder. Other functionality metrics are increasingly emphasized, such as the SF-36 for quality of life, Oswestry Disability Index (ODI) for ability to participate in activities, McGill Pain Questionnaire (MPQ) for quality/affective components, and the Beck Depression Inventory-II (BDI-II) for psychiatric contributions in addition to tracking medication use (particularly opioids) and healthcare costs (Kumar et al. 2007). Consensus guidelines emphasize appropriate patient selection from an indication standpoint while reinforcing the need for patients to have realistic, well-informed expectations free from psychological barriers (Deer et al. 2014). As chronic pain states are associated with psychiatric comorbidities and/or substance

use disorders, consideration for neuromodulation therapy must be provided in the context of a multidisciplinary care setting.

15.1.3 Electrical Stimulation Principles

It is helpful to understand the terminology of electrical stimulation. Frequency, measured in Hertz (Hz; second-1, "per second"), determines the spacing of electrical pulses/discharges. Therapeutic frequency in SCS can vary considerably from conventional low frequency stimulation (10–120 Hz), which is typically associated with paresthesia, to high frequency stimulation (1–10 kHz), which is paresthesia-free (Paz-Solís et al. 2022). Pulse width, measured in microseconds (μs), determines how long each electrical pulse lasts, generally much lower than the upper bound of 1/frequency. Wider pulse width is associated with higher energy delivered. Amplitude, or intensity, in the context of stimulation therapy can refer to the supplied voltage (V) or current (mA) of each pulse depending on the independent delivery metric chosen. Most often current is used given dynamic variations in resistance along the lead and at the tissue interface translated to voltage using Ohm's law (V = IR). The energy per pulse describes how much electrical charge (nanocoulombs) is deposited in the tissue with each pulse which is determined by the current integrated over the pulse duration; for square waves, this is simply a multiplication of the current and pulse width. The energy per pulse importantly relates to the degree of depolarization and concomitant activation of neuronal and non-neuronal cells, such as glial cells (e.g., astrocytes) and immune cells (e.g., microglia).

It is important to place the electrodes in proximity to the intended target either empirically (HF-10, paresthesia-free SCS) or guided by paresthesia in the painful areas of the body (paresthesia based SCS). Local tissue effects such as scarring or CSF between epidural leads and the spinal cord can lead to excessive charge dispersion and the need for greater intensity to meet therapeutic goals which can lead to suboptimal therapeutic effects and/or unintended side effects such as motor activation or stimulation over non-painful areas, as well as early battery depletion. To minimize charge buildup and electrochemical reactions at the lead-tissue interface, charge balancing can be achieved by allowing an equal amount of current passing in the opposite direction following each pulse or burst of pulses. This can be achieved passively, as is most common, or actively with generation of an inverted pulse (Miller et al. 2016).

Traditionally, neuromodulation has been titrated to effect by adjusting the frequency, pulse width, and intensity of a tonic train of pulses. Modern waveforms have emerged in recent years which utilize multiple overlapping frequencies, waveforms, or bursts of pulses (Fig. 15.1) (Paz-Solís et al. 2022). The total time that an electrical stimulation is on compared to off is its duty cycle, expressed in either percentage or ratio of time. For tonic stimulation, this can be calculated by multiplying the frequency and the pulse width; for instance, a 50 Hz signal with a 200 μs pulse width would have a duty cycle of 1%. SCS using duty cycles in excess of 20% is referred to as "high-dose" systems. Studies on the effects of decreasing duty cycle

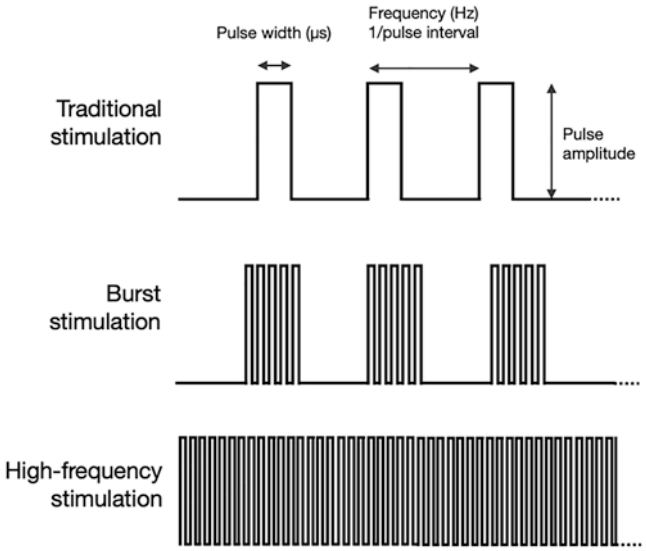

Fig. 15.1 Stimulation waveforms. Traditional stimulation utilizes low frequency pulses (generally around 50 Hz) which result in the feeling of paresthesias. Burst stimulation uses trains of higher frequency pulses repeated at a lower frequency which for most patients does not result in paresthesias. High-frequency stimulation (10 kHz) is paresthesia-free. For simplicity, charge balancing not illustrated

by incorporating intermittent stimulation have provided evidence of maintained therapeutic efficacy at substantially lower duty cycle and preserved battery life (Provenzano et al. 2022).

Electrodes with increasing number of contacts are developed and utilized clinically to account for heterogeneity in anatomy, potential lead migration, and the need for broad coverage. These contacts can be selectively modified or programmed in configuration and polarity (anodes or cathodes) for optimizing therapeutic effects. As this presents a dramatically large parameter space for configuration, finite element models have been developed to aid in "shaping" the complex electrical fields for optimizing spatial specificity and tissue penetration (Khadka et al. 2020).

15.2 Spinal Cord Stimulation

Spinal cord stimulation (SCS) involves stimulation along the dorsal column of the spinal cord (Fig. 15.2). The first use in humans was by Dr. Shealy in 1967 with a single monopolar lead implanted in the intrathecal space of a 70-year-old man with inoperable bronchogenic carcinoma demonstrating profound pain relief and production of a "buzzing" sensation over his trunk in his last few days before succumbing to his disease (Shealy et al. 1967). It was later demonstrated that stimulation of the dorsal column can be achieved with lead placement in the epidural space. The

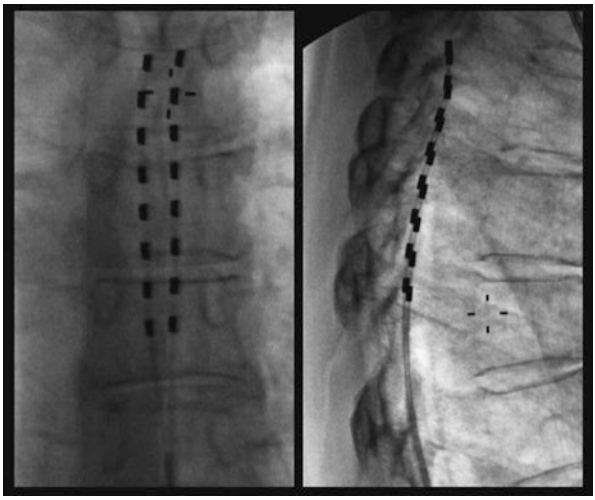

Fig. 15.2 Spinal cord stimulation involves placement of leads in the epidural space along the dorsal column. In this example, AP and lateral fluoroscopic images show two 8-contact percutaneous cylindrical leads in the thoracic spine for a patient with chronic low back pain

field has grown considerably in the decades since with over 50,000 SCS systems implanted in the US annually.

SCS has traditionally been paresthesia-based with the goal of stimulation to evoke a tingling sensation produced by tonic stimulation at lower frequencies (10–120 Hz, generally around 50 Hz) by activation of sensory afferents. Leads are placed with contacts over spinal levels based on coverage for the areas of pain. For instance, leads can be placed in the upper cervical epidural space for neck and upper extremity pain, or the thoracic region for low back and leg pain. Indications for SCS have expanded in recent years to include failed back surgery syndrome (FBSS), complex regional pain syndrome (CRPS) I and II, non-surgical refractory back pain (NSRBP), intractable angina, and painful diabetic neuropathy (PDN). With respect to FBSS alone, roughly 10 to 40% of patients who undergo low spine surgery have persistent or recurrent pain (North et al. 2005), presenting a large population of potential candidates for the therapy.

Appropriately selected patients through clinical and psychological evaluations will first go through a SCS trial of 1–2 weeks. In the trial period, one or two leads are placed percutaneously and connected to an external generator. The leads are placed in close proximity to the target segments guided by fluoroscopy either empirically, in the case of paresthesia-free SCS system, or with intra-procedure paresthesia mapping to ensure stimulation cover the painful region. The percutaneous leads are removed at the end of the trial period. If the degree of pain relief is sufficient to warrant permanent implant (at least 50% relief), then a permanent system can be implanted. Permanent leads can be placed either surgically with a laminotomy and manual placement of a paddle lead or percutaneously with threading of cylindrical leads. Paddle leads have the advantage of unidirectional current with less migration and are preferred in situations with difficult epidural access, while percutaneous

leads are minimally invasive and more easily revised. Compared to medical management alone, SCS has been shown to improve quality of life, functionality, patient satisfaction, and pain scores in appropriately selected patients while also being cost-effective taking into account quality adjusted life years (Farber et al. 2017; Zucco et al. 2015).

One of the earliest prospective randomized-controlled trials (RCT) with tonic SCS (tSCS) in 2005 demonstrates advantage of SCS therapy in 50 patients with persistent radicular pain following spine surgery (North et al. 2005). Patients were randomized to undergo neuromodulation with tSCS or repeat surgery with 3 year outcomes showing significantly more responders (≥50% relief) in the SCS group (47%) compared to the reoperation group (12%). Another RCT around that time compared SCS to conservative medical management in 100 patients with FBSS and predominantly neuropathic leg pain demonstrating a 48% responder rate in the SCS group compared to 9% in medical management group at 6 months (Kumar et al. 2005, 2007). More recent studies have focused on novel applications and novel waveform therapies including high-frequency stimulation, burst stimulation, differential targeted multiplex (DTM) stimulation, and closed-loop stimulation in comparison with traditional SCS. Additionally, stimulation at the dorsal root ganglion has presented a new target which has shown particular efficacy in CRPS I and II (causalgia).

High-frequency stimulation refers to the use of stimulation frequencies beyond >1 kHz, usually 10 kHz, which do not produce paresthesias. The multicenter, randomized, controlled, pivotal trial in 2015 consisted of 198 patients with chronic, intractable trunk and/or leg pain with subjects randomized 1:1 to tSCS or 10 kHz SCS (HFS) (Kapural et al. 2015). Lead position for the HFS group consisted of one with a distal tip at T8 and another over T9, while the conventional therapy group underwent paresthesia mapping with the average lead tip position near T7/T8. At the 3 months primary endpoint, the HFS group had significantly higher response rates for both back pain (84.5 vs. 43.8%) and leg pain (83.1 vs. 55.5%) with an overall response at 2 years of 76.5% in the HFS group compared to 49.3% in tSCS group (Kapural et al. 2016). However, a smaller sample size RCT of 55 patients with FBSS comparing HFS to tSCS did not significant differences in response between groups or across multiple outcome metrics (De Andres et al. 2017). Two more recent RCTs compared HFS to conventional medical management for non-surgical refractory back pain (NSRBP) (Kapural et al. 2022) and diabetic polyneuropathy (PDN) (Petersen et al. 2021) respectively and demonstrated significant improvements in the HFS group (85% response in PDN, 80.9% in NSRBP) compared to medical management alone (5% in PDN, 1.3% in NSRBP).

Burst Stimulation

As its name implies, burst stimulation refers to trains of pulses at a high frequency which are repeated at a lower frequency which is thought to reflect more endogenous encoding patterns of thalamic cells and engage additional supraspinal pathways (Sherman 2001). The current paradigm studied and used commercially consists of 5 pulses at 500 Hz repeated at 40 Hz. The first preliminary study with

burst stimulation consisted of 15 patients with chronic back and limb pain with a significant higher response for back pain in the burst group (51%) compared to the tonic group (30%) with non-significant differences in effect on limb pain. The follow-up RCT SUNBURST trial in 2017 consisted of 100 patients who received a permanent SCS implant randomized to 12 weeks of tSCS or burst stimulation followed by a 12 week crossover (Deer et al. 2018). In the trial, 60% of patients responded to burst stimulation compared to 51% in tonic stimulation, though notably response rate was defined as ≥30% change in VAS. When using ≥50% relief as a responder cutoff, response rates were 39% for burst and 32% for tonic stimulation maintaining significant difference across groups but a lower response in the tonic stimulation group than conventionally seen. The majority (61%) of patients experienced no paresthesias using burst stimulation. Patient preference was an additional endpoint in the study with 71% preferring burst, 19% preferring tonic, and 10% without preference. A follow-up study looked at "microdosing" burst therapy with on and off periods finding equivalent efficacy (Vesper et al. 2019).

Differential Targeted Multiplex Stimulation (DTM)

DTM refers to the use of synchronous overlapping waveforms (50–1200 Hz, 50–450 μs pulse widths) derived largely from animal studies that demonstrated frequency-dependent effects on neuronal-glial interactions and modulation of gene expression profiles (Vallejo et al. 2010, 2020). In a feasibility study comparing DTM to traditional therapy, 20 subjects with predominantly intractable low back pain underwent placement of trial leads with 3–5 days of tonic stimulation followed by 3–5 days of DTM stimulation with significantly higher response in the DTM group (80%) relative to the tSCS group (50%) and 85% of patients preferring DTM. A follow-up RCT recapitulated these early findings with 128 subjects randomized 1:1 to tSCS or DTM with 3 month follow-up demonstrating significantly higher response rates in the DTM group (80.1 vs. 51.2%). Notably there was also a higher proportion of profound responders (defined as ≥80% relief) with 69% in the DTM group compared to 35% in the control at 12 months.

Closed-Loop Stimulation

This method leverages stimulation-evoked potentials, termed evoked compound action potentials (ECAP), which are recorded through the unused lead contacts. The amplitude of the ECAP is then used to automate stimulus intensity adjustment in contrast with conventional open-loop therapy where energy output is fixed once the settings/intensity are manually adjusted. This is important as patient movement, postural changes, and even small physiologic perturbations like coughing all have effects on the current delivered from the electrodes to the spinal cord, which can change the experienced intensity of stimulation. The Evoke study in 2019 was the first double-blind RCT in 134 subjects diagnosed with intractable trunk and limb pain who were randomized 1:1 to ECAP or tSCS (Levy et al. 2019; Mekhail et al. 2020). Responder rates at 1 year was 83% for the ECAP group compared to 61% in the control group consistent across both back and leg pain. The number of profound responders (≥80% relief) was also significantly higher at 56% in the ECAP group compared to 37% in the control group. This modality of SCS is recently

FDA-approved for clinical use. Contrary to the SUNBURST trial, the Evoke trial had a higher proportion of responders to traditional therapy thought related to the use of ECAPs for setting program intensity during tSCS programming despite not incorporating real-time ECAP adjustments.

Dorsal Root Ganglion (DRG) Stimulation

DRG stimulation involves placing electrodes through the epidural space to an intervertebral foramen overlying the DRG, which contains the cell bodies of primary sensory neurons (Fig. 15.3). Similar to SCS, DRG is used for intractable neuropathic pain syndromes, such as CRPS. An early trial of 8 patients implanted with DRG stimulation for CRPS I & II demonstrated 6 patients having greater than 50% relief at 12 months (Van Buyten et al. 2015). The follow-up RCT, the ACCURATE trial in 152 patients with CRPS I or CRPS II (diagnosed through Budapest criteria) randomized 1:1 to DRG stimulation or traditional SCS, demonstrates significant difference in >50% pain relief responder rates—81.2% in the DRG group compared to 55.7% in the SCS group at 3 months which was maintained at 12 months (74.2 vs. 53%). The study also demonstrated improvements in QOL metrics in DRG stimulation compared to SCS and decreased postural effects. Similar to SCS, the lead position for DRG stimulation is based on the dermatomal innervations of spinal nerves such as targeting L4-S1 for foot pain, L3-L4 for knee pain, and L1 and S2 for pelvic pain. It is noteworthy that DRG stimulation is not FAD-approved for CRPS in the upper extremities despite CRPS is more common in the upper extremities than the low extremities.

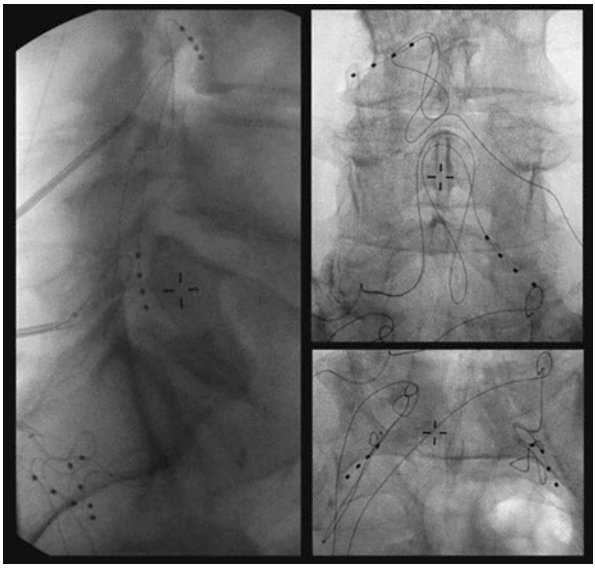

Fig. 15.3 Dorsal root ganglion (DRG) stimulation. In this example, a patient with chronic neuropathic pain in both lower extremities has 4-contact percutaneous DRG leads inserted along the left L3, right L5, and bilateral S1 foramen

15.3 Mechanisms of Neuromodulation

The first and most enduring proposed mechanism of neuromodulation through electrical stimulation was the gate control theory introduced by Melzack and Wall in 1965 (Melzack and Wall 1965). At the time, it was known that Aδ, C, and Aβ sensory fibers synapse at the dorsal horn. Theory postulates that mechanosensitive afferents from the large diameter, myelinated Aβ fibers suppress the nociceptive input from smaller diameter, unmyelinated C fibers and thinly myelinated Aδ fibers through circuit interactions as a result of co-synapsing on inhibitory interneurons and second order wide dynamic range (WDR) and nociceptor specific neurons. In other words, preferential stimulation of sensory fibers produce paresthesias which mask input from nociceptive fibers on a segmental basis in the dorsal horn. Outside of the patient experience of paresthesias, evidence for activation of sensory fibers includes detection of antidromic depolarization in the periphery, action potentials in the dorsal column (ECAPs), SSEP detection, and somatosensory cortex activation by fMRI (Sdrulla et al. 2018). However, the gate theory does not explain non-paresthesia-based stimulation or how stimulation effects persist following cessation, but it does offer a crude mechanistic approximation and highlights the role of central effects in pain modulation.

There has been a myriad of alternative or additional proposed mechanisms of stimulation including local and supraspinal effects. Local effects include changes in excitatory/inhibitory neurotransmitter release (↓glutamate, ↑GABA), effects on neuromodulatory transmitter systems (serotoninergic, cholinergic, adrenergic, opioid, cannabinoid), and effects on glial cells involved in neuro-glial communication and inflammation. Supraspinal considerations include effects along corticospinal and spinothalamic tracts communicating with brainstem nuclei which can lead to downstream inhibition and cortical regions involved in pain perception. For instance, with burst stimulation human PET scans and SSEP studies have suggested that the burst patterns activate medial pathways of the spinothalamic system which project to the anterior cingulate cortex involved in the affective response to pain, whereas tonic stimulation invokes the lateral pathway through the gracile nucleus projecting to the sensory cortex involved in discriminative pathways of pain leading to paresthesias (Chakravarthy et al. 2019). With high frequency stimulation, researchers have postulated effects such as desynchronization of maladaptive signals, conduction blockade through depolarizing or hyperpolarizing effects, or wind-up suppression. As will be explored in a later section, glial cells and immune cells have been found to have a significant role in the development, maintenance, and resolution of chronic pain with evidence for changes in response to neuromodulation (Vallejo et al. 2010, 2020).

15.4 Peripheral Nerve Stimulation and Vagal Nerve Stimulation

Peripheral Nerve Stimulation

When patients have unilateral, regional pain in distributions that follow patterns of peripheral nerves or a known nerve injury, peripheral nerve stimulation (PNS) can be an effective form of neuromodulation therapy. PNS offers advantages such as avoiding irreversible ablative procedures which can cause deafferentation pain, avoiding complex surgical nerve grafting, and providing much longer relief than peripheral blocks while still being reversible. PNS has found utility in numerous pain syndromes including neuropathic, ischemic, visceral, and post-traumatic/surgical pain (Chakravarthy et al. 2016).

The earliest trial of peripheral nerve stimulation was actually performed in 1967 by Wall and Sweet (Wall and Sweet 1967) involving 8 patients undergoing percutaneous or open placement of electrodes with sessions of 2 min of stimulation (100 Hz, 100 s pulse width) producing paresthesias over the involved area and reduced sensitivity to pressure. Half of the patients reported a preserved effect for 30 min after stopping stimulation. Later in 1976 Campbell and Long (Campbell and Long 1976) performed a percutaneous trial followed by surgical dissection and placement of a bipolar electrode wrapped around the affected nerve in 23 patients. Patients self-directed their stimulation and follow-up at 1.5 years showed 4 patients with excellent response (no analgesics, able to return to work), 5 with a moderate response, and the remaining with limited response. They also determined that the poor responders tended to be related to sites of stimulation that were distal to the origin of pain or site of injury.

The advances in technology since then, especially the development of ultrasound guidance, have fostered a resurgence in PNS interest with greater precision, ease, and speed of placement (Sivanesan and Gulati 2019). We have systematically reviewed clinical applications of PNS for pain management (Xu et al. 2021). Similar to SCS, the mechanism of peripheral stimulation is thought related to the gate control theory with the generation of paresthesias through activation of Aβ fibers during stimulation that inhibits nociceptive transmission. PNS is additionally thought to aid in nerve recovery by altering the ectopic discharge activity from an area that has sustained injury with reduction in peripheral sensitization effects, nociceptive threshold, and peripherally induced reconditioning of the CNS through an as yet to be determined mechanism (Deer et al. 2021). The most frequent complication following percutaneous placement tends to be lead migration due to difficulty in anchoring over mobile myofascial areas or lead fracturing due to small diameter contacts. PNS electrodes can either be implanted permanently with an implantable pulse generator (IPG) or placed temporarily (connected to an external generator) with FDA approval for up to 60 days. Coiled leads are used most often for percutaneous placement with a retrospective study demonstrating roughly 25 times less chance of infection with coiled leads compared with non-coiled (Ilfeld et al. 2017).

A 2016 prospective PNS RCT included 94 patients with intractable chronic pain of a peripheral nerve origin (27 lower extremity, 26 upper extremity, 41 trunk) who underwent PNS implant with a permanent system and 1:1 randomization to treatment or sham (Deer et al. 2016). At the 3 month primary endpoint, 38% of the stimulation group were considered responders (>50% improvement) compared to 10% in the control group with average pain score reductions of 27% in the treatment group and 2% in the control. A 2022 retrospective review of 747 patients who underwent 60-day temporary placement of a PNS system for similar indications showed a 60% response rate. Interestingly half of the responding group were found to be early responders (<2 weeks) and half were found to have a delayed response to therapy (Naidu et al. 2022). Many smaller studies have looked at specific sites/indications for PNS with benefits shown for cluster headaches with sphenopalatine ganglion stimulation (Goadsby et al. 2019), chronic migraines through occipital nerve stimulation (Mekhail et al. 2017), low back pain (McRoberts et al. 2013), and post-amputation pain (residual or phantom limb pain) (Gilmore et al. 2019). Certain peripheral nerves have also been found to have relief through unclear mechanisms such as tibial nerve stimulation providing relief of chronic pelvic pain (Hanyu-Deutmeyer and Pritzlaff 2020; Istek et al. 2014). Overall PNS technology continues to advance with increased adoption among providers.

Vagal Nerve Stimulation

The tenth cranial nerve, vagus nerve, is the predominant supply of the parasympathetic nervous system to the rest of the body with mixed sensory and motor fibers. Originating in the brainstem, the vagus nerve projects bilaterally in the neck along the carotids before branching into its terminal supply to the viscera including the heart, lungs, and gastrointestinal system. The descending effects of the vagus nerve include autonomic tone as well as effects on inflammation (Bonaz et al. 2013). Termed the inflammatory reflex (Pavlov and Tracey 2012), the vagus nerve includes afferents which respond to various changes including tissue inflammation and in turn activate an efferent cholinergic anti-inflammatory pathway that inhibits proinflammatory cytokine production and response in immune cells. This system has been shown to have a role in autoimmune disease and metabolic disorders offering additional opportunities for neuromodulation.

Vagal nerve stimulation (VNS) was in fact one of the earliest forms of electrical stimulation dated back to the 1880s by Corning to abort seizures which involved the application of a “carotid fork” that was applied to the neck with simultaneous electrical stimulation of the traversing vagus nerves and cervical sympathetic nerves (Lanska 2002). Due to inconsistent results, its use did not enter mainstream clinical application until almost 100 years later in 1988 with the first implantable vagal nerve stimulator used to treat refractory epilepsy. Its mechanism is still unknown but thought through activation of ascending pathway effects in the CNS. Since then VNS has been explored for a range of syndromes including migraines, cluster headaches, heart failure, fibromyalgia, depression, intractable hiccups, autoimmune disease, and even sepsis (Johnson and Wilson 2018).

The most common site for vagal stimulation is in the neck given easy access to a proximal aspect of the nerve. The left vagus nerve is generally utilized as the right innervates the sinoatrial node in the heart which when stimulated can cause bradycardia. Side effects of VNS stimulation largely fall to the recurrent laryngeal stimulation induced dysphonia, hoarseness, and cough. Frequencies for VNS tend to be lower (10–30 Hz) as studies have shown damage to the vagus nerve at frequencies above 50 Hz (Groves and Brown 2005).

As mentioned, VNS is currently being explored for its anti-inflammatory effects through activation of the cholinergic anti-inflammatory pathway (CAP). For instance, one pilot study for multidrug-refractory rheumatoid arthritis (Genovese et al. 2020) included 14 patients undergoing placement of a vagal nerve stimulator (10 Hz, 250 us pulse width) comparing 1 min of stimulation daily, 1 min four times daily, and sham. While safety and tolerance were the primary endpoint, five of the ten patients in the treatment groups met or exceeded the minimally clinically important difference (MCID) in disease activity score (DAS28CRP) after 12 weeks of stimulation compared to none in the sham control. Subjects also had blood drawn for in-vitro monocyte stimulation using LPS and measurement of the resulting cytokine levels. The results showed a substantial decrease (>30%) in pro-inflammatory cytokines IL1β, IL6, and tumor necrosis factor-alpha (TNF) in the treatment group following stimulation. While a pilot and underpowered study with limited stimulation time, it provides insight into the feasibility of using VNS for its anti-inflammatory effects. Overall the use of VNS for intractable chronic pain and inflammation-mediated pain is in its early stages with promising results and implications for otherwise difficult-to-manage conditions.

15.5 Modulation of Immune Function by Neuromodulation

The mechanisms and cumulative effects of stimulation therapy remain to be fully elucidated but there are demonstrated effects on immune function. This is particularly relevant as there is strong evidence that microglia and astrocytes are intimately involved in neuroinflammation following injury with downstream effects including neurotoxicity, hyperexcitability, and dysregulated synaptic communication (Milligan and Watkins 2009). Following activation, glial cells and immune cells release a number of proinflammatory signaling molecules including cytokines (interleukin-6 [IL-1b], IL-6, TNF-α), chemokines (CCL2, CXCL1), and matrix metalloproteases (MMP-2, MMP-9) with downstream activation of the inflammatory NF-κB pathway which has been implicated in the development of chronic pain states.

The population of glial cells and immune cells is very large, reflecting their important regulatory role. Studies on ex-vivo human spinal tissue have shown that there are as many as 6 times glial cells (astrocytes and microglia) as neurons. In the spinal cord roughly 13% of cells are neurons, 75% glial and immune cells, and 12% endothelial cells (Bahney and von Bartheld 2018). While astrocytes, the

predominant glial cells, do not generate action potentials themselves, they do have resting membrane potentials and exposure to oscillating electric fields with stimulation has been shown to have effects on their gene expression profiles and even receptor activation in the absence of ligand binding. Similarly and perhaps more importantly, microglia are the predominant immune cells with functionality similar to macrophages in the peripheral. Interactions between neurons, microglia, and astrocytes play a critical role in the development, maintenance, and resolution of chronic pain states (Buchheit et al. 2020).

The majority of research demonstrating profound effects on glial cells and immune function by stimulation come from animal studies. In a 2014 study using a spared nerve injury model, rats were exposed to epidural spinal cord stimulation 2 weeks following injury over 4 days at different frequencies (4 and 60 Hz), intensities (up to 90% of motor threshold), and times (30 min to 6 h). Analysis of the ex-vivo spinal cords revealed significantly increased glial immunoreactivity after the injury followed by significant decreases in biomarkers of glial and immune cell activation (GFAP, MCP-1, OX-42) in the dorsal horn following SCS therapy in a dose-dependent manner, which correlated with observed pain and threshold behavior (Sato et al. 2014). Vallejo's group further demonstrated frequency-dependent changes in transcriptomes (mRNA expression) and proteomics (Cedeño et al. 2020). In one study, they performed full-genome microarray analysis and weighted gene correlation network analysis and compared 72 h of continuous SCS (50 Hz) with sham control in rats with spared-nerve injury. Activation of glia and upregulated gene expression (Tlr2, Cxcl16, and Cd68) following SCS is associated with the beneficial responses such as neuroprotection and repair. In a follow-up study, the group performed proteomic analysis of dorsal spinal cord tissue following 72 h of SCS (10 Hz) in a rat model of nerve injury and found that 155 interconnected proteins significantly affected by SCS were associated with collagen-fibril organization, extracellular matrix integrity, redox pathways, and stress response (Tilley et al. 2021). Collectively, SCS following nerve injury appears to upregulate the restorative functions of activated glial and immune cells while downregulating their pro-inflammatory effects. These results have led to the development of DTM SCS. While difficult to carry out analogous studies in humans due to the need for tissue analysis, there have been studies that demonstrated increases in the anti-inflammatory cytokine IL-10 in the CSF and blood following burst SCS (Kinfe et al. 2017). It remains to be determined how electric fields specifically affect neuroinflammation; the findings of these studies offer insights into the potential pro- and anti-inflammatory balancing effects induced by neuromodulation therapy.

15.6 Conclusion

Neuromodulation has advanced considerably since its inception in the 1960s with new indications, targets, waveforms, and improvements in device safety, longevity, and ease of placement. We are in a stage of rapid evolution in technology and

high-quality clinical trials demonstrating strong evidence for neuromodulation therapy. The shift from traditional tonic stimulation to novel waveforms including high-frequency, burst, DTM, and the use of closed-loop systems has improved outcome measures in pain and functionality. Clinicians today can choose from a variety of modulation paradigms including SCS, DRG stimulation, PNS, and VNS that modulate neuroimmune functions at various sites of pain processing. While our understanding of the basis of stimulation has improved since the proposal of the gate control theory, the mechanisms and cumulative effects of neurostimulation remain to be fully characterized. A growing area of research is focused on the interplay between the immune system and nervous system in the context of pain generation, chronification, and resolution with early evidence showing benefits of stimulation with respect to mitigating effects of chronic inflammation.

References

Bahney J, von Bartheld CS. The cellular composition and glia-neuron ratio in the spinal cord of a human and a nonhuman primate: comparison with other species and brain regions. Anat Rec (Hoboken). 2018;301(4):697–710. https://doi.org/10.1002/ar.23728.

Bonaz B, Picq C, Sinniger V, Mayol JF, Clarençon D. Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. Neurogastroenterol Motil. 2013;25(3):208–21. https://doi.org/10.1111/nmo.12076.

Buchheit T, Huh Y, Maixner W, Cheng J, Ji RR. Neuroimmune modulation of pain and regenerative pain medicine. J Clin Invest. 2020;130(5):2164–76. https://doi.org/10.1172/jci134439.

Campbell JN, Long DM. Peripheral nerve stimulation in the treatment of intractable pain. J Neurosurg. 1976;45(6):692–9. https://doi.org/10.3171/jns.1976.45.6.0692.

Cedeño DL, Smith WJ, Kelley CA, Vallejo R. Spinal cord stimulation using differential target multiplexed programming modulates neural cell-specific transcriptomes in an animal model of neuropathic pain. Mol Pain. 2020;16:1744806920964360. https://doi.org/10.1177/1744806920964360.

Chakravarthy K, Nava A, Christo PJ, Williams K. Review of recent advances in peripheral nerve stimulation (PNS). Curr Pain Headache Rep. 2016;20(11):60. https://doi.org/10.1007/s11916-016-0590-8.

Chakravarthy K, Fishman MA, Zuidema X, Hunter CW, Levy R. Mechanism of action in burst spinal cord stimulation: review and recent advances. Pain Med. 2019;20(Suppl 1):S13–s22. https://doi.org/10.1093/pm/pnz073.

De Andres J, Monsalve-Dolz V, Fabregat-Cid G, Villanueva-Perez V, Harutyunyan A, Asensio-Samper JM, Sanchis-Lopez N. Prospective, randomized blind effect-on-outcome study of conventional vs high-frequency spinal cord stimulation in patients with pain and disability due to failed back surgery syndrome. Pain Med. 2017;18(12):2401–21. https://doi.org/10.1093/pm/pnx241.

Deer TR, Mekhail N, Provenzano D, Pope J, Krames E, Thomson S, North R. The appropriate use of neurostimulation: avoidance and treatment of complications of neurostimulation therapies for the treatment of chronic pain. Neuromodulation appropriateness consensus committee. Neuromodulation. 2014;17(6):571–97. https://doi.org/10.1111/ner.12206.

Deer T, Pope J, Benyamin R, Vallejo R, Friedman A, Caraway D, Mekhail N. Prospective, multicenter, randomized, double-blinded, partial crossover study to assess the safety and efficacy of the novel neuromodulation system in the treatment of patients with chronic pain of peripheral nerve origin. Neuromodulation. 2016;19(1):91–100. https://doi.org/10.1111/ner.12381.

Deer T, Slavin KV, Amirdelfan K, North RB, Burton AW, Yearwood TL, Wilson D. Success using neuromodulation with BURST (SUNBURST) study: results from a prospective, randomized controlled trial using a novel burst waveform. Neuromodulation. 2018;21(1):56–66. https://doi.org/10.1111/ner.12698.

Deer TR, Eldabe S, Falowski SM, Huntoon MA, Staats PS, Cassar IR, Boggs JW. Peripherally induced reconditioning of the central nervous system: a proposed mechanistic theory for sustained relief of chronic pain with percutaneous peripheral nerve stimulation. J Pain Res. 2021;14:721–36. https://doi.org/10.2147/jpr.S297091.

Farber SH, Han JL, Elsamadicy AA, Hussaini Q, Yang S, Pagadala P, Lad SP. Long-term cost utility of spinal cord stimulation in patients with failed Back surgery syndrome. Pain Physician. 2017;20(6):E797–e805.

Genovese MC, Gaylis NB, Sikes D, Kivitz A, Horowitz DL, Peterfy C. Safety and efficacy of neurostimulation with a miniaturised vagus nerve stimulation device in patients with multidrug-refractory rheumatoid arthritis: a two-stage multicentre, randomised pilot study. Lancet Rheumatol. 2020;2(9):e527–38. https://doi.org/10.1016/S2665-9913(20)30172-7.

Gilmore C, Ilfeld B, Rosenow J, Li S, Desai M, Hunter C, Boggs J. Percutaneous peripheral nerve stimulation for the treatment of chronic neuropathic postamputation pain: a multicenter, randomized, placebo-controlled trial. Reg Anesth Pain Med. 2019;44(6):637–45. https://doi.org/10.1136/rapm-2018-100109.

Goadsby PJ, Sahai-Srivastava S, Kezirian EJ, Calhoun AH, Matthews DC, McAllister PJ, Dodick DW. Safety and efficacy of sphenopalatine ganglion stimulation for chronic cluster headache: a double-blind, randomised controlled trial. Lancet Neurol. 2019;18(12):1081–90. https://doi.org/10.1016/s1474-4422(19)30322-9.

Groves DA, Brown VJ. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. Neurosci Biobehav Rev. 2005;29(3):493–500. https://doi.org/10.1016/j.neubiorev.2005.01.004.

Hanyu-Deutmeyer A, Pritzlaff SG. Peripheral nerve stimulation for the 21st century: sural, superficial peroneal, and Tibial nerves. Pain Med. 2020;21(Suppl 1):S64–s67. https://doi.org/10.1093/pm/pnaa202.

Ilfeld BM, Gabriel RA, Saulino MF, Chae J, Peckham PH, Grant SA, Boggs JW. Infection rates of electrical leads used for percutaneous neurostimulation of the peripheral nervous system. Pain Pract. 2017;17(6):753–62. https://doi.org/10.1111/papr.12523.

Istek A, Gungor Ugurlucan F, Yasa C, Gokyildiz S, Yalcin O. Randomized trial of long-term effects of percutaneous tibial nerve stimulation on chronic pelvic pain. Arch Gynecol Obstet. 2014;290(2):291–8. https://doi.org/10.1007/s00404-014-3190-z.

Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. J Inflamm Res. 2018;11:203–13. https://doi.org/10.2147/jir.S163248.

Kapural L, Yu C, Doust MW, Gliner BE, Vallejo R, Sitzman BT, Burgher AH. Novel 10-kHz high-frequency therapy (HF10 therapy) is superior to traditional low-frequency spinal cord stimulation for the treatment of chronic back and leg pain: the SENZA-RCT randomized controlled trial. Anesthesiology. 2015;123(4):851–60. https://doi.org/10.1097/aln.0000000000000774.

Kapural L, Yu C, Doust MW, Gliner BE, Vallejo R, Sitzman BT, Burgher AH. Comparison of 10-kHz high-frequency and traditional low-frequency spinal cord stimulation for the treatment of chronic back and leg pain: 24-month results from a multicenter, randomized. Controll Pivotal Trial Neurosurg. 2016;79(5):667–77. https://doi.org/10.1227/neu.0000000000001418.

Kapural L, Jameson J, Johnson C, Kloster D, Calodney A, Kosek P, Patel NP. Treatment of nonsurgical refractory back pain with high-frequency spinal cord stimulation at 10 kHz: 12-month results of a pragmatic, multicenter, randomized controlled trial. J Neurosurg Spine. 2022;1–12:188. https://doi.org/10.3171/2021.12.Spine211301.

Khadka N, Liu X, Zander H, Swami J, Rogers E, Lempka SF, Bikson M. Realistic anatomically detailed open-source spinal cord stimulation (RADO-SCS) model. J Neural Eng. 2020;17(2):026033. https://doi.org/10.1088/1741-2552/ab8344.

Kinfe TM, Muhammad S, Link C, Roeske S, Chaudhry SR, Yearwood TL. Burst spinal cord stimulation increases peripheral antineuroinflammatory interleukin 10 levels in failed back surgery syndrome patients with predominant back pain. Neuromodulation. 2017;20(4):322–30. https://doi.org/10.1111/ner.12586.

Kumar K, North R, Taylor R, Sculpher M, Van den Abeele C, Gehring M, Jacobs M. Spinal cord stimulation vs. conventional medical management: a prospective, randomized, controlled, multicenter study of patients with failed back surgery syndrome (PROCESS study). Neuromodulation. 2005;8(4):213–8. https://doi.org/10.1111/j.1525-1403.2005.00027.x.

Kumar K, Taylor RS, Jacques L, Eldabe S, Meglio M, Molet J, North RB. Spinal cord stimulation versus conventional medical management for neuropathic pain: a multicentre randomised controlled trial in patients with failed back surgery syndrome. Pain. 2007;132(1–2):179–88. https://doi.org/10.1016/j.pain.2007.07.028.

Lanska DJ. J.L. Corning and vagal nerve stimulation for seizures in the 1880s. Neurology. 2002;58(3):452–9. https://doi.org/10.1212/wnl.58.3.452.

Levy R, Deer TR, Poree L, Rosen SM, Kapural L, Amirdelfan K, Mekhail N. Multicenter, randomized, double-blind study protocol using human spinal cord recording comparing safety, efficacy, and neurophysiological responses between patients being treated with evoked compound action potential-controlled closed-loop spinal cord stimulation or open-loop spinal cord stimulation (the evoke study). Neuromodulation. 2019;22(3):317–26. https://doi.org/10.1111/ner.12932.

McRoberts WP, Wolkowitz R, Meyer DJ, Lipov E, Joshi J, Davis B, Barolat G. Peripheral nerve field stimulation for the management of localized chronic intractable back pain: results from a randomized controlled study. Neuromodulation. 2013;16(6):565–74. https://doi.org/10.1111/ner.12055.

Mekhail NA, Cheng J, Narouze S, Kapural L, Mekhail MN, Deer T. Clinical applications of neurostimulation: forty years later. Pain Pract. 2010;10(2):103–12. https://doi.org/10.1111/j.1533-2500.2009.00341.x.

Mekhail NA, Estemalik E, Azer G, Davis K, Tepper SJ. Safety and efficacy of occipital nerves stimulation for the treatment of chronic migraines: randomized, double-blind. Controll Single-Center Exper Pain Pract. 2017;17(5):669–77. https://doi.org/10.1111/papr.12504.

Mekhail N, Visnjevac O, Azer G, Mehanny DS, Agrawal P, Foorsov V. Spinal cord stimulation 50 years later: clinical outcomes of spinal cord stimulation based on randomized clinical trials-a systematic review. Reg Anesth Pain Med. 2018;43(4):391–406. https://doi.org/10.1097/aap.0000000000000744.

Mekhail N, Levy RM, Deer TR, Kapural L, Li S, Amirdelfan K, Poree L. Long-term safety and efficacy of closed-loop spinal cord stimulation to treat chronic back and leg pain (evoke): a double-blind, randomised, controlled trial. Lancet Neurol. 2020;19(2):123–34. https://doi.org/10.1016/s1474-4422(19)30414-4.

Melzack R, Wall PD. Pain mechanisms: a new theory. Science. 1965;150(3699):971–9. https://doi.org/10.1126/science.150.3699.971.

Miller JP, Eldabe S, Buchser E, Johanek LM, Guan Y, Linderoth B. Parameters of spinal cord stimulation and their role in electrical charge delivery: a review. Neuromodulation. 2016;19(4):373–84. https://doi.org/10.1111/ner.12438.

Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci. 2009;10(1):23–36. https://doi.org/10.1038/nrn2533.

Naidu R, Li S, Desai MJ, Sheth S, Crosby ND, Boggs JW. 60-day PNS treatment may improve identification of delayed responders and delayed non-responders to Neurostimulation for pain relief. J Pain Res. 2022;15:733–43. https://doi.org/10.2147/jpr.S349101.

North RB, Kidd DH, Farrokhi F, Piantadosi SA. Spinal cord stimulation versus repeated lumbosacral spine surgery for chronic pain: a randomized, controlled trial. Neurosurgery. 2005;56(1):98–106. https://doi.org/10.1227/01.neu.0000144839.65524.e0.

Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex—linking immunity and metabolism. Nat Rev Endocrinol. 2012;8(12):743–54. https://doi.org/10.1038/nrendo.2012.189.

Paz-Solís J, Thomson S, Jain R, Chen L, Huertas I, Doan Q. Exploration of high- and low-frequency options for subperception spinal cord stimulation using neural dosing parameter relationships: the HALO study. Neuromodulation. 2022;25(1):94–102. https://doi.org/10.1111/ner.13390.

Petersen EA, Stauss TG, Scowcroft JA, Brooks ES, White JL, Sills SM, Mekhail NA. Effect of high-frequency (10-kHz) spinal cord stimulation in patients with painful diabetic neuropathy: a randomized clinical trial. JAMA Neurol. 2021;78(6):687–98. https://doi.org/10.1001/jamaneurol.2021.0538.

Provenzano D, Tate J, Gupta M, Yu C, Verrills P, Guirguis M, Bradley K. Pulse dosing of 10-kHz paresthesia-independent spinal cord stimulation provides the same efficacy with substantial reduction of device recharge time. Pain Med. 2022;23(1):152–63. https://doi.org/10.1093/pm/pnab288.

Sato KL, Johanek LM, Sanada LS, Sluka KA. Spinal cord stimulation reduces mechanical hyperalgesia and glial cell activation in animals with neuropathic pain. Anesth Analg. 2014;118(2):464–72. https://doi.org/10.1213/ane.0000000000000047.

Sdrulla AD, Guan Y, Raja SN. Spinal cord stimulation: clinical efficacy and potential mechanisms. Pain Pract. 2018;18(8):1048–67. https://doi.org/10.1111/papr.12692.

Shealy CN, Mortimer JT, Reswick JB. Electrical inhibition of pain by stimulation of the dorsal columns: preliminary clinical report. Anesth Analg. 1967;46(4):489–91.

Sherman SM. Tonic and burst firing: dual modes of thalamocortical relay. Trends Neurosci. 2001;24(2):122–6. https://doi.org/10.1016/s0166-2236(00)01714-8.

Sivanesan E, Gulati A. Resurgence of peripheral nerve stimulation with innovation in device technologies. Reg Anesth Pain Med. 2019;44(6):615–6. https://doi.org/10.1136/rapm-2019-100488.

Tilley DM, Lietz CB, Cedeno DL, Kelley CA, Li L, Vallejo R. Proteomic modulation in the dorsal spinal cord following spinal cord stimulation therapy in an in vivo neuropathic pain model. Neuromodulation. 2021;24(1):22–32. https://doi.org/10.1111/ner.13103.

Vallejo R, Tilley DM, Vogel L, Benyamin R. The role of glia and the immune system in the development and maintenance of neuropathic pain. Pain Pract. 2010;10(3):167–84. https://doi.org/10.1111/j.1533-2500.2010.00367.x.

Vallejo R, Kelley CA, Gupta A, Smith WJ, Vallejo A, Cedeño DL. Modulation of neuroglial interactions using differential target multiplexed spinal cord stimulation in an animal model of neuropathic pain. Mol Pain. 2020;16:1744806920918057. https://doi.org/10.1177/1744806920918057.

Van Buyten JP, Smet I, Liem L, Russo M, Huygen F. Stimulation of dorsal root ganglia for the management of complex regional pain syndrome: a prospective case series. Pain Pract. 2015;15(3):208–16. https://doi.org/10.1111/papr.12170.

Vesper J, Slotty P, Schu S, Poeggel-Kraemer K, Littges H, Van Looy P, Van Havenbergh T. Burst SCS microdosing is as efficacious as standard burst SCS in treating chronic back and leg pain: results from a randomized controlled trial. Neuromodulation. 2019;22(2):190–3. https://doi.org/10.1111/ner.12883.

Wall PD, Sweet WH. Temporary abolition of pain in man. Science. 1967;155(3758):108–9. https://doi.org/10.1126/science.155.3758.108.

Xu J, Sun Z, Wu J, Rana M, Garza J, Zhu AC, Cheng J. Peripheral nerve stimulation in pain management: a systematic review. Pain Physician. 2021;24(2):E131–e152.

Zucco F, Ciampichini R, Lavano A, Costantini A, De Rose M, Poli P, Mantovani LG. Cost-effectiveness and cost-utility analysis of spinal cord stimulation in patients with failed back surgery syndrome: results from the PRECISE study. Neuromodulation. 2015;18(4):266–76. https://doi.org/10.1111/ner.12292.

Chapter 16
What Patients Need to Know About Pain Therapy?

Ellen W. K. Rosenquist

Abstract When you have pain, especially pain that is persistent, it can pervade every aspect of your life. Fear can start to build and anxiety may increase as you try to figure out where to begin to find help. As you start your search, you may feel overwhelmed by the seemingly endless options available to you. Where do you start? What are legitimate treatment options and what are not? Who's a credible provider and who is not? What can you do to help yourself? In this era of social media and the Internet, there are countless "experts" with "advice" about almost everything. But, how do you know what is fake and what is real? This chapter will help guide you to the most appropriate path for you to find the right provider for you and give you direction on how you can be an active participant in your journey to recovery.

Keywords Diagnosis · Provider · Traditional Chinese medicine (TCM)

16.1 Know Your Provider

There are several types of providers who offer pain management in our society.

- Traditional Chinese medicine providers
- Naturopathic physicians
- Chiropractors
- Osteopathic and allopathic physicians

Traditional Chinese medicine (TCM) providers utilize several approaches including, but not limited to, acupuncture, tai chi, and herbal products to help improve quality of life and treat some pain conditions. TCM has evolved over thousands of years. A TCM provider WITHOUT a recognized medical degree (DO, MD) can

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become a Doctor of Acupuncture and Oriental Medicine by obtaining an advanced clinical doctorate which requires 2 years of additional study beyond a master's degree. There are also a number of intensive training programs a provider can complete to earn a Doctor of Traditional Chinese Medicine which typically includes acupuncture training. TCM providers are not considered medical physicians in the United States.

According to the NIH National Center for Complementary and Integrative Medicine, "Chiropractic is a licensed health care profession that emphasizes the body's ability to heal itself. Treatment typically involves manual therapy, often including spinal manipulation. Other forms of treatment, such as exercise and nutritional counseling, may be used as well." (https://www.nccih.nih.gov/health/chiropractic-in-depth) Chronic low back pain or neck pain are common reasons why a patient may seek treatment from a chiropractor. Chiropractors may also treat other musculoskeletal related pains. In order to practice chiropractic in the United States, chiropractors must complete a Doctor of Chiropractic degree, pass a board examination, and have a state license.

According to the Association of Accredited Naturopathic Medical Colleges, established in 2001, "Naturopathic medicine is a distinct health care profession that combines the wisdom of nature with the rigors of modern science. Naturopathic physicians (NDs) are trained as primary care providers who diagnose, treat and manage patients with acute and chronic conditions, while addressing disease and dysfunction at the level of body, mind and spirit."(https://aanmc.org/naturopathic-medicine/) Naturopathic physicians focus on wellness through promotion of health and prevention of disease using natural means, if possible. Licensed naturopathic physicians complete a 4-year graduate-level degree in naturopathic medicine.

Osteopathic and allopathic physicians are licensed medical physicians who have completed extensive education and training in their chosen specialties. This typically includes a 4-year undergraduate degree, a 4-year medical degree, 3–5 years of training through a residency program, followed by an additional 1–3 years post-residency fellowship focused on specialty training, that is, pain management. There are slight differences in philosophy and training between osteopathic and allopathic physicians. For instance, osteopathic physicians are trained in osteopathic manipulation (OMM) which is excluded from allopathic training programs. Many medical physicians obtain additional training in acupuncture and other complimentary medical techniques that can be used to treat pain.

When you are searching for a provider to address your pain management needs, you must first determine what approach you personally feel comfortable with and can have confidence in. It is important to understand that while some conditions can be treated with natural or holistic methods, many conditions do require the advances of medical science and should be addressed by a medical physician. No matter what route you decide to take, you must make sure that the provider you choose to establish a clinical relationship with is well educated, adequately trained, and board certified if applicable. The task of searching for a medical physician can seem daunting. Online search engines can provide you with a plethora of information about any given provider. However, the accuracy of the information can come into question.

There are numerous online sources that allow individuals to submit reviews about providers that they may or may not have actually interacted with. Just like online voting for a friend's pet for a contest, often times the value of a provider's rating can be skewed and be misleading. A patient who may not have agreed with what the provider has offered as treatment options or a patient who is angry because the provider would not prescribe inappropriate medications that the patient desired may provide a bad review on one of these review sites. That same patient can ask their friends and family to also submit bad reviews to the same review site in order to purposely reduce the overall rating of that provider. Therefore, it is not recommended to utilize these sources as your primary source of information regarding a potential provider.

Every state has a state board of medicine which has a publicly accessible website. You can search for a particular provider to determine if they are board certified. It is not required that a physician is board certified to practice medicine in the United States. However, having board certification means that the physician has met all of the expert requirements of their specialty and continues to meet the requirements for continued medical education and training needed to keep them up to date with advanced knowledge in their particular field of medicine. You can also determine if there have been any complaints filed against that provider or if there has been any disciplinary action taken against the provider.

Another reliable source of information regarding a potential provider is the official website of the healthcare system or practice where the provider is employed. Many of these websites provide a professional summary of each provider and what specific conditions the physician treats. There is also usually a section containing patient reviews, including comments. Even with all of these various resources available, none can be considered to be 100% accurate and you ultimately have to combine all of the information you have gathered and determine your confidence in that provider's ability to address your needs.

16.2 Know Your Diagnosis

Once you have seen your physician about your condition, you may be given a diagnosis or a differential diagnosis. What is a differential diagnosis? It is the process that your physician goes through to differentiate between conditions that share similar signs or symptoms. More testing or diagnostic procedures may need to be done before a specific diagnosis can be provided. If this is your situation, you may be given a list of additional tests or procedures that your provider wants you to do which will aid them in determining what the source of your pain is and how to manage it optimally. Even if your physician explains in detail what they are ordering or why they are ordering it, you still may have questions when you walk out the door. Where can you find additional information about these tests and procedures that are accurate? The Internet is the most common and utilized source for information in today's world. While there are countless sites you can refer to, below are just a few

examples of resources with the highest standards of reliability and accuracy. It is important to know that most major healthcare systems in the United States have publicly available information regarding specific tests and procedures.

https://medlineplus.gov/: MedlinePlus is a service of the National Library of Medicine (NLM), the world's largest medical library, which is part of the National Institutes of Health (NIH). Our mission is to present high-quality, relevant health and wellness information that is trusted, easy to understand, and free of advertising, in both English and Spanish. Anywhere, anytime, on any device—for free.(https://medlineplus.gov/about/)

https://www.mayoclinic.org/tests-procedures
https://my.clevelandclinic.org/health/treatments

16.3 Know Your Treatment Options and Participate in Formulating Your Treatment Plan

Once your physician has determined your diagnosis, treatment options will be offered to you. It is important for you to work with your physician as a team to develop a plan of care. However, it is vital that you keep an open mind and listen to all of the treatment options that the provider offers to you. There may be options offered to you that you have tried in the past that were not successful or you know of someone who has had that treatment before and it didn't work for them. You should not dismiss these options immediately. You are unique and your body and symptoms do change with time. Therefore, something that may not have worked before may help at this stage in your life. Also, everyone responds to treatments differently. Your response to a treatment will not be the exact same as a friend who has had the same treatment. It is equally important for your physician to understand your priorities and goals for treatment of your condition. What may be the physician's priority in your treatment plan may not be your priority, so you want to make sure both of you are in alignment with the overall treatment plan.

16.4 Know Your Specific Treatment Modalities

There are many modalities for treating pain. Most pain management providers consider a multimodal approach to treatment.

- Physical therapy and aqua therapy
- Acupuncture or other complimentary medical techniques
- Pharmacologic agents
- Interventional pain procedures (injections, minimally invasive surgeries)
- Psychology based treatments (cognitive behavioral feedback, biofeedback)

Physical therapy is a modality that utilizes methods such as massage, heat, and stretches and exercise to help reduce pain by improving flexibility, mobility, strength, and dynamic function of the body. Treatments are individualized for each patient after a physical therapist has evaluated you. The stretches, exercises, and techniques learned during your physical therapy exercises should be performed on a consistent basis even after you have completed your course of therapy in order to maintain the improvements your body has achieved. It is important to understand that change takes time with improvements in function of our bodies and sometimes training our bodies to do different things than what it is used to can create some increased pain.

There are a number of complimentary medical techniques available that can be integrated as part of a multidisciplinary treatment plan. Acupuncture, frequency specific microcurrent treatment, Reiki, and electromagnetic therapy are several examples. Most providers who offer these complimentary techniques have completed additional training to become adept at these techniques. You should confirm with the provider that they do have adequate training if offered on of these treatment options.

There are many different pharmacologic agents that have been utilized for the treatment of different types of pain. Opioid medications had long been used widely to treat all types of pain. However, with new knowledge of how these medications work in the human body, the use of these medications for the treatment of chronic non-cancer pain has fallen out of favor. There are medications that treat inflammatory pain like arthritis. There are medications that treat nerve related pain like diabetic neuropathy. There are medications that treat pain from muscle spasms. Your physician may prescribe a combination of different categories of medications depending on the complexity and the origin of your pain complaints.

With advances in medical science and technology, there are many different interventional treatments that can be utilized to treat pain. It is very important to keep in mind that an injection is just one type of treatment for specific types of pain. There is not an injection that will help with every type of pain. Injections do not take the place of other categories of treatment. Not every patient with the same diagnosis is an appropriate candidate for the same injection. Just because you know someone who had an injection that worked for them does not mean that the same injection will work for you. Each individual patient is unique with unique considerations that your physician will account for when developing your unique treatment plan.

Psychology-based treatments have become more acceptable forms of treatment as society has overcome a lot of the stigma surrounding psychological disorders. It is widely accepted that a person's biology, psychological state, and social environment all contribute to an individual's chronic pain experiences. There is a great deal of complexity related to the psychology of chronic pain and several different psychology-based treatment modalities have been developed and proven very successful in the treatment of the psychological aspects of chronic pain (Psychol Res Behav Manag, 2014).

16.5 Know How to Monitor Your Treatment Responses

Once a treatment plan has been implemented, it is important to monitor your body for any type of response to treatment—good or bad. It may be helpful to you to start a journal to record your daily pain experiences in order to be able to notice subtle improvement trends or any unwanted side effects of a given treatment. Be careful not to overanalyze, however, because excessive attention to your pain can actually cause your pain to worsen (pain catastrophizing) (Schutze et al., 2020). Review your journal before each follow-up appointment so that you are able to provide your physician with accurate recall of any changes in your condition. This will help your physician to adjust your treatment plan as necessary.

16.6 Know How to Report and Mitigate Side Effects and Adverse Events

If you do notice side effects that can be confidently related to a specific treatment, it is important to inform your physician as soon as possible. When reporting the side effect(s), provide detailed and specific information that can help your physician determine any other appropriate alternative treatment options that may be available to you. If you feel that the side effects that you are experiencing are serious and you are not able to reach your physician, you should present to the nearest urgent care or emergency room for an evaluation.

16.7 Know How Your Lifestyle Can Exacerbate or Mitigate Your Pain

Many lifestyle choices and habits can affect your pain. Smoking, sleep hygiene, nutrition, and ergonomics are a few examples of modifiable factors that can influence chronic pain.

Multiple epidemiological and clinical studies have suggested that the prevalence of smoking among persons in pain may be up to twice that observed in the general population. In a study looking at the relationship between chronic pain, cigarette smoking, and nicotine dependence, it was found that “the prevalence of current smoking was: 42% among persons experiencing medically unexplained chronic pain in the past year; 35% among persons reporting medically unexplained chronic pain in their lifetime; and 30% among persons reporting past year or lifetime chronic neck or back pain. Furthermore, after adjusting for a host of highly relevant psychosocial factors (i.e., age, marital status, income, education, race, gender, and the presence of any lifetime mood, anxiety, or substance use disorder), persons who met criteria for past year nicotine dependence were almost twice as likely to report past

year medically-unexplained chronic pain (OR = 1.83; 95% CI = 1.15–2.90) and past year chronic neck or back pain (OR = 1.95; 95% CI = 1.41–2.68)" (Zvolensky et al. 2009).

Some studies conducted in recent years suggest that sleep disturbances may contribute to the development and maintenance of chronic pain, including endogenous pain inhibition and joint pain (Finan et al. 2013) One study concluded that "short-term (2-month) improvements in sleep predicted long-term (9- and 18-month) improvements for multiple measures of sleep, chronic pain, and fatigue. These findings are consistent with benefits of improved sleep for chronic pain and fatigue among older persons with osteoarthritis pain and co-morbid insomnia if robust improvements in sleep are achieved and sustained" (Viteillo et al. 2014). Therefore, improving sleep hygiene could potentially result in a reduction in chronic pain complaints.

What you eat can affect how your body feels. In recent years, it has been suggested that certain foods we eat can increase chronic pain. Foods containing high-fructose corn syrup or that are high in sugar content, artificial trans fats, vegetable oils, refined carbohydrates, excessive alcohol, and processed meats are commonly thought to promote inflammation, thereby promoting chronic pain through low level inflammation throughout the body. Many practitioners will promote anti-inflammatory diets such as a "Mediterranean diet." The American Heart Association promotes this diet because it "emphasizes vegetables, fruits, whole grains, beans and legumes; includes low-fat or fat-free dairy products, fish, poultry, non-tropical vegetable oils and nuts; and limits added sugars, sugary beverages, sodium, highly processed foods, refined carbohydrates, saturated fats, and fatty or processed meats"(https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/nutrition-basics/mediterranean-die).

Simple ergonomic corrections in your daily routine can help to mitigate chronic daily pain such as working on your posture, having good ergonomics when using a computer or reading a book, and limiting the use of handheld devices (as they can put a strain on your neck and joints of your hands).

16.8 Know How to Build and Nurture a Trusting Patient-Clinician Relationship for Best Possible Outcomes

Open and honest communication is the best way to build a trusting patient-clinician relationship which will lead to the best possible outcomes for you as well as the physician treating you. One thing to remember when interacting with your physician is that they are human just like you. Despite having very similar educational backgrounds, every physician is a unique individual with a unique personality. Every patient is a unique individual with a unique personality. Every person can have a good day or a bad day the day. I would encourage you to discuss any

concerns you may have with your physician in a sincere and honest fashion. Be open minded about the information and advice your physician provides to you even if it is not what you want to hear. Do not go on the defensive immediately if their ideas are not your ideas. At the end of the day, your physician just wants to help you get better.

References

Finan PH, Goodin BR, Smith MT. The association of sleep and pain: an update and a path forward. J Pain. 2013;14(12):1539–52. https://doi.org/10.1016/j.jpain.2013.08.007.

https://aanmc.org/naturopathic-medicine/

https://medlineplus.gov/about/

https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/nutrition-basics/mediterranean-die https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/nutrition-basics/mediterranean-diet

https://www.nccih.nih.gov/health/chiropractic-in-depth

Schutze R, Rees C, Smith A, Slater H, O'Sullivan P. Metacognition, perseverative thinking, and pain catastrophizing: A moderated-mediation analysis. Eur J Pain. 2020;24(1):223–33.

Sturgeon JA. Psychological therapies for the management of chronic pain. Psychol Res Behav Manag. 2014;7:115–124. https://doi.org/10.2147/PRBM.S44762. Epub 2014 Apr 10.

Viteillo MV, McCurry SM, Shortreed SM, Baker LD, Rybarczyk BD, Keefe FJ, Von Korff M. Short-term improvement in insomnia symptoms predicts long-term improvements in sleep, pain, and fatigue in older adults with comorbid osteoarthritis and insomnia. Pain. 2014;155(8):1547–54. https://doi.org/10.1016/j.pain.2014.04.032.

Zvolensky MJ, McMillan K, Gonzalez A, Asmundson GJG. Chronic pain and cigarette smoking and nicotine dependence among a representative sample of adults. Nicotine Tob Res. 2009;11(12):1407–14.

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https://doi.org/10.1007/978-3-031-29231-6