Neuroimmune Interactions in Chronic Pain
Review

OPEN

PAIN REPORTS®

Neuroimmune interactions and osteoarthritis pain: focus on macrophages

Terese Geraghty[a], Deborah R. Winter[b], Richard J. Miller[c], Rachel E. Miller[a], Anne-Marie Malfait[a,*]

Abstract

Bidirectional interactions between the immune system and the nervous system are increasingly appreciated as playing a pathogenic role in chronic pain. Unraveling the mechanisms by which inflammatory pain is mediated through communication between nerves and immune cells may lead to exciting new strategies for therapeutic intervention. In this narrative review, we focus on the role of macrophages in the pathogenesis of osteoarthritis (OA) pain. From regulating homeostasis to conducting phagocytosis, and from inducing inflammation to resolving it, macrophages are plastic cells that are highly adaptable to their environment. They rely on communicating with the environment through cytokines, growth factors, neuropeptides, and other signals to respond to inflammation or injury. The contribution of macrophages to OA joint damage has garnered much attention in recent years. Here, we discuss how macrophages may participate in the initiation and maintenance of pain in OA. We aim to summarize what is currently known about macrophages in OA pain and identify important gaps in the field to fuel future investigations.

Keywords: Osteoarthritis, Pain, Macrophages, Inflammation, Neuroimmunity, Animal models

1. Introduction

1.1. Chronic pain in osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis. It is a painful, chronic disease of the synovial joints that primarily affects the knees, hips, hands, and small joints in the spine. A major cause of chronic pain and disability worldwide,[48] OA presents a highly significant socioeconomic burden because of health care costs and work loss.[115] There are no pharmacological treatments available to slow or blunt the progressive joint degeneration that characterizes OA, ie, disease-modifying OA drugs.[110] Joint pain drives patients with OA to seek medical help. Nonpharmacological strategies such as weight loss and exercise play a major role in managing OA pain.[61] Although a variety of oral, intra-articular, and topical pharmacological analgesic strategies are available as standard-of-care, including nonsteroidal anti-inflammatory drugs and intra-articular glucocorticosteroids,[61] they often do not provide adequate pain relief, and many knee and hip OA patients end up undergoing total joint replacement. The strongest risk factors for the development of OA are age, history of joint injury, and obesity. With the prevalence of OA and obesity increasing, and with the ageing of the population, safe and efficacious targeted therapies to manage OA pain are urgently needed.[106,149]

Pain is the feature that characterizes clinical OA, and its quality and severity vary among patients and can change over time.[79] Osteoarthritis pain ranges from "aching,c "dull" pain in the affected joint to less localized periarticular and referred pain. In early OA, pain has a highly mechanical nature and is mainly felt during an activity or when using the joint—eg, the knee hurts when climbing stairs. As the disease advances, pain can become more widespread and can also occur at rest.[106] In recent years, many studies in patients with painful OA have identified a strong component of peripheral and central sensitization. In people with knee OA, quantitative sensory testing reveals evidence of widespread hypersensitivity to mechanical pressure, including hyperalgesia ("increased pain from a stimulus that normally provokes pain") and allodynia ("pain due to a stimulus that does not normally provoke pain"), both at the affected joint and at sites remote from the joint.[13] Patients with OA also show facilitated temporal summation and impaired conditioned pain modulation, evidence for central sensitization.[74]

Osteoarthritis is a classical biomechanically driven disease[31] which also seems to involve a component of low-grade inflammation.[124] The precise pathways of the innate immune and inflammatory responses that are operative at the onset and during the progression of OA joint damage are being unravelled with increasing precision (reviewed in Ref. 124). By contrast,

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

[a] *Division of Rheumatology, Department of Internal Medicine, Rush University Medical Center, Chicago, IL, USA,* [b] *Division of Rheumatology, Department of Medicine, Northwestern University, Chicago, IL, USA,* [c] *Department of Pharmacology, Northwestern University, Chicago, IL, USA*

**Corresponding author. Address: Rush University Medical Center, Division of Rheumatology 1611 W Harrison Street, suite 510 Chicago IL 60612, USA. Tel.: (312)-563-2925. E-mail address: anne-marie_malfait@rush.edu (A.-M. Malfait).*

PR9 6 (2021) e892

http://dx.doi.org/10.1097/PR9.0000000000000892

although pain (*dolor*)—in addition to redness (*rubor*), heat (*calor*), swelling (*tumor*), and loss of function (*functio laesa*)—is one of the 5 cardinal signs of inflammation, as defined by Celsus in the first century AD, our understanding of the role inflammation may play specifically in the genesis of OA pain is much more limited. Although several studies in people with OA have described associations between synovitis detected by MRI and pain or sensitization,[107] targeting specific inflammatory mediators (eg, neutralizing IL-1 or TNFα with monoclonal antibodies) has been largely disappointing.[113] Nonetheless, the different cell types that make up the innate immune system, including monocytes, granulocytes, macrophages, mast cells, and dendritic cells, situated in tissues throughout the body as well as in circulation,[89,90] can all contribute to the genesis and maintenance of pain. How they may contribute to pain specifically in OA is a topic of great interest, since it can be expected that a deeper understanding of the interrelationships between the innate immune system and neuronal system may open avenues to novel interventional strategies (reviewed in Refs. 15 and 19).

1.2. Genesis and maintenance of pain requires more than the nervous system

The genesis and maintenance of pain is fundamentally a neurobiological process, mediated by specialized cells and regions of the peripheral nervous system and central nervous system (CNS).[9] The first step in pain generation is nociception, in which specialized sensory afferents, called nociceptors, detect potentially damaging chemical, mechanical, or thermal stimuli. Nociceptors convert the signal into action potentials, transmitted to the dorsal root ganglia (DRG), which contain the cell bodies of all sensory neurons, including nociceptors. From the cell body, sensory neurons extend a central axon that relays the painful stimuli to the dorsal horn of the spinal cord and then to higher levels of the neuraxis where they are decoded as conscious aspects of pain.[9]

In recent years, a growing body of literature has identified multiple nodes of interaction between the immune system and the nervous system, and the bidirectional communication between these 2 systems may provide many potential opportunities for targeted, mechanism-based therapeutic interventions for autoimmune and inflammatory diseases (reviewed in Ref. 19). Neuroimmune interactions are also increasingly gaining attention in the field of chronic pain.[51] Like the immune system, nociceptors are key for defending the body against potential danger. Indeed, the International Association for the Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage."[116] In keeping with this warning function of the nociceptive system, pattern recognition receptors (PRRs), which recognize danger and pathogen signals that are released from injured or dying cells, are not only expressed by innate immune cells but also by sensory neurons.[56] Prolonged tissue damage results in multiple innate immune and nervous system interactions, at the crossroads of which are cytokines and chemokines that contribute to pain. There is a large tapestry of cells that can contribute to the secretion of inflammatory and nociceptive mediators in OA, including neuronal, immune, and joint tissue cells such as chondrocytes and various bone cell types. Macrophages in particular secrete many mediators that can aid in both the induction and resolution of inflammation and pain. In this narrative review, we aim to discuss what is currently known about the role of macrophages in OA pain, both in experimental models and in patients, based on a PubMed search for key terms, including but not limited to: "osteoarthritis,s "pain,a "macrophages,a "microglia,i "neuro-immunity,e "inflammation,n "animal models,n "chronic pain,h "neuro-immune interactions,e and "inflammatory pain." We will first discuss the neuroimmune interactome, and then, we will detail the various ways in which macrophages may contribute to pain in OA.

2. Neuroimmune interactions in maintenance and disruption of tissue homeostasis

Experiments dating back to 1874 by Goltz and Bayliss demonstrated that electrically stimulating dorsal roots induced skin vasodilation, which led these scientists to conclude that an inflammatory response could be produced that was independent of the immune system.[10,40] This led to the concept of "neurogenic inflammation," which is mediated by neuropeptides (bioactive peptides released from the peripheral terminals of sensory afferents) such as substance P and calcitonin gene-related peptide (CGRP).[18,29,86] The latter is a potent vasodilator that can act directly on endothelial and smooth muscle cells. In animal models of rheumatoid arthritis (RA), denervation of the joint was shown to drive attenuation of inflammation in a manner that was dependent on neuronal expression of substance P.[72,73] Clearly, neuroimmune communications play major roles in the pathophysiology of arthritis.

As the nervous system and immune system comprise complex biological entities, with distinct structures and functions, they have traditionally been studied separately. However, these 2 systems are highly integrated and function together to coordinate host defense.[18,142] Neuroimmunity exists as an evolutionary adaptation to detect and react to dangers in the environment.[18,142] This requires fine-tuned interactions between the 2 systems, to respond quickly and effectively. Examples of interactions and concerted actions between the nervous and the immune system are far reaching. These include observations that (1) both sensory neurons and immune cells can directly detect and respond to bacteria and danger signals through PRR expression; (2) immune cells can increase nociceptor sensitivity through the action of cytokines; (3) nociceptors can, in turn, alter immune functions; and (4) autonomic neurons can globally suppress inflammation.[4,17,142,155]

Nonsynaptic neuroimmune communication occurs through the innervation of lymphoid tissues, as well as when immune cells infiltrate the peripheral nervous system, including sensory and sympathetic ganglia. It has long been known that the sympathetic nervous system plays an important role in pain. In people and experimental animals with neuropathic pain, sympathetic neurons sprout in the DRG and form basket-like structures around the cell bodies of sensory neurons.[88,133] Sympathetic-sensory coupling has in general been shown to be excitatory and pronociceptive.[158] In addition, it is now also becoming clear that the sympathetic nervous system interacts with the immune system, and that the exchange between the immune, sensory, and sympathetic nervous systems can all modulate pain pathways (as reviewed in Ref. 139). Joints are densely innervated with sympathetic neurons,[14,62] and in models of inflammatory arthritis, it has been described that sympathetic neurons sprout in the joint and the overlying skin (Refs. 33 and 75). However, there are no reports yet of macrophage-sympathetic neuron interactions in models of arthritis, including OA, and this may be a fruitful direction for future research.

There are many types of immune cells that can play a role in neuroimmune crosstalk and function,[142] including dendritic cells, neutrophils, macrophages, mast cells, and T cells. It is clear that interactions between the nervous and immune systems are

extremely common, and several recent reviews have discussed the implications of specific interactions for tissue homeostasis, inflammation, infection, and allergy.[38,54,142] In the context of OA pain, however, they have hardly been studied and only a small number of reports, generally focused on macrophages, are just starting to percolate, but already suggest a rich substrate for potential therapeutic interventions. As discussed, pain is an important signaling mechanism to prompt behavior that avoids tissue damage or allows healing of injured tissues. Thus, pain is vital for the maintenance and restoration of tissue homeostasis. It may be considered the "first responder" in host defense because the rapidity of responses to painful stimuli is an order of magnitude higher than the speed at which the innate immune system responds.[18] The classical inflammatory response induces immune cells to eliminate or remove pathogens, and damaged cells and debris are cleared during the proinflammatory response, during which immune cells are recruited and release proinflammatory mediators. After the proinflammatory phase, local tissue repair and restoration of homeostasis occur during the resolution phase, in which the appropriate release of anti-inflammatory mediators is critical. In OA, there is a prolonged proinflammatory phase and lack of tissue repair and remodeling that is necessary for resolution of inflammation,[125] and this may be a contributing factor to persistent inflammatory pain. Whether resolution happens or not depends for a large part on the participation of macrophages.[152] In models of neuropathic and inflammatory pain, specialized proresolving mediators (SPMs) have been shown to be important for resolution of pain. Specialized proresolving mediators are a superfamily of endogenous chemical mediators that promote the resolution of inflammation (reviewed in Ref. 131). Among those SPMs, resolvins are oxygenated metabolites of omega-3-fatty acids, and some resolvins, including resolvins D1, D2, and D5, have shown analgesic actions in models of neuropathic, inflammatory, or postoperative pain (Refs. 78, 112, and 164). Another group of SPMs, maresins, which are produced by macrophages, have shown pain-relieving properties in a model of inflammatory arthritis.[30] Of interest, in patients with RA, it has been shown that synovial fluid (SF) levels of SPMs were negatively associated with pain.[35] Furthermore, a meta-analysis of trials in patients with inflammatory arthritis revealed that supplementation with omega-3 fatty acids for 3 to 4 months reduced patient-reported joint pain intensity and consumption of nonsteroidal anti-inflammatory drugs.[39] These interesting observations suggest that SPMs may represent a potential therapeutic approach to pain relief and this topic needs further study in OA pain. A recent study showed that exogenously administered, 17(R)-hydroxy-docosahexaenoic acid (17[R]-HDoHE), a D-series resolvin precursor, reversed established pain behavior in 2 rat models of OA, without affecting joint pathology, and this was associated with elevated plasma levels of resolvin D2.[52]

The accumulating evidence that macrophages contribute to the pathogenesis of pain in OA disease will be summarized below. First, we present a brief overview of macrophages and their homeostatic functions.

3. Role of macrophages in osteoarthritis pain

3.1. Homeostatic functions of macrophages

Macrophages are present in almost every tissue of the body, where they are critical for maintaining a state of homeostasis.[25] At steady state, macrophages perform different functions depending on their tissue of residence.[3,65] For example, brain-resident macrophages, known as microglia, prune synapses in development, and red pulp macrophages in the spleen recycle heme from erythrocytes.[61–64] Thus, different tissue-resident macrophage populations exhibit different phenotypes and patterns of gene expression.[32,66] More than 1 macrophage population may coexist in a single tissue, such as alveolar and interstitial macrophages in the lung, and are believed to fulfill different roles in tissue homeostasis.[63,161] Tissue-resident macrophages have 2 possible ontogenies: They may be derived from embryonic precursors or from adult hematopoiesis through monocytes.[36,41,44,50,127,132] Many macrophage populations are believed to be a combination of both, with monocytes differentiating into macrophages and replacing the original cells over time.[45,47,57,101,147,159] In these cases, the monocyte-derived macrophages eventually become phenotypically identical to the embryonic derived cells they replace.[121,128] This is possible through tissue-specific transcription factors that reprogram macrophages in response to environmental signals.[42,66] The same process is likely to play a role in development as tissue and cell-type factors work in combination to specify the macrophage fate.[43,85] Macrophages have also been shown to be altered towards an inflammatory phenotype as tissues age, which may be a natural part of aging.[27,109,137] Although much of our knowledge concerning macrophages is based on mouse models, there is evidence to suggest that macrophage ontogeny and function is conserved in humans.[7,11] Macrophages are considered plastic, because they can adapt to different tissues, and heterogeneous, because different populations may be found in a single tissue.

In addition to homeostatic functions, macrophages are well known for their role in inflammation. In many models of tissue inflammation, circulating monocytes infiltrate the tissue and differentiate into proinflammatory macrophages.[45,67,98,103] These monocyte-derived macrophages are distinct from those that exist at steady state since they are specified by stimulus-specific factors.[34,49,55,111] They often maintain their monocyte identity longer and are slow to demonstrate the tissue-resident phenotype.[99,130] Infiltrating macrophages have been shown to be a major driver of fibrosis and tissue damage resulting from inflammation.[22,141,157] For example, recruitment of monocyte-derived macrophages worsens outcome in mouse models of acute lung injury and post-traumatic brain injury.[21] By contrast, macrophages exhibiting a tissue-resident phenotype are often decreased in number during tissue inflammation and then expand during resolution.[67,165] It is unclear exactly what mechanisms control this process or whether the eventual expansion pattern is a result of proliferation or recruitment. It has been suggested that tissue-resident macrophages exhibit an anti-inflammatory role and promote recovery from inflammation resulting in opposing interactions between different macrophage populations in the tissue. This is in line with in vitro studies suggesting a model for macrophage polarization from inflammatory (M1) to alternative activation (M2).[5,68,96] However, since in vitro macrophages fail to recapitulate the complexity of in vivo macrophages, this model has fallen out of favor.[83,105] Instead, current studies tend to focus on characterizing macrophages in an unbiased manner to account for a spectrum of activation along multiple dimensions.

Recent insight into the heterogeneity of macrophages has greatly expanded our understanding of their role in RA, a common autoimmune disease, where destruction of synovial joints is characterized by chronic synovitis[87] that includes influx of inflammatory macrophages.[82,146] In particular, macrophages are found in the synovial lining as well as the sublining layer.[58,146] In the murine K/BxN serum transfer model of RA, it has been suggested that there are at least 2 subpopulations of synovial

macrophages in the joint: MHCII$^+$ monocyte-derived and MHCII$^-$ tissue-resident populations.[98] It is possible that MHCII+ cells are relatively recently recruited cells that have yet to fully differentiate into tissue-resident cells. A recent article showed that CX3CR1$^+$ cells are the macrophages that form the synovial lining in the joints of mice.[23] These cells are critical to joint homeostasis, and their loss leads to the development of inflammatory arthritis.[23] Although Misharin and et al. did not distinguish macrophages by CX3CR1, the study by Culemann et al.[23] suggests that CX3CR1$^+$ synovial lining macrophages are more likely to be MHCII$^-$. Therefore, the MHCII$^+$ macrophages from Misharin et al. may align with a subpopulation of interstitial macrophages described by Culemann et al.

By contrast, a decrease in the number of sublining macrophages is a biomarker of response to treatment.[12,151] Sublining macrophages can be further subdivided into additional subpopulations. The Accelerating Medicine Partnership performed single-cell RNA-seq on synovial tissue from patients with OA and RA.[162] They identified 4 clusters of myeloid cells that differed in their composition across patient groups. In particular, the second cluster, NUPR1+ cells, was found in greater proportions in patients with OA and its gene expression profile suggests it may correspond to the human equivalent of the CX3CR1$^+$ synovial lining macrophages observed in mice. Another study described RNA-seq data from macrophages isolated from synovial biopsy tissue obtained from RA patients.[82] Certain gene expression modules were found to be associated with clinical characteristics such as tender joint count and medications status. This study suggested that the transcriptional profile of macrophages may be used as a biomarker of disease state.

Finally, a recent single cell transcriptomics study profiled synovial tissue macrophages from RA patients and reported distinct subpopulations associated with distinct clinical states.[2] Importantly, this study identified 2 subpopulations with unique molecular signatures that were enriched in negative regulators of inflammation, and a low proportion of one of those subpopulations was associated with increased risk of diseases flare of cessation of treatment.

Thus, increasingly, transcriptomic profiling of synovial macrophages in RA is revealing phenotypes that are associated with specific disease states and characteristics. Comprehensive studies such as those in RA have yet to be performed in OA. A recent report compared synovial macrophages from patients with OA to those from patients with inflammatory arthritis.[154] Within the OA samples, the authors found 2 distinct subpopulations, which they named classical OA macrophages that had cartilage remodeling features, and inflammatory-like OA (iOA) macrophages, which were similar to macrophages from patients with inflammatory OA, and had more proliferative capacity. Detailed phenotyping studies such as these should greatly contribute to our understanding of OA pathogenesis, and how macrophages may contribute to pain phenotypes in this disease.

3.2. Macrophages modulate pain pathways through bidirectional interactions with nociceptors

Recent years have seen the publication of many studies that explore the bidirectional communication between nociceptors and macrophages (for a recent review on this topic, see Ref. 16). In brief, several communication mechanisms have been discovered both in vitro and in a variety of in vivo models. First, macrophages can release an array of mediators that bind receptors expressed by nociceptors, activating signaling pathways that lead to increased excitability of neurons and hypersensitivity to pain stimuli (sensitization). These mediators include cytokines such as IL-1 and TNFα, as well as the neurotrophin, nerve growth factor, which acts on nociceptors through TrkA and increases their excitability.[28] Furthermore, reactive oxygen species can activate transient receptor potential ankyrin 1 expressed by nociceptors.[26] Second, nociceptors in turn produce a range of molecules that can affect macrophages. In particular, macrophages express receptors for neuropeptides that are released by activated nociceptors, and the result is a modification of their phenotype. For example, binding of substance P to its receptor, neurokinin-1, expressed on macrophages leads to an NFκB-driven production of IL-1 and TNFα.[140] By contrast, neuronally derived CGRP seems to exert anti-inflammatory actions. Calcitonin gene-related peptide modulates TLR4-stimulated macrophages by enhancing regulatory cytokines such as IL-10 and inhibits macrophage proinflammatory cytokine production in vitro in a cAMP-response element binding protein-dependent manner.[8] This suggests neuroimmune communication through CGRP that promotes a regulatory macrophage phenotype even in a proinflammatory microenvironment,[8] and this may have implications in chronic pain pathways.

In the context of tissue injury, nociceptors can also produce and respond to a large range of proinflammatory chemokines—in particular, alarmins such as S100A8/9 that are generated during tissue injury bind PRRs expressed by nociceptors and cause them to produce chemokine (C-C motif) ligand 2 (CCL2).[91] Chemokine (C-C motif) ligand 2 is a powerful chemotactic signal for monocytes/macrophages, and, as we will discuss below, this specific communication loop may be an important proalgesic mechanism in the context of OA. Third, microRNAs have recently been implicated in pain, with a potential role for microRNA-containing exosomes to communicate between nociceptors and macrophages; eg, a recent study described that nociceptors can secrete exosomes containing miR-21 in response to TRPV1 activation, and phagocytosis of miR-21-exosomes by macrophages affected their phenotype. Interestingly, miR-21 deletion in sensory neurons inhibited macrophage infiltration and neuropathic pain.[134] Finally, as we already mentioned, macrophages are critical for the resolution of inflammation and pain. IL-1β– and carrageenan-induced hyperalgesia are significantly prolonged in LysM-GRK2$^{+/-}$ mice, which have reduced levels of the G-protein-coupled receptor kinase 2 (GRK2) in LysM$^+$ myeloid cells.[153] Furthermore, adoptive transfer of wildtype, but not of GRK2$^{+/-}$ bone marrow–derived monocytes normalizes the resolution of IL-1β–induced hyperalgesia in LysM-GRK2$^{+/-}$ mice.[152] The authors also showed that GRK2$^{+/-}$ macrophages had diminished IL-10 production in vitro. Together, these results suggest an important role for IL-10 signaling in macrophage control of transient inflammatory pain.

3.3. Macrophages: contribution to osteoarthritis pain

In the context of OA, very few studies have specifically explored direct macrophage–nociceptor interactions. Studies dealing with macrophages have largely focused on macrophages in the affected joints, particularly the synovium. In addition, however, macrophages are present in many organs, including in nerves and in DRG. Furthermore, microglia, the resident macrophages of the CNS, have received much attention for their modulating role in chronic pain.[114] Below, we summarize studies that have addressed the role of macrophages in OA pain in synovium, DRG, and dorsal horn, respectively (Tables 1 and 2).

3.3.1. Synovial macrophages

3.3.1.1. Human studies

It is becoming increasingly accepted that synovial macrophages play a role in onset and progression of OA joint pathology, as has been discussed in several excellent recent reviews (**Table 1**).[77,156,163] Much less is known about the contribution of synovial macrophages to OA pain,[95] but several recent studies in human subjects have suggested a correlation between synovial macrophages and pain. In 1 study, activated macrophages detected in vivo by etarfolatide, an agent that binds to folate-receptor-β, were found in 76% of symptomatic OA knees (n = 50 knees).[64] The presence of joint pain at fingers, wrists, ankles, and great toes was also linked to the presence of activated macrophages at these joints. In another study, several SF biomarkers that were associated with radiographic progression and symptom severity were highly correlated with SF levels of the macrophage markers, CD163 and CD14.[46] Levels of soluble CD14 in SF were positively correlated with severity of joint space narrowing and osteophyte formation in 2 cohorts with radiographic knee OA.[24] In addition, SF and plasma CD14 levels were positively associated with self-reported knee pain severity.[24] Stoppiello et al.[138] evaluated the synovium and medial tibial plateaux from subjects with OA harvested at time of total knee replacement surgery (advanced OA group) and compared them with postmortem donors who had not sought medical attention for knee pain during the last year of life, but who had the same extent of chondropathy (asymptomatic chondropathy group) (n = 29 per group). They reported that subjects in the advanced knee OA group displayed increased synovitis and synovial NGF levels compared with the nonsymptomatic group. Interestingly, synovial NGF expression was localized mainly to fibroblasts, but also to some macrophages. Altogether, these results suggest that soluble macrophage biomarkers and macrophage expression profiles exist that may predict OA disease severity and pain and suggest that macrophages and associated pathways may provide a source of targets for OA pain.

3.3.1.2. Studies in experimental models

Very few studies in experimental models of OA have explored exactly how synovial macrophages might contribute to pain

Table 1

Clinical studies examining association between macrophages and osteoarthritis pain severity.

Patient population	Methods	Results	Reference
Patients with OA undergoing TKR surgery (advanced OA group) compared with postmortem subjects who had not sought medical help for knee pain in the last year of life (non-OA group) (n = 26 per group); symptomatic chondropathy vs asymptomatic chondropathy (n = 29 per group).	Medial tibial plateaus and synovium collected for histology; CD68 and NGF were histologically evaluated and quantified	Advanced OA subjects showed more severe synovitis, increased synovial NGF and CD68+ macrophages, and cartilage loss; NGF was localized to fibroblasts and some macrophages; symptomatic chondropathy group had increased synovitis and synovial NGF compared with the asymptomatic chondropathy group	Stoppiello et al. (2014). Arthritis Rheumatol.
Radiographic knee OA patients (n = 25 cohort 1; n = 159 cohort 2)	SPECT-CT imaging (n = 25); n = 159: soluble CD14 and CD163 levels in synovial fluid and blood evaluated; patient-reported outcomes for knee pain (NHANES-I)	Synovial fluid CD14, CD163, and serum CD163 were associated with abundance of activated MQs in knee joint capsule and synovium; synovial fluid CD14 levels correlated positively with self-reported knee pain severity and joint space narrowing	Daghestani et al. (2015). Arthritis Rheumatol.
Symptomatic knee OA patients (n = 161); healthy controls (n = 138)	Serum samples collected from OA or healthy controls; synovial fluid samples from patients with OA; patient-reported outcomes (WOMAC) in patients with OA	CCL2 (MCP1) concentrations in synovial fluid (not serum) of patients with OA were independently and positively associated with self-reported greater pain and physical disability by WOMAC assessment; patients with OA had higher serum levels of CCL2 compared with healthy controls	Li et al. (2015). Ann Clin Biochem.
Symptomatic knee OA patients (n = 25)	SPECT-CT imaging after etarfolatide (detects activated macrophages)	Activated macrophages found in 76% of OA knees; quantity of activated macrophages associated with OA pain severity (R = 0.60, P < 0.0001) and radiographic OA severity (R = 0.66, P < 0.001); Haraden et al. showed that several synovial fluid inflammatory biomarkers were associated with OA pain and radiographic severity as well as synovial fluid levels of macrophage markers CD163 and CD14.	Kraus et al. (2016). Osteoarthritis Cartilage. Haraden et al. (2019). Arthritis Res Ther.
Symptomatic knee OA patients (n = 86)	Synovial fluid levels of soluble receptors and chemokines measured by immunoassays; patient-reported outcomes (KOOS and WOMAC)	Synovial fluid macrophages were most abundant of all leukocytes; CD14+ CD16+ macrophages correlated with synovial fluid CCL2 levels but not sCD163 or sCD14; the percentage of CD14+ CD16− and the percentage of CD14+ CD16+ macrophages were correlated with overall KOOS and WOMAC scores; CD14lowCD16+ SF MQs were not associated with pain	Gomez-Aristizabal et al. (2019). Arthritis Res Ther.

CCL2, chemokine (C-C motif) ligand 2; OA, osteoarthritis; NGF, nerve growth factor; TKR, total knee replacement.

(**Table 2**). Nonetheless, macrophage infiltration into the synovium has been documented in several experimental models of OA, albeit to different degrees.[53,95,123,148] In a widely used surgical model of OA in mice, induced by destabilization of the medial meniscus (DMM),[37] whole joint levels of *Cd68* mRNA, a macrophage marker, were significantly suppressed in *Ccl2*$^{-/-}$ and *Ccr2*$^{-/-}$ joints compared with wildtype mice, 6 hours after surgery. These knock-out mice subsequently developed less pain than wildtype mice, despite developing comparable joint damage, indirectly suggesting a role for CD68 macrophages in pain development.[97]

An interesting recent study examined involvement of synovial macrophages in the rat monoiodoacetate-induced (MIA) model of advanced knee OA.[122] Monoiodoacetate-induced rats exhibited severe cartilage damage and synovitis, with an influx of synovial macrophages identified by Iba1+ staining. In this model, cyclooxygenase (Cox) inhibitors such as celecoxib and naproxen and the steroid, dexamethasone, were ineffective, but an opioid and an anti-NGF antibody were effective in reversing pain as assessed by grip strength and weight-bearing deficits. Intravenous injection of clodronate-laden liposomes (4 weeks after 5-mg MIA) depleted synovial macrophages (decreased to 7.3% CD11b+ CD45+ macrophages in the synovium compared with 15% in the vehicle group), which decreased the levels of IL1β and NGF in the synovium and the fat pad of the knee, leading to pain suppression in advanced OA. The authors concluded that synovial macrophages may be involved in advanced knee OA pain which was resistant to COX inhibitors, and therefore, drugs targeting synovial macrophages might have significant analgesic effects.[122] It should, however, be noted that the authors did not assess whether macrophage depletion affected the severity of joint damage.

Studies by Lee et al. demonstrated that mice deficient in *Irf4*, *Ccl17*, and *Ccr4*, but not *TNF* genes showed decreased cartilage destruction, osteophyte size, and pain (measured as weight distribution in the inflamed limb relative to noninflamed limb) in the collagenase-induced OA (CiOA) model.[70] Neutralization of CCL17 and Jmjd3 attenuated both joint damage and pain in this model. After sorting out synovial cells into neutrophils, macrophages, fibroblasts, endothelial cells, and other cell types and evaluating gene expression, *Ccl17* mRNA expression was found only in macrophages in wildtype mice, but was absent in macrophages from *Irf4*$^{-/-}$ and *GM-CSF*$^{-/-}$ mice. Thus, CCL17 promotion of joint damage and pain is likely controlled by GM-CSF and IRF4 signaling, and CCL17 may provide a target for OA.

3.3.2. Macrophages in the dorsal root ganglia

In many models of disease, including OA, neuroimmune crosstalk in the DRG has been shown to contribute to maintenance of pain (for reviews, see Refs. 16, 117, and 126). This has been best described in models of neuropathic pain. Interestingly, a recent study using a nerve injury model found that DRG macrophages play a primary role in both the initiation and maintenance of mechanical hypersensitivity as opposed to macrophages at the nerve injury site.[160] In addition, a role for macrophage infiltration into the DRG has been described in models of inflammatory arthritis as well as OA. For example, in the rat antigen-induced arthritis (AIA) model, a large increase in ED1+ macrophages was observed bilaterally in knee-innervating lumbar level DRG but not in the thoracic DRG 3 days after model induction.[129] Blocking TNFα with etanercept reduced the DRG infiltration. A follow-up study, again using the rat AIA model at the 3-day time point, characterized these macrophages as displaying a proinflammatory (LPS/IFNγ-TNFα-IL1) phenotype.[84] In addition, bone marrow derived macrophages were stimulated in vitro by various factors to determine which activator induced a similar expression pattern as seen in vivo. The authors found that TNFα-stimulated macrophages showed the most marked similarity to the AIA DRG, correlating with their previous findings demonstrating that etanercept could reduce macrophage infiltration.[84]

Macrophage infiltration of the DRG has also been reported in experimental OA induced by DMM, starting around 8 weeks after surgery.[93,94] This coincided with increased gene expression of *Ccl2* and *Ccr2* mRNA in the DRG, and onset of movement-provoked pain behaviors.[93] *Ccr2* null mice, while developing early-onset mechanical allodynia, did not show sustained allodynia past week 8 after surgery, and did not develop movement-associated pain behaviors 8 to 16 weeks after DMM, in the presence of joint damage that was comparable with wild type mice.[93,97] These *Ccr2* null mice did not display macrophage infiltration in the DRG,[93] suggesting a role for DRG macrophages in the persistence of pain in this model. This is compatible with the finding that pharmacological inhibition of CCR2 in early stages of the model was able to reverse weight-bearing deficits in late-stage disease.[76] More recently, pathway analysis of a microarray study of knee-innervating DRG after DMM surgery suggested that distinct molecular pathways drive early vs persistent pain and also suggested a role for immune cell recruitment.[94] Immune pathways became activated in early OA pain, and continued to develop during later phases of the model, when persistent pain behaviors developed. Several genes (*Ccl2*, *Cx3cr1*, *Ngf*, and *Tlr1*) were identified as being associated with neuroinflammation, and these genes have been previously linked to OA pain.[20, 28, 76, 92, 93, 97]

3.3.3. Microglia in osteoarthritis pain

As mentioned above, microglia are the resident macrophages of the CNS, and they have received much attention for their modulating role in chronic pain.[114] Indeed, targeting glial activation through minocycline or propentofylline has reduced experimental neuropathic pain.[69] In recent years, several studies have described microglia activation in experimental models of OA. In the MIA model in mice or rats, microglial activation in the dorsal horn develops as early as day 7, accompanying development of mechanical allodynia,[71,104,108,120,136,144] while the upregulation of glial fibrillary acidic protein+ astrocytes has been reported in some studies later in the model by day 28.[119,120] Specific ablation of spinal microglia through intrathecal injections of the immunotoxin, saporin, conjugated to the Mac1 antibody (Mac1-saporin), attenuated mechanical allodynia by days 5 and 7 after MIA.[104] The potent microglia inhibitor, minocycline, selectively inhibits proinflammatory macrophage activation state in vitro,[60] and oral minocycline treatment beginning on day 14 of the MIA model attenuated mechanical allodynia and microglial activation by day 28.[120]

The MIA model is a rapidly progressive model of OA,[80] and microglia activation occurs early in disease. It is important to note that in more slowly progressive models, microglia activation only appear in later stages of the disease. For example, in the CiOA model, increases in microglial numbers in the dorsal horn become apparent by 6 weeks after model induction, and inhibition of glial activation by intrathecal injection of fluorocitrate at that time acutely reversed pain behaviors (knee bend test and gait deficits).[1]

The surgical DMM model is an exceptionally slowly progressive model of OA joint damage, and here, we reported microglial activation beginning 8 weeks after surgery, associated with onset of late-stage pain behaviors.[145] The effects of specifically targeting spinal microglia has not yet been tested in a surgical model of OA, but drugs known to target glial activation may warrant further investigation for OA pain.[6] These studies also further highlight the temporal regulation of neuroimmune changes in the neuraxis after

Table 2

Preclinical osteoarthritis models examining relationship between macrophages and pain.

OA model	Time point(s)	Results	Reference
Rat MIA	2 or 4 wk after MIA	Pain behaviors in 5-mg MIA rats were resistant to COX inhibitors and a steroid, but sensitive to anti-NGF and morphine; MIA induced macrophage infiltration in the synovium associated with high IL1b, NGF, NOS2, and COX2 expression in knee joint; clodronate depletion reduced macrophage numbers and pain behavior (grip strength and weight-bearing)	Sakurai et al., (2019). PAIN.
Mouse MIA	7 d after MIA	TrkA knock-in mouse joints had significant leukocyte infiltration and mast cells; Prostaglandin D2 synthase inhibitor prevented MIA-mechanical hypersensitivity in TrkA KI mice at doses ineffective in WT mice; microglial activation was observed 8 d after MIA	Sousa-Valente et al., (2018). Osteoarthritis Cartilage.
Rat MIA	3 wk after MIA	Increased ipsilateral expression of Cd11b+ microglia, but not astrocytes, in MIA rats	Lee et al., (2011). Mol Pain.
Mouse MIA	7 and 28 d after MIA	Increased ipsilateral microglial activation (Iba1+) by day 7 and significantly by day 28. GFAP expression in the dorsal horn was not changed.	Ogbonna et al., (2013). Eur J Pain.
Rat MIA	7, 14, 21, and 28 d after MIA	Microglia in the ipsilateral spinal cord were activated by day 7 and continued through day 28. Bilateral spinal GFAP (astrocytes) increases were seen at day 28, but not at earlier time points. Inhibition of glial activation by either nimesulide or minocycline attenuated pain behavior, activation of microglia in the ipsilateral spinal cord, and numbers of activated microglia and GFAP immunofluorescence.	Sagar et al., (2011). Mol Pain.
MIA	7 d after MIA	2-mg MIA, but not 1-mg MIA, induced microglial activation in both the ipsilateral dorsal and ventral horn by day 7	Thakur et al., (2012). PLoS One.
Rat MIA	7 d after MIA	Increased microglia in the ipsilateral and contralateral dorsal horn by day 7; specific ablation of spinal microglia through intrathecal injections of the immunotoxin, saporin, conjugated to the Mac1 antibody (Mac1-saporin), attenuated mechanical allodynia by days 5 and 7 after MIA	Mousseau 2018 Science Advances
Mouse DMM	4, 8, and 16 wk after DMM	CCR2 null mice develop mechanical allodynia at 4 wk, but resolved by 16 wk; CCR2 null mice lacked movement-provoked pain at 8 wks; macrophages infiltrate DRG at 8 wk maintained up to 16 wk after DMM; CCR2 null mice have no macrophage infiltration in DRG	Miller et al., (2012). PNAS.
Mouse DMM	4, 8, and 16 wk after DMM	Increased activated CX3CR1+ and Iba1+ microglia at 8 and 16 wk in WT mice, but not 4 wk; DRG cultures have increased CX3CL1 levels at 8 and 16 wk; Adamts5 null mice do not develop mechanical allodynia up to 16 wk after DMM and do not have increased CX3CL1 levels.	Tran et al., (2017). Osteoarthritis Cartilage.
Mouse DMM	16 wk after DMM	S100A8 and a2-macroglobulin treatment in DRG cultures stimulated MCP-1 release; TLR4 inhibition reversed this effect; TLR4 null mice were not protected from mechanical allodynia or joint damage in DMM	Miller et al., (2015). Arthritis Rheumatol.
Mouse CiOA	Day 20–42 after CiOA	Irf4, CCl17, and CCR4, but not TNF knock-out mice showed decreased cartilage destruction, osteophyte size, and weight-bearing pain behavior; CCL17 and Jmjd3 neutralization attenuated both joint destruction and pain; Ccl17 mRNA expression was only found in macrophages and was controlled by GMCSF and IRF4 signaling	Lee et al., (2018). Arthritis Res Ther.
Rat CiOA	6 wk after CiOA	GFAP expression on satellite glial cells in the DRG and on astrocytes in the dorsal horn was significantly increased after CIOA; Iba1+ microglia were also upregulated in the dorsal horn after CIOA; inhibition of glial activation by fluorocitrate improved pain behaviors (knee-bend test and gait deficits) in CIOA mice	Adaes et al., (2017). Molecular Pain.

CiOA, collagenase-induced OA; DMM, destabilization of the medial meniscus; GFAP, glial fibrillary acidic protein; MIA, monoiodoacetate-induced; OA, osteoarthritis, WT, wild type.

the induction of joint damage, where temporally defined changes progress from the periphery (DRG) to the spinal cord. This implies that different interventions may be effective at different time points.

Although most studies report higher upregulation in the ipsilateral dorsal horn, some studies have reported that the contralateral dorsal horn still has higher levels of microglia compared with controls.[104,120,145] More work is required to understand the significance of contralateral dorsal horn changes, but future studies should report both ipsilateral and contralateral data.

4. Future directions

4.1. Sexual dimorphism in osteoarthritis-related pain: a role for macrophages?

There are significant differences in underlying mechanisms driving immune responses between the sexes, and this likely contributes to the observed sex differences in pain.[59,118] Studies that address the role of microglial activation in chronic pain have largely been conducted in male animals.[100] Interestingly, experimental nerve injury results in mechanical allodynia and microglial activation in mice of both sexes, but inhibitors of microglial activation block allodynia only in males. By contrast, female mice were found to rely on T cells in the spinal cord to establish pain, and this was hormonally dependent since female mice still used microglia to establish pain when testosterone levels were elevated.[135] In the DRG, macrophage expansion occurs in both male and female mice after nerve injury, but is more pronounced in male mice. Depletion of macrophages from the DRG is, however, effectively reduces mechanical hypersensitivity in both sexes.[160]

In the context of OA, it is well known that there is a sexual dimorphism in susceptibility to joint damage in different experimental models, and more recently differences in pain-related behaviors between male and female mice have been described.[143,150]

However, there are no studies yet that explore the role of macrophages and their crosstalk with nociceptors in this observed sexual dimorphism in OA-associated pain. It is noteworthy that although there was no significant difference in macrophage infiltration in the knee joints between male and female mice 3 days after CiOA, there was a lower fold change (day 0–day 3) of macrophage expansion in females compared with males (5- vs 8-fold).[102] Altogether, these studies highlight it will be important that future studies on neuroimmune interactions and OA pain will be conducted in models that allow for evaluation of both sexes.[81]

4.2. Conclusions

Accumulating evidence from human studies and experimental models in animals increasingly suggests that macrophages contribute to the generation and maintenance of pain in OA. They can do this through secretion of soluble factors that modify nociceptor activity, and, in turn, they can respond to nociceptor-derived signals. Pain signaling and the immune system both strive to maintain the homeostasis of organisms. It is therefore not surprising that these 2 systems are constantly interacting with one another and current techniques, such as single-cell sqRNA, have demonstrated this type of temporal plasticity. Indeed, in the words of the philosopher Heraclitus living in Greece in the fifth century BCE—"You cannot step into the same river twice"—interacting nociceptors and macrophages continuously alter one another's transcriptional and functional identities in response to external influences. There really is no such thing as a single nociceptor or macrophage phenotype. Bidirectional communication between the 2 systems can occur at different levels of the pain neuraxis: in the affected joint (mainly in the synovium) where free nerve endings of nociceptors and tissue macrophages interact, in the DRG which can become infiltrated by macrophages in response to peripheral inflammation, and in the dorsal horn, where microglia can modify synapses between nociceptors and second-order neurons. Hence, in the context of a chronic progressive disease such as OA, the interactions between these cells at these different levels may provide an ever-changing network of signals that are important in perpetuating pain. It is currently unknown which subtypes of macrophages may be optimal targets for intervention and this question warrants further study. Several transcriptomic studies are beginning to elucidate the different subpopulations of macrophages that exist in association with distinct clinical states, but little work has been performed in targeting these populations. One could speculate that increasing proresolving, proremodeling macrophage numbers and/or activity, or—inversely—hindering proinflammatory macrophages may constitute formidable approaches for modifying pain.

In summary, the neuroimmune interactome clearly merits detailed investigation, and it can be anticipated that a thorough exploration of the communication between sensory neurons, immune cells, and resident tissue cells (**Fig. 1**) will uncover new strategies to alleviate and/or prevent chronic OA pain.

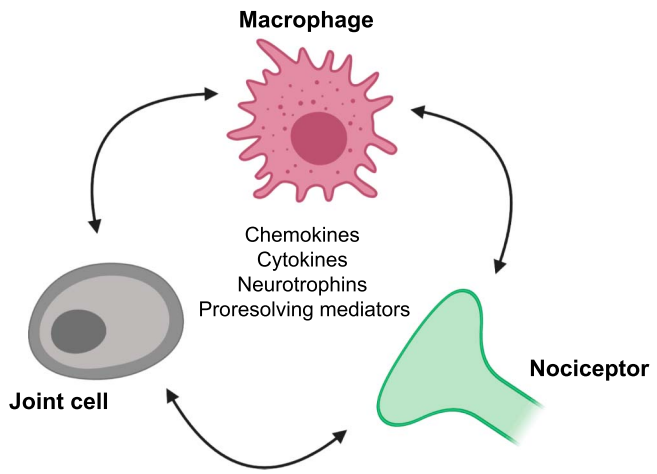

Figure 1. In the context of a chronic progressive disease such as osteoarthritis, the interactions between macrophages, tissue resident cells, and nociceptors at the level of the joint, DRG, and spinal cord may provide an ever-changing network of signals that are important in perpetuating pain. These signaling molecules include chemokines, cytokines, neurotrophins, and proresolving mediators, among others.

Disclosures

A.-M. Malfait has received consulting fees from Eli Lilly, Pfizer, Vizuri, and Ceva. The remaining authors have no conflicts of interest to declare.

Acknowledgements

The authors are grateful for the support of the National Institutes of Health (National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS]) (grant numbers K01AR070328 to R.E. Miller, R01AR060364 and R61AR073576 to A.-M. Malfait, R01AR064251 to A.-M. Malfait and R.J. Miller, T32AR073157 to T. Geraghty). A.-M. Malfait is supported by the George W. Stuppy, MD, Chair of Arthritis at Rush University.

Article history:
Received 8 September 2020
Received in revised form 1 December 2020
Accepted 6 December 2020
Available online 9 March 2021

References

[1] Adaes S, Almeida L, Potes CS, Ferreira AR, Castro-Lopes JM, Ferreira-Gomes J, Neto FL. Glial activation in the collagenase model of nociception associated with osteoarthritis. Mol Pain 2017;13:1744806916688219.

[2] Alivernini S, MacDonald L, Elmesmari A, Finlay S, Tolusso B, Gigante MR, Petricca L, Di Mario C, Bui L, Perniola S, Attar M, Gessi M, Fedele AL, Chilaka S, Somma D, Sansom SN, Filer A, McSharry C, Millar NL, Kirschner K, Nerviani A, Lewis MJ, Pitzalis C, Clark AR, Ferraccioli G, Udalova I, Buckley CD, Gremese E, McInnes IB, Otto TD, Kurowska-Stolarska M. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020;26:1295–306.

[3] Amit I, Winter DR, Jung S. The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. Nat Immunol 2016;17:18–25.

[4] Andersson U, Tracey KJ. Reflex principles of immunological homeostasis. Annu Rev Immunol 2012;30:313–35.

[5] Antonio S, Alberto M. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 2012;122:787–95.

[6] Appleton CT. What's pain (sensitization) got to do with it? Microgliosis may be a treatment target in osteoarthritis-related pain sensitization. Osteoarthritis Cartilage 2017;25:613–15.

[7] Bajpai G, Schneider C, Wong N, Bredemeyer A, Hulsmans M, Nahrendorf M, Epelman S, Kreisel D, Liu Y, Itoh A, Shankar TS, Selzman CH, Drakos SG, Lavine KJ. The human heart contains distinct macrophage subsets with divergent origins and functions. Nat Med 2018;24:1234–45.

[8] Baliu-Pique M, Jusek G, Holzmann B. Neuroimmunological communication via CGRP promotes the development of a regulatory phenotype in TLR4-stimulated macrophages. Eur J Immunol 2014;44: 3708–16.

[9] Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell 2009;139:267–84.

[10] Bayliss WM. On the origin from the spinal cord of the vaso-dilator fibres of the hind-limb, and on the nature of these fibres. J Physiol 1901;26: 173–209.

[11] Bharat A, Bhorade SM, Morales-Nebreda L, McQuattie-Pimentel AC, Soberanes S, Ridge K, DeCamp MM, Mestan KK, Perlman H, Budinger GR, Misharin AV. Flow cytometry reveals similarities between lung macrophages in humans and mice. Am J Respir Cell Mol Biol 2016;54: 147–9.

[12] Bresnihan B, Gerlag DM, Rooney T, Smeets TJ, Wijbrandts CA, Boyle D, Fitzgerald O, Kirkham BW, McInnes IB, Smith M, Ulfgren AK, Veale DJ, Tak PP. Synovial macrophages as a biomarker of response to therapeutic intervention in rheumatoid arthritis: standardization and consistency across centers. J Rheumatol 2007;34:620–2.

[13] Carlesso LC, Neogi T. Identifying pain susceptibility phenotypes in knee osteoarthritis. Clin Exp Rheumatol 2019;37(suppl 120)(5):96–9.

[14] Catre MG, Salo PT. Quantitative analysis of the sympathetic innervation of the rat knee joint. J Anat 1999;194(Pt 2):233–9.

[15] Chavan SS, Pavlov VA, Tracey KJ. Mechanisms and therapeutic relevance of neuro-immune communication. Immunity 2017;46: 927–42.

[16] 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.

[17] 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; 501:52–7.

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

[19] Chu C, Artis D, Chiu IM. Neuro-immune interactions in the tissues. Immunity 2020;52:464–74.

[20] Clark AK, Staniland AA, Malcangio M. Fractalkine/CX3CR1 signalling in chronic pain and inflammation. Curr Pharm Biotechnol 2011;12: 1707–14.

[21] Coates BM, Staricha KL, Koch CM, Cheng Y, Shumaker DK, Budinger GRS, Perlman H, Misharin AV, Ridge KM. Inflammatory monocytes drive influenza A virus–mediated lung injury in juvenile mice. J Immunol 2018; 200:2391–404.

[22] Cohen M, Matcovitch O, David E, Barnett-Itzhaki Z, Keren-Shaul H, Blecher-Gonen R, Jaitin DA, Sica A, Amit I, Schwartz M. Chronic exposure to TGFβ1 regulates myeloid cell inflammatory response in an IRF7-dependent manner. EMBO J 2014;33:2906–21.

[23] Culemann S, Grüneboom A, Nicolás-Ávila JÁ, Weidner D, Lämmle KF, Rothe T, Quintana JA, Kirchner P, Krljanac B, Eberhardt M, Ferrazzi F, Kretzschmar E, Schicht M, Fischer K, Gelse K, Faas M, Pfeifle R, Ackermann JA, Pachowsky M, Renner N, Simon D, Haseloff RF, Ekici AB, Bäuerle T, Blasig IE, Vera J, Voehringer D, Kleyer A, Paulsen F, Schett G, Hidalgo A, Krönke G. Locally renewing resident synovial macrophages provide a protective barrier for the joint. Nature 2019;572: 670–5.

[24] Daghestani HN, Pieper CF, Kraus VB. Soluble macrophage biomarkers indicate inflammatory phenotypes in patients with knee osteoarthritis. Arthritis Rheumatol 2015;67:956–65.

[25] Davies L, Jenkins S, Allen J, Taylor P. Tissue-resident macrophages. Nat Immunol 2013;14:986–95.

[26] De Logu F, Nassini R, Materazzi S, Carvalho Goncalves M, Nosi D, Rossi Degl'Innocenti D, Marone IM, Ferreira J, Li Puma S, Benemei S, Trevisan G, Souza Monteiro de Araujo D, Patacchini R, Bunnett NW, Geppetti P. Schwann cell TRPA1 mediates neuroinflammation that sustains macrophage-dependent neuropathic pain in mice. Nat Commun 2017;8:1887.

[27] Deczkowska A, Matcovitch-Natan O, Tsitsou-Kampeli A, Ben-Hamo S, Dvir-Szternfeld R, Spinrad A, Singer O, David E, Winter DR, Smith LK, Kertser A, Baruch K, Rosenzweig N, Terem A, Prinz M, Villeda S, Citri A, Amit I, Schwartz M. Mef2C restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner. Nat Commun 2017;8:717.

[28] Denk F, Bennett DL, McMahon SB. Nerve growth factor and pain mechanisms. Annu Rev Neurosci 2017;40:307–25.

[29] Edvinsson L, Ekman R, Jansen I, McCulloch J, Uddman R. Calcitonin gene-related peptide and cerebral blood vessels: distribution and vasomotor effects. J Cereb Blood Flow Metab 1987;7:720–8.

[30] Fattori V, Pinho-Ribeiro FA, Staurengo-Ferrari L, Borghi SM, Rossaneis AC, Casagrande R, Verri WA. The specialised pro-resolving lipid mediator maresin 1 reduces inflammatory pain with a long-lasting analgesic effect. Br J Pharmacol 2019;176:1728–44.

[31] Felson DT. Osteoarthritis as a disease of mechanics. Osteoarthritis Cartilage 2013;21:10–15.

[32] Gautier E, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, Helft J, Chow A, Elpek K, Gordonov S, Mazloom A, Ma'ayan A, Chua WJ, Hansen T, Turley S, Merad M, Randolph G; Immunological Genome C. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol 2012;13:1118–28.

[33] Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Coughlin KA, Kaczmarska MJ, Castaneda-Corral G, Bloom AP, Kuskowski MA, Mantyh PW. Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint. Arthritis Rheum 2012;64: 2223–32.

[34] Ghisletti S, Barozzi I, Mietton F, Polletti S, De Santa F, Venturini E, Gregory L, Lonie L, Chew A, Wei C-L, Ragoussis J, Natoli G. Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. Immunity 2010;32:317–28.

[35] Giera M, Ioan-Facsinay A, Toes R, Gao F, Dalli J, Deelder AM, Serhan CN, Mayboroda OA. Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC-MS/MS. Biochim Biophys Acta 2012;1821:1415–24.

[36] Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 2010;330:841–5.

[37] Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage 2007;15:1061–9.

[38] Godinho-Silva C, Cardoso F, Veiga-Fernandes H. Neuro-immune cell units: a new paradigm in physiology. Annu Rev Immunol 2019;37: 19–46.

[39] Goldberg RJ, Katz J. A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. PAIN 2007;129:210–23.

[40] Goltz F. Uber gefasserweiternde nerven. Pflueger Arch Ges Physiol 1874;9:185.

[41] Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, Garner H, Trouillet C, de Bruijn MF, Geissmann F, Rodewald HR. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 2015;518:547–51.

[42] Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, Stender JD, Chun HB, Garner H, Geissmann F, Glass CK. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 2014;159:1327–40.

[43] Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, Jaeger BN, O'Connor C, Fitzpatrick C, Pasillas MP, Pena M, Adair A, Gonda DD, Levy ML, Ransohoff RM, Gage FH, Glass CK. An environment-dependent transcriptional network specifies human microglia identity. Science 2017;356:eaal3222.

[44] Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, Deswarte K, Malissen B, Hammad H, Lambrecht BN. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med 2013;210: 1977–92.

[45] Guilliams M, Scott CL. Does niche competition determine the origin of tissue-resident macrophages? Nat Rev Immunol 2017;17:451–60.

[46] Haraden CA, Huebner JL, Hsueh MF, Li YJ, Kraus VB. Synovial fluid biomarkers associated with osteoarthritis severity reflect macrophage and neutrophil related inflammation. Arthritis Res Ther 2019;21:146.

[47] Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, Becker CD, See P, Price J, Lucas D, Greter M, Mortha A, Boyer SW, Forsberg EC, Tanaka M, van Rooijen N, Garcia-Sastre A, Stanley ER, Ginhoux F, Frenette PS, Merad M. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 2013;38:792–804.

[48] Hay SI, Collaborators. Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1260–344.

[49] Heinz S, Benner C, Spann N, Bertolino E, Lin Y, Laslo P, Cheng J, Murre C, Singh H, Glass C. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 2010;38:576–89.
[50] Hoeffel G, Chen J, Lavin Y, Low D, Almeida Francisca F, See P, Beaudin Anna E, Lum J, Low I, Forsberg EC, Poidinger M, Zolezzi F, Larbi A, Ng Lai G, Chan Jerry KY, Greter M, Becher B, Samokhvalov Igor M, Merad M, Ginhoux F. C-Myb+ erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunity 2015;42:665–78.
[51] Hore Z, Denk F. Neuroimmune interactions in chronic pain—an interdisciplinary perspective. Brain Behav Immun 2019;79:56–62.
[52] Huang J, Burston JJ, Li L, Ashraf S, Mapp PI, Bennett AJ, Ravipati S, Pousinis P, Barrett DA, Scammell BE, Chapman V. Targeting the D series resolvin receptor system for the treatment of osteoarthritis pain. Arthritis Rheumatol 2017;69:996–1008.
[53] Jackson MT, Moradi B, Zaki S, Smith MM, McCracken S, Smith SM, Jackson CJ, Little CB. Depletion of protease-activated receptor 2 but not protease-activated receptor 1 may confer protection against osteoarthritis in mice through extracartilaginous mechanisms. Arthritis Rheumatol 2014;66:3337–48.
[54] Kabata H, Artis D. Neuro-immune crosstalk and allergic inflammation. J Clin Invest 2019;129:1475–82.
[55] Kaikkonen M, Spann N, Heinz S, Romanoski C, Allison K, Stender J, Chun H, Tough D, Prinjha R, Benner C, Glass C. Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol Cell 2013;51:310–25.
[56] Kato J, Agalave NM, Svensson CI. Pattern recognition receptors in chronic pain: mechanisms and therapeutic implications. Eur J Pharmacol 2016;788:261–73.
[57] Kelly LM, Englmeier U, Lafon I, Sieweke MH, Graf T. MafB is an inducer of monocytic differentiation. EMBO J 2000;19:1987–97.
[58] Kennedy A, Fearon U, Veale DJ, Godson C. Macrophages in synovial inflammation. Front Immunol 2011;2:52.
[59] Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016;16:626–38.
[60] Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, Hirakawa A, Takeuchi H, Suzumura A, Ishiguro N, Kadomatsu K. Minocycline selectively inhibits M1 polarization of microglia. Cell Death Dis 2013;4:e525.
[61] Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J, Callahan L, Copenhaver C, Dodge C, Felson D, Gellar K, Harvey WF, Hawker G, Herzig E, Kwoh CK, Nelson AE, Samuels J, Scanzello C, White D, Wise B, Altman RD, DiRenzo D, Fontanarosa J, Giradi G, Ishimori M, Misra D, Shah AA, Shmagel AK, Thoma LM, Turgunbaev M, Turner AS, Reston J. 2019 American College of Rheumatology/arthritis foundation guideline for the management of osteoarthritis of the hand, hip, and knee. Arthritis Rheumatol 2020;72:220–33.
[62] Koopman FA, Stoof SP, Straub RH, Van Maanen MA, Vervoordeldonk MJ, Tak PP. Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. Mol Med 2011;17:937–48.
[63] Kopf M, Schneider C, Nobs SP. The development and function of lung-resident macrophages and dendritic cells. Nat Immunol 2015;16:36–44.
[64] Kraus VB, McDaniel G, Huebner JL, Stabler TV, Pieper CF, Shipes SW, Petry NA, Low PS, Shen J, McNearney TA, Mitchell P. Direct in vivo evidence of activated macrophages in human osteoarthritis. Osteoarthritis Cartilage 2016;24:1613–21.
[65] Lavin Y, Merad M. Macrophages: gatekeepers of tissue integrity. Cancer Immunol Res 2013;1:201–9.
[66] Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S, Amit I. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 2014;159:1312–26.
[67] Lavine KJ, Epelman S, Uchida K, Weber KJ, Nichols CG, Schilling JD, Ornitz DM, Randolph GJ, Mann DL. Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. Proc Natl Acad Sci U S A 2014;111:16029–34.
[68] Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 2011;11:750–61.
[69] LeBlanc BW, Zerah ML, Kadasi LM, Chai N, Saab CY. Minocycline injection in the ventral posterolateral thalamus reverses microglial reactivity and thermal hyperalgesia secondary to sciatic neuropathy. Neurosci Lett 2011;498:138–42.
[70] Lee MC, Saleh R, Achuthan A, Fleetwood AJ, Forster I, Hamilton JA, Cook AD. CCL17 blockade as a therapy for osteoarthritis pain and disease. Arthritis Res Ther 2018;20:62.
[71] Lee Y, Pai M, Brederson JD, Wilcox D, Hsieh G, Jarvis MF, Bitner RS. Monosodium iodoacetate-induced joint pain is associated with increased phosphorylation of mitogen activated protein kinases in the rat spinal cord. Mol Pain 2011;7:39.
[72] Levine JD, Clark R, Devor M, Helms C, Moskowitz MA, Basbaum AI. Intraneuronal substance P contributes to the severity of experimental arthritis. Science 1984;226:547–9.
[73] Levine JD, Khasar SG, Green PG. Neurogenic inflammation and arthritis. Ann N Y Acad Sci 2006;1069:155–67.
[74] Lluch E, Torres R, Nijs J, Van Oosterwijck J. Evidence for central sensitization in patients with osteoarthritis pain: a systematic literature review. Eur J Pain 2014;18:1367–75.
[75] Longo G, Osikowicz M, Ribeiro-da-Silva A. Sympathetic fiber sprouting in inflamed joints and adjacent skin contributes to pain-related behavior in arthritis. J Neurosci 2013;33:10066–74.
[76] Longobardi L, Temple JD, Tagliafierro L, Willcockson H, Esposito A, D'Onofrio N, Stein E, Li T, Myers TJ, Ozkan H, Balestrieri ML, Ulici V, Loeser RF, Spagnoli A. Role of the C-C chemokine receptor-2 in a murine model of injury-induced osteoarthritis. Osteoarthritis Cartilage 2017;25:914–25.
[77] Lopes EBP, Filiberti A, Husain SA, Humphrey MB. Immune contributions to osteoarthritis. Curr Osteoporos Rep 2017;15:593–600.
[78] 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 2019;10:745.
[79] Malfait AM, Block JA. Osteoarthritis. In: M Parnham, editor. Encyclopedia of Inflammatory Diseases. Basel, Switzerland: Birkhäuser, 2015.
[80] Malfait AM, Little CB. On the predictive utility of animal models of osteoarthritis. Arthritis Res Ther 2015;17:225.
[81] Malfait AM, Miller RE. Why we should study osteoarthritis pain in experimental models in both sexes. Osteoarthritis Cartilage 2020;28:397–9.
[82] Mandelin AM II, Homan PJ, Shaffer AM, Cuda CM, Dominguez ST, Bacalao E, Carns M, Hinchcliff M, Lee J, Aren K, Thakrar A, Montgomery AB, Bridges SL Jr, Bathon JM, Atkinson JP, Fox DA, Matteson EL, Buckley CD, Pitzalis C, Parks D, Hughes LB, Geraldino-Pardilla L, Ike R, Phillips K, Wright K, Filer A, Kelly S, Ruderman EM, Morgan V, Abdala-Valencia H, Misharin AV, Budinger GS, Bartom ET, Pope RM, Perlman H, Winter DR. Transcriptional profiling of synovial macrophages using minimally invasive ultrasound-guided synovial biopsies in rheumatoid arthritis. Arthritis Rheumatol 2018;70:841–54.
[83] Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014;6:13.
[84] Massier J, Eitner A, Segond von Banchet G, Schaible HG. Effects of differently activated rodent macrophages on sensory neurons: implications for arthritis pain. Arthritis Rheumatol 2015;67:2263–72.
[85] Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada González F, Perrin P, Keren-Shaul H, Gury M, Lara-Astaiso D, Thaiss CA, Cohen M, Bahar Halpern K, Baruch K, Deczkowska A, Lorenzo-Vivas E, Itzkovitz S, Elinav E, Sieweke MH, Schwartz M, Amit I. Microglia development follows a stepwise program to regulate brain homeostasis. Science 2016;353:aad8670.
[86] McCormack DG, Mak JC, Coupe MO, Barnes PJ. Calcitonin gene-related peptide vasodilation of human pulmonary vessels. J Appl Physiol (1985) 1989;67:1265–70.
[87] McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365:2205–19.
[88] McLachlan EM, Janig W, Devor M, Michaelis M. Peripheral nerve injury triggers noradrenergic sprouting within dorsal root ganglia. Nature 1993;363:543–6.
[89] Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev 2000;173:89–97.
[90] Medzhitov R, Janeway C Jr. Innate immunity. N Engl J Med 2000;343:338–44.
[91] Miller RE, Belmadani A, Ishihara S, Tran PB, Ren D, Miller RJ, Malfait AM. Damage-associated molecular patterns generated in osteoarthritis directly excite murine nociceptive neurons through Toll-like receptor 4. Arthritis Rheumatol 2015;67:2933–43.
[92] Miller RE, Ishihara S, Tran PB, Golub SB, Last K, Miller RJ, Fosang AJ, Malfait AM. An aggrecan fragment drives osteoarthritis pain through Toll-like receptor 2. JCI Insight 2018;3:e95704.

[93] Miller RE, Tran PB, Das R, Ghoreishi-Haack N, Ren D, Miller RJ, Malfait AM. CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. Proc Natl Acad Sci U S A 2012;109:20602–7.
[94] Miller RE, Tran PB, Ishihara S, Syx D, Ren D, Miller RJ, Valdes AM, Malfait AM. Microarray analyses of the dorsal root ganglia support a role for innate neuro-immune pathways in persistent pain in experimental osteoarthritis. Osteoarthritis Cartilage 2020;28:581–92.
[95] Miller RJ, Malfait AM, Miller RE. The innate immune response as a mediator of osteoarthritis pain. Osteoarthritis Cartilage 2020;28: 562–71.
[96] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 2000;164: 6166–73.
[97] Miotla Zarebska J, Chanalaris A, Driscoll C, Burleigh A, Miller RE, Malfait AM, Stott B, Vincent TL. CCL2 and CCR2 regulate pain-related behaviour and early gene expression in post-traumatic murine osteoarthritis but contribute little to chondropathy. Osteoarthritis Cartilage 2017;25:406–12.
[98] Misharin AV, Cuda CM, Saber R, Turner JD, Gierut AK, Haines GK III, Berdnikovs S, Filer A, Clark AR, Buckley CD, Mutlu GM, Budinger GR, Perlman H. Nonclassical Ly6C(-) monocytes drive the development of inflammatory arthritis in mice. Cell Rep 2014;9:591–604.
[99] Misharin AV, Morales-Nebreda L, Reyfman PA, Cuda CM, Walter JM, McQuattie-Pimentel AC, Chen CI, Anekalla KR, Joshi N, Williams KJN, Abdala-Valencia H, Yacoub TJ, Chi M, Chiu S, Gonzalez-Gonzalez FJ, Gates K, Lam AP, Nicholson TT, Homan PJ, Soberanes S, Dominguez S, Morgan VK, Saber R, Shaffer A, Hinchcliff M, Marshall SA, Bharat A, Berdnikovs S, Bhorade SM, Bartom ET, Morimoto RI, Balch WE, Sznajder JI, Chandel NS, Mutlu GM, Jain M, Gottardi CJ, Singer BD, Ridge KM, Bagheri N, Shilatifard A, Budinger GRS, Perlman H. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. J Exp Med 2017;214:2387–404.
[100] Mogil JS, Chanda ML. The case for the inclusion of female subjects in basic science studies of pain. PAIN 2005;117:1–5.
[101] Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, Pinto AR, Klapproth K, Henri S, Malissen B, Rodewald HR, Rosenthal NA, Bajenoff M, Prinz M, Jung S, Sieweke MH. Progressive replacement of embryo-derived cardiac macrophages with age. J Exp Med 2014;211: 2151–8.
[102] Montgomery A, Fahy N, Hamilton S, Eckman B, De Almeida L, Ishihara S, Mayr MG, Chen SY, Gadhvi G, Cuda C, Malfait AM, Bastiaansen-Jenniskens Y, Winter DR. Macrophages drive the inflammatory phase in experimental osteoarthritis. bioRxiv 2020:2020.2005.2028.122408.
[103] Morias Y, Abels C, Laoui D, Van Overmeire E, Guilliams M, Schouppe E, Tacke F, deVries CJ, De Baetselier P, Beschin A. Ly6C- monocytes regulate parasite-induced liver inflammation by inducing the differentiation of pathogenic Ly6C+ monocytes into macrophages. PLoS Pathog 2015;11:e1004873.
[104] Mousseau M, Burma NE, Lee KY, Leduc-Pessah H, Kwok CHT, Reid AR, O'Brien M, Sagalajev B, Stratton JA, Patrick N, Stemkowski PL, Biernaskie J, Zamponi GW, Salo P, McDougall JJ, Prescott SA, Matyas JR, Trang T. Microglial pannexin-1 channel activation is a spinal determinant of joint pain. Sci Adv 2018;4:eaas9846.
[105] Nahrendorf M, Swirski FK. Abandoning M1/M2 for a network model of macrophage function. Circ Res 2016;119:414–17.
[106] Neogi T. The epidemiology and impact of pain in osteoarthritis. Osteoarthritis Cartilage 2013;21:1145–53.
[107] Neogi T. Structural correlates of pain in osteoarthritis. Clin Exp Rheumatol 2017;35(suppl 107):75–8.
[108] Ogbonna AC, Clark AK, Gentry C, Hobbs C, Malcangio M. Pain-like behaviour and spinal changes in the monosodium iodoacetate model of osteoarthritis in C57Bl/6 mice. Eur J Pain 2013;17:514–26.
[109] Oishi Y, Manabe I. Macrophages in age-related chronic inflammatory diseases. NPJ Aging Mech Dis 2016;2:16018.
[110] Oo WM, Hunter DJ. Disease modification in osteoarthritis: are we there yet? Clin Exp Rheumatol 2019;37(suppl 120):135–40.
[111] Ostuni R, Piccolo V, Barozzi I, Polletti S, Termanini A, Bonifacio S, Curina A, Prosperini E, Ghisletti S, Natoli G. Latent enhancers activated by stimulation in differentiated cells. Cell 2013;152:157–71.
[112] 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 2011;31:18433.
[113] Persson MSM, Sarmanova A, Doherty M, Zhang W. Conventional and biologic disease-modifying anti-rheumatic drugs for osteoarthritis: a meta-analysis of randomized controlled trials. Rheumatology (Oxford) 2018;57:1830–7.
[114] Prinz M, Jung S, Priller J. Microglia biology: one century of evolving concepts. Cell 2019;179:292–311.
[115] Puig-Junoy J, Ruiz Zamora A. Socio-economic costs of osteoarthritis: a systematic review of cost-of-illness studies. Semin Arthritis Rheum 2015;44:531–41.
[116] 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;161:1976–82.
[117] Raoof R, Willemen H, Eijkelkamp N. Divergent roles of immune cells and their mediators in pain. Rheumatology (Oxford) 2018;57:429–40.
[118] Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. J Neurosci Res 2017;95:500–8.
[119] Sagar DR, Ashraf S, Xu L, Burston JJ, Menhinick MR, Poulter CL, Bennett AJ, Walsh DA, Chapman V. Osteoprotegerin reduces the development of pain behaviour and joint pathology in a model of osteoarthritis. Ann Rheum Dis 2014;73:1558–65.
[120] Sagar DR, Burston JJ, Hathway GJ, Woodhams SG, Pearson RG, Bennett AJ, Kendall DA, Scammell BE, Chapman V. The contribution of spinal glial cells to chronic pain behaviour in the monosodium iodoacetate model of osteoarthritic pain. Mol Pain 2011;7:88.
[121] Sakai M, Troutman TD, Seidman JS, Ouyang Z, Spann NJ, Abe Y, Ego KM, Bruni CM, Deng Z, Schlachetzki JCM, Nott A, Bennett H, Chang J, Vu BT, Pasillas MP, Link VM, Texari L, Heinz S, Thompson BM, McDonald JG, Geissmann F, Glass CK. Liver-derived signals sequentially reprogram myeloid enhancers to initiate and maintain kupffer cell identity. Immunity 2019;51:655–70.e658.
[122] Sakurai Y, Fujita M, Kawasaki S, Sanaki T, Yoshioka T, Higashino K, Tofukuji S, Yoneda S, Takahashi T, Koda K, Asaki T, Hasegawa M, Morioka Y. Contribution of synovial macrophages to rat advanced osteoarthritis pain resistant to cyclooxygenase inhibitors. PAIN 2019; 160:895–907.
[123] Sambamurthy N, Zhou C, Nguyen V, Smalley R, Hankenson KD, Dodge GR, Scanzello CR. Deficiency of the pattern-recognition receptor CD14 protects against joint pathology and functional decline in a murine model of osteoarthritis. PLoS One 2018;13:e0206217.
[124] Scanzello CR. Role of low-grade inflammation in osteoarthritis. Curr Opin Rheumatol 2017;29:79–85.
[125] Scanzello CR, Plaas A, Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr Opin Rheumatol 2008;20:565–72.
[126] Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci 2007;10:1361–8.
[127] Schulz C, Perdiguero EG, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, Prinz M, Wu B, Jacobsen SEW, Pollard JW, Frampton J, Liu KJ, Geissmann F. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 2012;336:86–90.
[128] Scott CL, Zheng F, De Baetselier P, Martens L, Saeys Y, De Prijck S, Lippens S, Abels C, Schoonooghe S, Raes G, Devoogdt N, Lambrecht BN, Beschin A, Guilliams M. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. Nat Commun 2016;7:10321.
[129] Segond von Banchet G, Boettger MK, Fischer N, Gajda M, Brauer R, Schaible HG. Experimental arthritis causes tumor necrosis factor-alpha-dependent infiltration of macrophages into rat dorsal root ganglia which correlates with pain-related behavior. PAIN 2009;145:151–9.
[130] Seidman JS, Troutman TD, Sakai M, Gola A, Spann NJ, Bennett H, Bruni CM, Ouyang Z, Li RZ, Sun X, Vu BT, Pasillas MP, Ego KM, Gosselin D, Link VM, Chong LW, Evans RM, Thompson BM, McDonald JG, Hosseini M, Witztum JL, Germain RN, Glass CK. Niche-specific reprogramming of epigenetic landscapes drives myeloid cell diversity in nonalcoholic steatohepatitis. Immunity 2020;52:1057–74.e7.
[131] Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 2018;128:2657–69.
[132] Sheng J, Ruedl C, Karjalainen K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. Immunity 2015;43:382–93.
[133] Shinder V, Govrin-Lippmann R, Cohen S, Belenky M, Ilin P, Fried K, Wilkinson HA, Devor M. Structural basis of sympathetic-sensory coupling in rat and human dorsal root ganglia following peripheral nerve injury. J Neurocytol 1999;28:743–61.
[134] 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;8:1778.

[135] 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.
[136] Sousa-Valente J, Calvo L, Vacca V, Simeoli R, Arevalo JC, Malcangio M. Role of TrkA signalling and mast cells in the initiation of osteoarthritis pain in the monoiodoacetate model. Osteoarthritis Cartilage 2018;26:84–94.
[137] Stahl EC, Haschak MJ, Popovic B, Brown BN. Macrophages in the aging liver and age-related liver disease. Front Immunol 2018;9:2795.
[138] Stoppiello LA, Mapp PI, Wilson D, Hill R, Scammell BE, Walsh DA. Structural associations of symptomatic knee osteoarthritis. Arthritis Rheumatol 2014;66:3018–27.
[139] Strong JA, Zhang JM, Schaible HG. The sympathetic nervous system and pain. In: JN Wood, editor. The Oxford Handbook of the Neurobiology of Pain. New York, NY: Oxford University Press, 2020.
[140] Sun J, Ramnath RD, Zhi L, Tamizhselvi R, Bhatia M. Substance P enhances NF-kappaB transactivation and chemokine response in murine macrophages via ERK1/2 and p38 MAPK signaling pathways. Am J Physiol Cell Physiol 2008;294:C1586–1596.
[141] Tacke F, Zimmermann HW. Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 2014;60:1090–6.
[142] Talbot S, Foster SL, Woolf CJ. Neuroimmunity: physiology and pathology. Annu Rev Immunol 2016;34:421–47.
[143] Temp J, Labuz D, Negrete R, Sunkara V, Machelska H. Pain and knee damage in male and female mice in the medial meniscal transection-induced osteoarthritis. Osteoarthritis Cartilage 2020;28:475–85.
[144] Thakur M, Rahman W, Hobbs C, Dickenson AH, Bennett DL. Characterisation of a peripheral neuropathic component of the rat monoiodoacetate model of osteoarthritis. PLoS One 2012;7:e33730.
[145] Tran PB, Miller RE, Ishihara S, Miller RJ, Malfait AM. Spinal microglial activation in a murine surgical model of knee osteoarthritis. Osteoarthritis Cartilage 2017;25:718–26.
[146] Udalova IA, Mantovani A, Feldmann M. Macrophage heterogeneity in the context of rheumatoid arthritis. Nat Rev Rheumatol 2016;12:472–85.
[147] van de Laar L, Saelens W, De Prijck S, Martens L, Scott CL, Van Isterdael G, Hoffmann E, Beyaert R, Saeys Y, Lambrecht BN, Guilliams M. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. Immunity 2016;44:755–68.
[148] van Lent PL, Blom AB, Schelbergen RF, Sloetjes A, Lafeber FP, Lems WF, Cats H, Vogl T, Roth J, van den Berg WB. Active involvement of alarmins S100A8 and S100A9 in the regulation of synovial activation and joint destruction during mouse and human osteoarthritis. Arthritis Rheum 2012;64:1466–76.
[149] Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol 2018;30:160–7.
[150] von Loga IS, Batchelor V, Driscoll C, Burleigh A, Chia SL, Stott B, Miotla-Zarebska J, Riley D, Dell'Accio F, Vincent TL. Does pain at an earlier stage of chondropathy protect female mice from structural progression after surgically induced osteoarthritis? Arthritis Rheumatol 2020;72:2083–93.
[151] Wijbrandts CA, Vergunst CE, Haringman JJ, Gerlag DM, Smeets TJ, Tak PP. Absence of changes in the number of synovial sublining macrophages after ineffective treatment for rheumatoid arthritis: implications for use of synovial sublining macrophages as a biomarker. Arthritis Rheum 2007;56:3869–71.
[152] Willemen HL, Eijkelkamp N, Garza Carbajal A, Wang H, Mack M, Zijlstra J, Heijnen CJ, Kavelaars A. Monocytes/Macrophages control resolution of transient inflammatory pain. J Pain 2014;15:496–506.
[153] Willemen HL, Eijkelkamp N, Wang H, Dantzer R, Dorn GW II, Kelley KW, Heijnen CJ, Kavelaars A. Microglial/macrophage GRK2 determines duration of peripheral IL-1beta-induced hyperalgesia: contribution of spinal cord CX3CR1, p38 and IL-1 signaling. PAIN 2010;150:550–60.
[154] Wood MJ, Leckenby A, Reynolds G, Spiering R, Pratt AG, Rankin KS, Isaacs JD, Haniffa MA, Milling S, Hilkens CMU. Macrophage proliferation distinguishes 2 subgroups of knee osteoarthritis patients. JCI Insight 2019;4:e125325.
[155] Woolf CJ, Ma Q. Nociceptors—noxious stimulus detectors. Neuron 2007;55:353–64.
[156] Wu CL, Harasymowicz NS, Klimak MA, Collins KH, Guilak F. The role of macrophages in osteoarthritis and cartilage repair. Osteoarthritis Cartilage 2020;28:544–54.
[157] Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016;44:450–62.
[158] Xie W, Strong JA, Zhang JM. Increased excitability and spontaneous activity of rat sensory neurons following in vitro stimulation of sympathetic fiber sprouts in the isolated dorsal root ganglion. PAIN 2010;151:447–59.
[159] Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, Strauss-Ayali D, Viukov S, Guilliams M, Misharin A, Hume DA, Perlman H, Malissen B, Zelzer E, Jung S. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 2013;38:79–91.
[160] 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;11:264.
[161] Yu YR, Hotten DF, Malakhau Y, Volker E, Ghio AJ, Noble PW, Kraft M, Hollingsworth JW, Gunn MD, Tighe RM. Flow cytometric analysis of myeloid cells in human blood, bronchoalveolar lavage, and lung tissues. Am J Respir Cell Mol Biol 2016;54:13–24.
[162] Zhang F, Wei K, Slowikowski K, Fonseka CY, Rao DA, Kelly S, Goodman SM, Tabechian D, Hughes LB, Salomon-Escoto K, Watts GFM, Jonsson AH, Rangel-Moreno J, Meednu N, Rozo C, Apruzzese W, Eisenhaure TM, Lieb DJ, Boyle DL, Mandelin AM II, Boyce BF, DiCarlo E, Gravallese EM, Gregersen PK, Moreland L, Firestein GS, Hacohen N, Nusbaum C, Lederer JA, Perlman H, Pitzalis C, Filer A, Holers VM, Bykerk VP, Donlin LT, Anolik JH, Brenner MB, Raychaudhuri S. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. Nat Immunol 2019;20:928–42.
[163] Zhang H, Cai D, Bai X. Macrophages regulate the progression of osteoarthritis. Osteoarthritis Cartilage 2020;28:555–61.
[164] 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.
[165] Zigmond E, Samia-Grinberg S, Pasmanik-Chor M, Brazowski E, Shibolet O, Halpern Z, Varol C. Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. J Immunol 2014;193:344–53.