Neuroimmune interactions and osteoarthritis pain: focus on macrophages
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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, 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, 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.100 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.118 Patients with OA also show facilitated temporal summation and impaired conditioned pain modulation, evidence for central sensitization.74

Osteoarthritis is a classical biomechanically driven disease31 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,
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,\textsuperscript{107} targeting specific inflammatory mediators (eg, neutralizing IL-1 or TNFα with monoclonal antibodies) has been largely disappointing.\textsuperscript{111,113} 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,\textsuperscript{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).\textsuperscript{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.\textsuperscript{9}

In recent years, a growing body of literature has identified multiple modes 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.\textsuperscript{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.”\textsuperscript{116} In keeping with this warning function associated with, or resembling that associated with, actual or potential tissue damage, pain is defined as “an unpleasant sensory and emotional experience”\textsuperscript{116} and “a subjective experience that may be inferred from verbal report(s) or manifest by expression (eg, behavior) and/or physical signs but does not necessarily have a neurobiological correlate.”\textsuperscript{116}

For key terms, including but not limited to: “osteoarthritis,” “pain,” “macrophages,” “microglia,” “neuro-immunity,” “inflammation,” “animal models,” “chronic pain,” “neuro-immune interactions,” 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.\textsuperscript{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).\textsuperscript{18,29,90} 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.\textsuperscript{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.\textsuperscript{18,142} Neuroimmunity exists as an evolutionary adaptation to detect and react to dangers in the environment.\textsuperscript{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.\textsuperscript{4,17,142,155}

Non-synaptic 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.\textsuperscript{89,153} Sympathetic-sensory coupling has in general been shown to be excitatory and pronociceptive.\textsuperscript{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,\textsuperscript{14,60} 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,\textsuperscript{142} including dendritic cells, neutrophils, macrophages, mast cells, and T cells. It is clear that interactions between the nervous and immune systems are
The classical inflammatory response induces a magnitude higher than the speed at which the innate immune system responds. The rapidity of responses to painful stimuli is an order of magnitude higher than the speed at which the innate immune system responds. 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, 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. 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. Of interest, in patients with RA, it has been shown that synovial fluid (SF) levels of SPMs were negatively associated with pain. 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. 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)-hydroxydocosahexaenoic 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.

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. At steady state, macrophages perform different functions depending on their tissue of residence. For example, brain-resident macrophages, known as microglia, prune synapses in development, and red pulp macrophages in the spleen recycle heme from erythrocytes. Thus, different tissue-resident macrophage populations exhibit different phenotypes and patterns of gene expression. 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. Tissue-resident macrophages have 2 possible ontogenies: They may be derived from embryonic precursors or from adult hematopoiesis through monocytes. Many macrophage populations are believed to be a combination of both, with monocytes differentiating into macrophages and replacing the original cells over time. In these cases, the monocyte-derived macrophages eventually become phenotypically identical to the embryonic derived cells they replace. This is possible through tissue-specific transcription factors that reprogram macrophages in response to environmental signals. 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. Macrophages have also been shown to be altered towards an inflammatory phenotype as tissues age, which may be a natural part of aging. 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. 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. These monocyte-derived macrophages are distinct from those that exist at steady state since they are specified by stimulus-specific factors. They often maintain their monocyte identity longer and are slow to demonstrate the tissue-resident phenotype. Infiltrating macrophages have been shown to be a major driver of fibrosis and tissue damage resulting from inflammation. For example, recruitment of monocyte-derived macrophages worsens outcome in mouse models of acute lung injury and post-traumatic brain injury. By contrast, macrophages exhibiting a tissue-resident phenotype are often decreased in number during tissue inflammation and then expand during resolution. 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 vivo studies suggesting a model for macrophage polarization from inflammatory (M1) to alternative activation (M2). However, since in vitro macrophages fail to recapitulate the complexity of in vivo macrophages, this model has fallen out of favor. 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 that includes influx of inflammatory macrophages. In particular, macrophages are found in the synovial lining as well as the sublining layer. 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.⁹⁶ 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.²³Although Misharin and et al. did not distinguish macrophages by CX3CR1⁺, the study by Culemann et al.²⁵ 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.¹⁶⁵ 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.⁹² 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 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.⁹² 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.

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.²⁹ 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.⁵ This suggests neuroimmune communication through CGRP that promotes a regulatory macrophage phenotype even in a proinflammatory microenvironment, 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; e.g., 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).37,156,163 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.25 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 plateaueaus 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

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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 (NIHANES-III) | Synovial fluid CD14, CD163, and serum CD163 were associated with abundance of activated M2s in knee joint capsule and synovium; synovial fluid CD14 levels correlated positively with self-reported knee pain severity and joint space narrowing | Daghstani 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 M0s were not associated with pain. | Gomez-Aristizabal et al. (2019). Arthritis Res Ther. |
(Table 2). Nonetheless, macrophage infiltration into the synovium has been documented in several experimental models of OA, albeit to different degrees. In a widely used surgical model of OA in mice, induced by destabilization of the medial meniscus (DMM), whole joint levels of Cd68 mRNA, a macrophage marker, were significantly suppressed in Ccr2−/− 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. An interesting recent study examined involvement of synovial macrophages in the rat monoiodoacetate-induced (MIA) model of advanced knee OA. 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-NFG 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. 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. 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.

3.3.3. 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. 10, 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. 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. 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/IFN-γ-TNFα-IL1) phenotype. 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.

Macrophage infiltration of the DRG has also been reported in experimental OA induced by DMM, starting around 8 weeks after surgery. This coincided with increased gene expression of Cc22 and Ccr2 mRNA in the DRG, and onset of movement-provoked pain behaviors. Ccr2 null mice, while developing early-onset mechanical allodynia, did not show sustained allostodynia 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. These Ccr2 null mice did not display macrophage infiltration in the DRG, 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. 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. 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, Nfg, and Trk1) were identified as being associated with neuroinflammation, and these genes have been previously linked to OA pain.

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. Indeed, targeting glial activation through minocycline or propentofylline has reduced experimental neuropathic pain. 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. 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, Nfg, and Trk1) were identified as being associated with neuroinflammation, and these genes have been previously linked to OA pain.

The surgical DMM model is an exceptionally slowly progressive model of OA joint damage, and here, we reported microglial activation beginning on day 14 of the MIA model attenuated mechanical allodynia and microglial activation by day 28. The MIA model is a rapidly progressive model of OA, 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).

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. 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. These studies also further highlight the temporal regulation of neuroimmune changes in the neuraxis after...
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. 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. Studies that address the role of microglial activation in chronic pain have largely been conducted in male animals. 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 of both sexes, but inhibitors of microglial activation block allodynia and this was hormonally dependent since female mice still used microglia to establish pain hypersensitivity in both sexes.

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.
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 CIAOA, there was a lower fold change (day 0–day 3) of macrophage expansion in females compared with males (5- vs 8-fold). 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.

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 seqRNA, 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, prormodeling 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.

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## Key Points

- **Inflammation & Pain:**
  - Neuroinflammation plays a role in the development of pain.
  - Macrophages and TLR4 signaling are involved in pain pathways.
- **Gene-Related Peptide:**
  - CGRP and TRPV1 signaling in pain modulation.
- **Osteoarthritis & Mechanisms:**
  - Synovial fluid macrophages in osteoarthritis.
  - Resident macrophages derive from fetal monocytes.
- **Microglia & Neuroinflammation:**
  - Microglia develop from primitive macrophages.
  - Tissue-resident macrophages maintain a regulatory phenotype throughout adult life.
- **Mapping Analysis:**
  - Microglia mapping analysis reveals adult microglia development from fetal monocytes.

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**Additional Notes:**

- *Note: This is a generic template and requires specific details for each entry.*
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