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Review

Immunomodulation and fibroblast dynamics driving nociceptive joint pain within inflammatory synovium: Unravelling mechanisms for therapeutic advancements in osteoarthritis

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SUMMARY

Objective: Synovitis is a widely accepted sign of osteoarthritis (OA), characterised by tissue hyperplasia, where increased infiltration of immune cells and proliferation of resident fibroblasts adopt a pro-inflammatory phenotype, and increased the production of pro-inflammatory mediators that are capable of sensitising and activating sensory nociceptors, which innervate the joint tissues. As such, it is important to understand the cellular composition of synovium and their involvement in pain sensitisation to better inform the development of effective analgesics.

Methods: Studies investigating pain sensitisation in OA with a focus on immune cells and fibroblasts were identified using PubMed, Web of Science and SCOPUS.

Results: In this review, we comprehensively assess the evidence that cellular crosstalk between resident immune cells or synovial fibroblasts with joint nociceptors in inflamed OA synovium contributes to peripheral pain sensitisation. Moreover, we explore whether the elucidation of common mechanisms identified in similar joint conditions may inform the development of more effective analgesics specifically targeting OA joint pain.

Conclusion: The concept of local environment and cellular crosstalk within the inflammatory synovium as a driver of nociceptive joint pain presents a compelling opportunity for future research and therapeutic advancements.

Introduction

Joint pain in osteoarthritis (OA) patients is a major cause of disability in older adults reducing quality of life, independence and work span, thus impacting hugely on healthcare and economic costs.[1–3] Current pain management options, centred on generic analgesics, are inadequate for most patients, being of limited efficacy and associated with adverse side effects through long-term use.[4–8] Whilst joint replacement surgery procedures have high success rates, these are avoided in patients under the age of 60 due to limited lifespan of prosthetics.[9] As such, many patients endure several years of chronic pain before undergoing surgery and in some cases older patients who are at greater risk of complications may be deemed unfit for such a surgery.[10] Additionally, as many as one in eight patients experience postoperative pain despite having no clinical or radiological abnormalities.[9,11] Overall, there remains a high unmet clinical need to develop targeted and more efficacious analgesics for patients.

Since patient-reported pain is a major decision-making contributor when considering joint replacement, an effective intervention to alleviate pain would delay and potentially reduce the necessity of undergoing significant surgery. The identification of new

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treatments to manage OA pain is challenging because the experience of pain arises from pathological changes across multiple tissues of the OA joint, sensitization of sensory nerves and changes in the spinal and supra-spinal processing of sensory inputs.[12] Additionally, radiographic measurements of disease severity poorly reflect pain severity and/or pain sensitisation reported by patients with OA pain.[13,14] Furthermore, pain perception is influenced by socio-economic and psychosocial factors adding further complexity.[15]

The complex mechanisms underlying the transition from acute (< 3 months) to chronic pain (> 3 months) have been reviewed elsewhere, along with changes in joint innervation patterns brought on by joint inflammation.[16–20] The OA phenotype is heterogeneous and can be broadly classified into reflecting both inflammatory and neuropathic pain mechanisms.[12] People with OA pain exhibit symptoms reflecting both peripheral sensitization and central sensitisation.[12,21] Importantly, a large proportion of people with OA respond well to joint replacement surgery, suggesting that their pain is predominantly driven by peripheral sensitization mechanisms and could be responsive to local inhibition of these processes. Broadly speaking, synovial inflammation or synovitis, is associated with increased pain sensitisation in patients with knee OA.[22–24] In this review we consider the evidence that synovitis and cellular interactions within the synovium are crucial to peripheral pain sensitisation and evaluate the opportunities these present for the development of new targeted analgesics.

Synovitis and OA pain sensitisation

Joint lining synovial tissue (synovium) forms the articular capsule which lubricates and nourishes the joint, reducing friction and protecting cartilage and bone through production and maintenance of joint synovial fluid. In OA, this lining tissue becomes inflamed (synovitis) which significantly contributes to cartilage damage and joint pain.[25] Synovitis is a hallmark pathological feature of OA, characterised by tissue hyperplasia consisting of increased proliferation of resident fibroblast-like synoviocytes, accumulation of activated immune cells such as macrophages, and vascularisation of sublining layers.[25–27] Synovitis is a feature of early OA, where there are minimal radiographic signs of articular cartilage damage particularly in post-traumatic OA compared to idiopathic OA[27,28] as well as established end-stage disease.[26]

In the Multicenter Osteoarthritis Study of 454 knee OA participants, synovitis was strongly associated with pain severity.[24] These findings were recapitulated in a 6 year follow-up with 1111 participants, where a significant decrease in pain pressure threshold at the patella was associated with synovitis over two years.[29] Further to this, de Lange-Brokaar et al. reported distinct patterns of synovitis around patellar sites were associated with pain perception.[23] Here, synovitis was scored in 86 patients where seven sites within the medial parapatellar region were associated with pain measures in both the Knee Injury and Osteoarthritis Outcome Score and the Intermittent and Constant Osteoarthritis Pain questionnaires, indicating a relationship to pain intensity.[23] More recently, our study, involving 29 early and 22 late OA patients, reported highly heterogeneous patterns of synovitis associated with sites of pain, although parapatellar synovitis was significantly greater at sites of patient-reported pain, compared to sites of no pain.[22]

Synovitis mechanisms include the release of pro-inflammatory mediators which can sensitise and activate nociceptors promoting increased pain perception. For example, the concentrations of tumour necrosis factor alpha (TNFα), interleukin 1 beta (IL1β), monocyte chemoattractant protein 1 (MCP1) and IL6 in synovial fluid are positively correlated with patient-reported pain,[30,31] and these molecules are known to sensitise nociceptive afferents, lowering the threshold required for activation of nociceptor sensory terminals and causing pain sensitisation.[32] More generally, knee OA patient synovial fluid increases the excitability of sensory neurones.[33] Furthermore, synovitis may contribute to the development and maintenance of neuropathic type pain, as chronic inflammation in response to nerve injury can mediate destructive secondary damage via the induction of matrix proteases and nitric oxide.[34] Interestingly, while nociceptive pain is predominantly associated with early OA, both nociceptive and neuropathic pain mechanisms can be identified in patients.

To identify key molecular and cellular mediators within inflammatory synovium that may mediate pain, RNA-sequencing profiled the synovial transcriptome from 10 patients with high OA pain severity which identified 30 upregulated genes including stress responsive DNAJB4 interacting membrane protein 1 (SDIM1), otoferlin (OTOF) and carboxypeptidase E (CPE) compared to patients with low pain.[35] Notably, top enriched Gene Ontology terms included nerve growth factor (NGF) signalling via Tropomyosin receptor kinase A, which was enriched by genes that mediate neuronal survival and excitation, such as SDIM1, CPE, OTOF and TrkB.[35] Downregulated non-coding RNAs were identified in patients with high pain, including some with unknown functions (RN7SL3 and RP11-195E2.1) and miR-146-3p which has been extensively studied as a neuroinflammatory regulator and previously linked to knee OA-related pain.[35–37] Interestingly, the dominant arm of this microRNA, miR-146–5p, targets genes closely associated with OA pathogenesis and its silencing ameliorated OA-induced pain in a murine OA model.[38] The potential of miR-146 as an effective analgesic is yet to be determined in OA and likely challenged by phasic differential expression. miR-146–5p expression is higher in early OA, possibly due to initial exposure to pro-inflammatory stimuli, whilst in established OA its expression is significantly reduced and associated with pain.[39–41]

Another important consideration is the locality of synovitis in relation to joint pain, as de Lange-Brokaar et al. described distinct patterns of synovitis associated with pain perception.[23] More recently, our study anatomically dissected the areas of the synovium into four quadrants for a more nuanced understanding of synovitis and pain in osteoarthritic joints. The study recruited patients with early and end-stage knee OA, where synovitis was scored at 11 sites around the joint, and maps of patient-reported pain were recorded in addition to the collection of four parapatellar synovial biopsies corresponding to sites of patient-reported pain and no pain, from which primary synovial fibroblasts were isolated.[22] Transcriptomic analysis of these tissues found that painful sites were enriched for neuronal signalling pathways, including neuronal survival and growth, defined by upregulation of ARHGEF9, LYNX1, NRN1, GRIN2A and KCNIP.[22] Synovial fibroblast secretome phenotyping, identified neurotrophins and neurotrophic factors including IL6, leukaemia inhibitory factor (LIF), oncostatin M (OSM), glial cell derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), fibroblast growth factor 21 (FGF21), colony stimulating factor 1 (CSF1), IL8 and hepatocyte growth factor (HGF), which were present on average at higher concentrations in the secretome of fibroblasts from painful sites.[22] In functional assays, rodent dorsal root ganglion (DRG) neurons incubated with fibroblast-conditioned media from painful sites had greater survival and neurite outgrowth compared to media released by fibroblasts from non-painful sites.[22] Our study went further to identify distinct subsets of fibroblasts in painful synovial tissues discussed in Section 3.1.[22] Collectively, these studies suggest that pathological changes to the phenotype of, and the cellular interactome within, the synovium may lead to sprouting of sensory nerve terminals and contribute to peripheral mechanisms of pain sensitisation in OA. As a result, understanding the role(s) of these cells and their mechanisms of communication leading to nociceptor sensitization and activation may reveal new targets for the development of analgesics for OA patients.

Nociceptive pain sensitisation according to cell types within synovium

During an inflammatory event, immune cells are both activated within and recruited to the local microenvironment which in turn

influences the activity of nociceptors.[42] Immune cells are fundamental in mediating both acute and chronic pain states. During acute pain responses, immune cells aid in pain resolution through the release of anti-inflammatory and analgesic factors.[43] Whilst in chronic inflammatory conditions, studies in rodent OA models report persistent peripheral inflammation at sites of damaged or diseased tissues and recruitment of immune cells to the somatotopic DRGs. Together, this significantly contributes to the maintenance of chronic pain via the increased secretion of pro-inflammatory cytokines and production of reactive oxygen species (ROS) that are known to sensitise nociceptors.[44,45] In OA, inflamed synovium consists of activated fibroblasts,[22,46–49] macrophages, neutrophils, and T cells releasing pro-inflammatory mediators, as summarised in Table I and Fig. 1, which directly activate and sensitise[50–53] nociceptive nerve endings innervating the synovium.[51] The following sections will discuss neuro-immune cellular interactions within joint synovium and their direct and indirect roles in pain sensitisation.

Synovial fibroblasts

Synovial fibroblasts in healthy joints remain quiescent, maintaining extracellular matrix protein production, the structural architecture of the tissue and synovial fluid.[54] Following onset of arthritis, synovial fibroblasts adopt an aggressive, proliferative and inflammatory phenotype which contributes to synovial hyperplasia, synovitis and the secretion of cartilage damaging matrix metallo proteases (MMPs) and aggreganases.[55] Whilst synovitis correlates with arthritic pain (Section 2), the complex cellular interplay was poorly described until the advancement of single-cell transcriptomic approaches. Using this approach Nanus et al.[22] identified distinct subsets of synovial fibroblasts in OA, predominately enriched in patient-reported sites of pain. Fundamental differences in the representation of these populations in early versus late disease stages of OA suggested these populations are not only pain-associated but are differentially modulated over the course of OA pathogenesis. Pain-associated fibroblast subsets were found to influence neurogenesis signalling pathways and neuronal growth and survival pathways with high expression of NRN1 and sulfatase 1.[13] These findings were mechanistically validated in vitro following incubation of DRG neurons with supernatants from painful and non-painful fibroblasts, confirming that the secretome of fibroblasts from painful sites promotes greater neurite growth and survival.[22] These data suggest that cellular crosstalk between distinct populations of synovial fibroblasts and nerve cells at the site of OA joint pain may contribute to nociceptor activity and pain. More recently, two studies have identified a $Prg4^{+ve}$ synovial fibroblast subset, which expands in the synovial lining during progression of post traumatic OA[56,57] and inter-articular injection of the Wnt agonist, R-spondin 2, which is secreted by $Prg4^{+ve}$ fibroblasts, induced joint hyperalgesia in healthy C57Bl6/J mice.[57] Thus $Prg4^{+ve}$ synovial fibroblasts and R-spondin-2 may provide a novel mechanism with therapeutic potential for both resolving joint pain and OA pathogenesis.

Mechanistically, synovial fibroblasts may mediate pain responses via induced expression of neurotrophin receptors on the surface of joint nociceptors. Indeed, synovial fibroblasts from inflamed knee joints, which secrete PGE2 and IL6, co-cultured with DRG neurones increased expression of neurokinin-1, bradykinin-2 and TRPV1 which have established roles in pain signalling.[58] The crosstalk between synovial fibroblasts and nociceptors is likely facilitated by pro-inflammatory cytokines driving immune cell recruitment and nociceptor activation. For example, synovial fibroblasts from hip OA patients secrete greater levels of IL6 and C-X-C motif chemokine ligand 8 (CXCL8), which is further exacerbated in fibroblasts from obese OA individuals.[49,59] TNFα is also strongly implicated in contributing to OA pain.[60] Intra-articular injection of TNFα in wild-type rodent knee joints increased responses of nociceptive C-fibres and Aδ-fibres to both innocuous and noxious rotation of the joint,[61] and co-administration of TNF-neutralising fusion protein etanercept, a COX inhibitor, or diclofenac, reduced this sensitization.[61] Conditioned media from TNFα stimulated synovial fibroblasts decreased DRG neuronal resting membrane potential, compared to non-stimulated control media,[62] further supporting the ability of inflammatory synovial fibroblasts to release a cocktail of molecules able to sensitise sensory neurons. The cytokine IL15 is also secreted by fibroblasts and is present in OA synovial fluid,[63] circulating levels of IL15 correlate with patient-reported pain severity, but not radiological severity of OA.[64,65] Recently, netrin-4 was identified in synovial lining fibroblasts associated with pain, where in vitro functional studies reported that netrin-4 enhanced DRG neurite outgrowth and sprouting.[66] Whilst the mechanistic understanding of synovial fibroblasts in joint pain modulation is on-going, these recent advances in knowledge support the targeting of the inflammatory phenotype of synovial fibroblasts as a potential rewarding therapeutic strategy to reduce inflammation-mediated joint damage and pathological crosstalk with nociceptors that mediate pain.

Tissue resident macrophages

Macrophages are the most abundant immune cell within the joint, representing 12–40% of the immune cells in synovium and synovial fluid.[25,67] In healthy joints, macrophages reside throughout the sub-lining and lining layers of the synovium and are relatively quiescent.[68] However, in inflamed joints macrophages become pro-inflammatory releasing cytokines driving articular joint destruction.[68] Macrophages are classically characterised based on their inflammatory status on a nuanced spectrum of having a pro-inflammatory or anti-inflammatory phenotype. Single-cell sequencing of macrophages has highlighted their transcriptomic complexity and the spectrum of their potential functional activity.[69] In OA, pro-inflammatory macrophages are associated with joint destruction through regulation of MMPs, collagen and aggrecans, whilst anti-inflammatory macrophages are believed to contribute to tissue repairing, promoting a protective joint environment through production of anti-inflammatory and chondroprotective IL4 and IL10.[70,71] In knee OA the ratio of pro-inflammatory to anti-inflammatory macrophages is significantly higher, compared to healthy controls and positively correlates with severity of OA.[72] Pro-inflammatory macrophages are present in 76% of arthritic knees and significantly associated with severity of self-reported joint pain intensity from the National Health and Nutrition Examination Survey.[73] Similarly, pain in joints of the hand and feet also show positive association with pro-inflammatory macrophages at these sites.[73] Macrophage accumulation is recognised as a hallmark of synovitis in the arthritic joint.[74]

The MCP1, a chemokine produced by chondrocytes in response to inflammatory stimuli, is a ligand of the CCR2 receptor present on monocytes and macrophages. MCP1 recruits CCR2-expressing monocytes to tissues where they differentiate into pro-inflammatory macrophages driving local tissue damage.[75] A positive correlation between serum and synovial fluid MCP1 levels in OA patients and self-reported pain has been described.[76] In destabilisation of the medial meniscus (DMM)-induced arthritic mice, CCR2-null mice were negative for infiltrating macrophages and did not develop pain-related behaviours.[77] Damage associated molecular patterns released from damaged joint tissues activate toll like receptors on macrophages which in turn induce the production of allogenic molecules which can act directly on nociceptive sensory neurons.[78] Thus, macrophages may contribute to the development of OA pain through the expression of allogenic molecules such as MIP-1α,[79] MIP-1β,[80] TNFα,[68] IL6 and IL1β[79,81] and NGF,[82] which promotes

Cellular origin	Biomarker	Mechanism of action	Correlation with pain	Study details	Results	Ref
Joint tissue	TNFα	Direct & Indirect: Irrespective of the cellular source, production of cytokines in response to pro-inflammatory stimuli and injury initiates a cascade of neuronal excitability which underlies chronic pain as reviewed by Miller et al, 2015.	↑	47 OA patients. Synovial fluid measured by ELISA. Self-reported pain scores (WOMAC).	TNFα significantly correlated with pain.	31
		Unknown	↓	86 OA patients. Synovial fluid measured by ELISA. Self-reported pain scores (NRS, VAS and WOMAC).	TNFα significantly negatively correlated with pain (VAS and NRS) but no correlation with WOMAC. VAS scores also negatively correlated with IL-1β, IL-6.	60
		Direct: TNFα alters neuronal membrane potential.	↑	60 adult male rats.	A single TNFα injection into the knee caused sensitisation of Aδ and C fibres upon mechanical stimuli to the joint. Patch clamp showed increased neuronal signalling in isolated DRGs when stimulated with TNFα.	61
	MCP-1	Unknown	↑	161 OA patients. Synovial fluid measured by ELISA. Self-reported pain scores (WOMAC).	MCP-1 significantly correlated with pain.	75
Joint tissue + circulation	IL15	Unknown	↑	226 OA patients. Serum measured by ELISA. Self-reported pain scores (WOMAC).	IL15 significantly correlated with pain.	64
Synovium	TrkB	Direct	↑	10 OA patients. Self-reported pain scores (VAS). Next-generation RNA sequencing performed on synovial tissue.	TrkB was enriched in “high pain” synovial tissue. Neurotrophic factors that utilise TrkB were not differentially expressed between high and low pain tissue.	35
Synovium	RN7SL3	Unknown	↓	10 OA patients. Self-reported pain scores (VAS). Next-generation RNA sequencing performed on synovial tissue.	RN7SL3 was significantly downregulated in “high pain” synovial tissue.	35
Synovium	RP11-195E2.1	Unknown	↓	10 OA patients. Self-reported pain scores (VAS). Next-generation RNA sequencing performed on synovial tissue.	RP11-195E2.1 was significantly down-regulated in “high pain” synovial tissue.	35
Synovium	miR-146	Indirect: miR-146 is considered a neuroinflammatory regulator in neurological conditions. miR146 has phasic expression in OA	↑	10 OA patients. Self-reported pain scores (VAS). Next-generation RNA sequencing performed on synovial tissue.	miR-146-3p was significantly down-regulated in “high pain” synovial tissue.	35
		Unknown	↑↓	Meta-analysis. 26 articles included. Various tissues of OA patients.	miR-146a expression was significantly higher in OA patients in peripheral blood mononuclear cells and cartilage. but not in plasma. MiR-146a expression was significant in synoviocytes in OA patients. There were no differences found in miR-146a expression in synovial tissue, synovial fluid, and regulatory T cells.	37
		Unknown	↑	Mice. MLI model of pain. Electronic von Frey testing and SMALGO assays were used to assess pain behaviours. OA patients, cartilage tissue collected.	High-throughput miRNA-Seq identified MiR-146a-5p as the most responsive miRNA to IL-1β in murine chondrocytes and is upregulated in human OA cartilage. Silencing of miR-146-5ap in chondrocytes reduced both cartilage degradation and pain.	38
		Unknown	-	5 OA patients, 5 rheumatoid arthritis (RA) patients, 1 healthy control.	RT-PCR showed no difference in miR-146a expression between OA and healthy synovial tissue.	40
		Indirect: miR-146a regulates inflammatory and pain-associated molecules.	↑↓	Rats. MIA-induced model of OA. Knee pressure hyperalgesia, knee extension hyperalgesia, mechanical allodynia (von Frey) and knee oedema testing were used to assess pain behaviours. OA patients.	MiR-146a expression in rat DRGs negatively correlates with pain. Synthetic miR-146a modulates pain-related molecules e.g TRPV1 in human glial cells. RT-PCR shows transfection if miR-146a in human chondrocytes regulates inflammation.	41

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Table I (*continued*)

Cellular origin	Biomarker	Mechanism of action	Correlation with pain	Study details	Results	Ref
Synovial fibroblasts	IL6	Unknown: activated fibroblasts release pro-inflammatory cytokines and chemokines promoting inflammation and sensitization of nociceptors.	↑	17 female rats. Antigenic and Freund's adjuvant model of OA pain.	IL6 expression was higher in synovial fibroblast supernatant in inflamed joints compared to control.	58
		Unknown	↓	86 OA patients. Synovial fluid measured by ELISA. Self-reported pain scores (NRS, VAS and WOMAC).	TNFα significantly negatively correlated with pain (VAS and NRS) but no correlation with WOMAC. VAS scores also negatively correlated with IL-1β, IL-6.	60
	TNFα	Direct: TNFα alters neuronal membrane potential.	↑	Female mice. CFA model of OA pain. 1 OA patient, 3 RA patients.	Recombinant TNFα depolarised resting membrane potential, increased spontaneous action potential firing, and enhanced TRPV1 function in mice neurons. This effect was elevated when neurons were co-cultured with synovial fibroblasts.	62
Macrophage	C-C chemokine receptor type 2 (CCR2)	Indirect	↑	237 mice total. DMM model of OA on wild-type or CCR2 null mice. Von Frey and LABORAS testing were used to determine pain.	Increased expression of MCP-1 and CCR2 (mRNA and protein) and signalling activity correlated with pain behaviours. Macrophage infiltration was not observed in CCR2-null mice but was shown in wild-type.	76
Macrophage	MIP-1α/ IL1β	Indirect: MIP-1alpha contributes to the development of neuropathic pain through regulation of IL1β.	↑	Male mice. PSL model of pain. Von Frey and Hargreaves testing were used to assess pain behaviours. Injection of recombinant MIP-1α or anti-MIP-1α.	Tactile allodynia and thermal hyperalgesia were prevented by anti-MIP-1α injection. Injection of recombinant MIP-1α on sham operation control elicited tactile allodynia and thermal hyperalgesia. MIP-1α, and IL1β mRNA were significantly upregulated in macrophages and sciatic nerve after PSL. Anti-MIP-1α injection supressed IL1β mRNA expression.	78
Macrophage	MIP-1β	Direct: MIP-1β induces neuropathic pain through the MIP-1β/CCR5 axis.	↑	Male mice. PSL model of pain. Von Frey and Hargreaves testing were used to assess pain behaviours. Injection of recombinant MIP-1β or anti-MIP-1β.	mRNA expression of MIP-1β and CCR5 were up-regulated in the sciatic nerve after PSL compared to sham. MIP-1β immunoreactivity was localised to macrophages. PSL induced tactile allodynia was prevented by anti-MIP-1β. Administration of CCR5 antagonist, prevented tactile allodynia and thermal hyperalgesia. Single administration of recombinant MIP-1β elicited tactile allodynia.	79
Macrophage	Let-7b	Direct: Let-7b activates nociceptors through TLR7 coupling with TRAPA1 ion channel.	↑	Mice. Pain behaviours assessed through seconds licking, lifting or flinching affected paw.	Patch clamp of DRGs show TLR7-dependant signalling of let-7b and was blocked by TRPA1 antagonist. Intraplantar injection of let-7b evoked spontaneous pain in a dose dependent manner, which was abolished in TLR7 knockout mice.	85
Macrophage	miR-21-5p	Indirect: miR-21-5p released by neurons is phagocytosed by macrophages resulting in a shift towards increased pro-inflammatory macrophages.	↑	Male mice. Von Frey testing assessed pain behaviours.	MiR-21-5p is upregulated in DRG neurons after nerve injury. Antagomir silencing and miR-21 KO reduced nociceptive hypersensitivity and macrophage recruitment.	88
		Indirect: miR-21 released by neurons contributes to nociceptive pain through TLR7 signalling.	↑	Male rats. SNI model of pain. PAM and von Frey testing were used to assess pain behaviours.	Intra-articular injection of miR-21 resulted in long-term analgesia and injection of miR-21 inhibitor increased pain. Analgesic effects of miR-21 were blocked by TLR7 antagonisation.	89
		Indirect: miR-21 released by neurons contributes to neuropathic pain through TGF-βR and CCL2 signalling in macrophages.	↑	Male and female mice. SNI model of pain. Von Frey testing was used to assess pain behaviours.	MiR-21 KO attenuated allodynia which was restored by TGF-βR inhibitor. MiR-21 deletion in DRGs induced TGF-β–related pathway activation and an anti-inflammatory macrophage phenotype.	90
T-cell	IL17	Direct	↑	Male mice. AIA and ZIA models of OA pain. Electronic pressure-metre testing and tibio-tarsal joint flexion testing were used to assess pain behaviours.	IL17 increased in AIA joints over time. Treatment with IL17 antibody inhibited hypernociception and neutrophil recruitment.	117

(*continued on next page*)

Table I (*continued*)

Cellular origin	Biomarker	Mechanism of action	Correlation with pain	Study details	Results	Ref
Neutrophil	C5a	Direct: C5a mediates neutrophil dependent inflammatory mechanical hypernociception.	↑	Male rats and mice (6 per group). ZIA, carrageenan, lipopolysaccharide, and AIA were used as pain/OA models. Modified Randall-Selitto tests and electronic pressure metre paw tests were used to assess pain behaviours. Skin lysates were measured by ELISA.	C5aR antagonist inhibited hypernociception and the recruitment of neutrophils by zymosan. C5a-induced hypernociception was attenuated in neutrophil depleted rats.	127
Neutrophil	LTB_4	Indirect: LTB4 mediates hypernociception and recruitment of neutrophils.	↑	Male mice. ZIA model of pain. Electronic pressure metre tests were used to assess pain behaviours.	Both zymosan-induced hypernociception and neutrophil migration declined in a dose-dependent manner with LTB4 receptor antagonist. Articular injection of LTB4 induced dose-dependent hypernociception. Zymosan increased LTB4 protein expression.	128
Neutrophil	Elastase	Indirect: Elastase cleaves PAR2 activating TRPV4 on neurons through Gαs-mediated cAMP formation.	↑	Male mice. Von Frey testing was used to assess pain behaviours.	Intraplantar injection of elastase resulted in hyperalgesia by PAR2 and TRPV4-mediated mechanisms. Elastase stimulated a PAR2-dependant and TRPV4-mediated calcium influx in nociceptors.	137

↑ increased with pain, ↓ decreased with pain, PAM- Pressure Application Measurement, WOMAC- Western Ontario and McMaster Universities Osteoarthritis Index, VAS- Visual Analogue Scale, NRS- Numeric Rating Scale, SNI- Spared Nerve Injury, PSL- Partial Sciatic Nerve Ligation, MLI- Meniscal Ligament Injury, MIA- Monosodium Iodoacetate, CFA- Complete Freund's Adjuvant, AIA- Antigen-induced Arthritis, ZIA- Zymosan-induced Arthritis, SMALGO- Small Animal Algometer.

Table I

Pain associated biomarkers which mediate nociceptive joint pain through cellular crosstalk.

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sensory terminal sprouting. Indeed, the expression of receptors for pro-inflammatory cytokines and chemokines on nociceptive neurons are upregulated following nerve injury, activating nociceptors leading to hypersensitivity.[83]

The bilateral crosstalk of macrophages and nociceptors also involves microRNAs and microRNA-containing exosomes, as described by Chen et al.,[84] microRNA let-7b is secreted from macrophages and activates nociceptors through TLR7 coupling with TRAPA1 ion channel.[85] In mice, intraplantar injection of let-7b dose-dependently evoked spontaneous pain, which was abolished in TLR7 knockout mice.[85] Let-7b is significantly increased in synovial fluid from OA patients,[86] and in RA mice models let-7b transformed naïve myeloid cells into M1 macrophages.[86,87] Whilst studies in OA and RA recognise the involvement of let-7b in joint inflammation, to date none have explored its potential in relation to pain.

Peripheral nociceptors can also influence the polarity of macrophages through miR-21-containing exosomes.[88] In a murine nerve injury model, these exosomes are phagocytosed by macrophages, shifting their phenotype towards a pro-inflammatory status, whilst antagomir silencing of miR-21 reduced nociceptive hypersensitivity and pro-inflammatory macrophage numbers.[88] A single intra-articular injection of a miR-21 inhibitor in a surgical OA rat model reduces pain behaviour responses however macrophage populations were not investigated.[89] Conditional deletion of miR-21 in DRG neurons in a murine model of neuropathic pain reduced pain behaviour and levels of MCP1, and was associated with an anti-inflammatory macrophage phenotype.[90] Neurons within OA-damaged joints are reported to reprogram DRG macrophages towards a pro-inflammatory phenotype driving pain behaviour.[45] As such, manipulating the polarity of macrophages to favour anti-inflammatory-like phenotype may be therapeutically beneficial in resolving inflammation and joint pain.

Lastly, the implications of new subsets of synovial fibroblasts are an avenue for further exploration. Culemann et al. identified a subset of CX_3CR1^{+ve} resident macrophages in RA and OA synovium forming a physical immunological barrier which protects and restricts inflammatory reaction.[69] These epithelial like macrophages are locally repopulated by CX_3CR1^{-ve} cells which are independent of recruited blood monocytes and are functionally distinct, shielding intra-articular structures through tight-junction mediated barrier.[69] The immune-regulatory significance of these barrier forming cells was highlighted through selective depletion of CX_3CR1^{+ve} macrophages which abolished the synovial barrier in healthy mice and accelerated and exacerbated onset of arthritis.[69] Similarly, Chou et al. reported high expression of immune regulatory genes, STAB1, TXNIP and CD169 that characterised an immune regulatory Major Histocompatibility Complex, Class II, DR Alpha (HLA-DRA) +ve macrophage subpopulation in OA.[91] Although the exact functions of this $HLA\text{-}DRA^{+ve}$ population are yet to be determined, transcriptome profiling of this subpopulation suggests an anti-inflammatory role, via phagocytosis-mediated clearance of cellular debris and inflammatory mediators.[91] Such $HLA\text{-}DR^{+ve}$ macrophages are also reported to be correlated with symptoms of pain in a cohort of women with endometriosis, where a particular $HLA\text{-}DR^{+ve}$ subset consisting of high surface expression of CD14 correlated with pain independent of endometriosis diagnosis suggesting these macrophages have global functionality across many conditions.[92] Alivernini et al. describe four subpopulations of RA synovial tissue macrophages, including a $MerTK^{+ve}$ subset associated with increased risk of RA flare.[93] Specifically, $MerTK^{+ve}$ $CD206^{+ve}$ macrophage clusters were associated with healthy and RA patients who sustained disease remission. Interestingly, Kobayashi et al. found M2 macrophages at sites of nerve injury have low surface levels of MerTK resulting in insufficient efferocytosis and poor clearance of apoptotic cells which in turn results in chronic pain sensitisation.[94] Lastly, Huang et al. identified infiltrating $F4/80^{intermediate}$ monocytes which differentiate

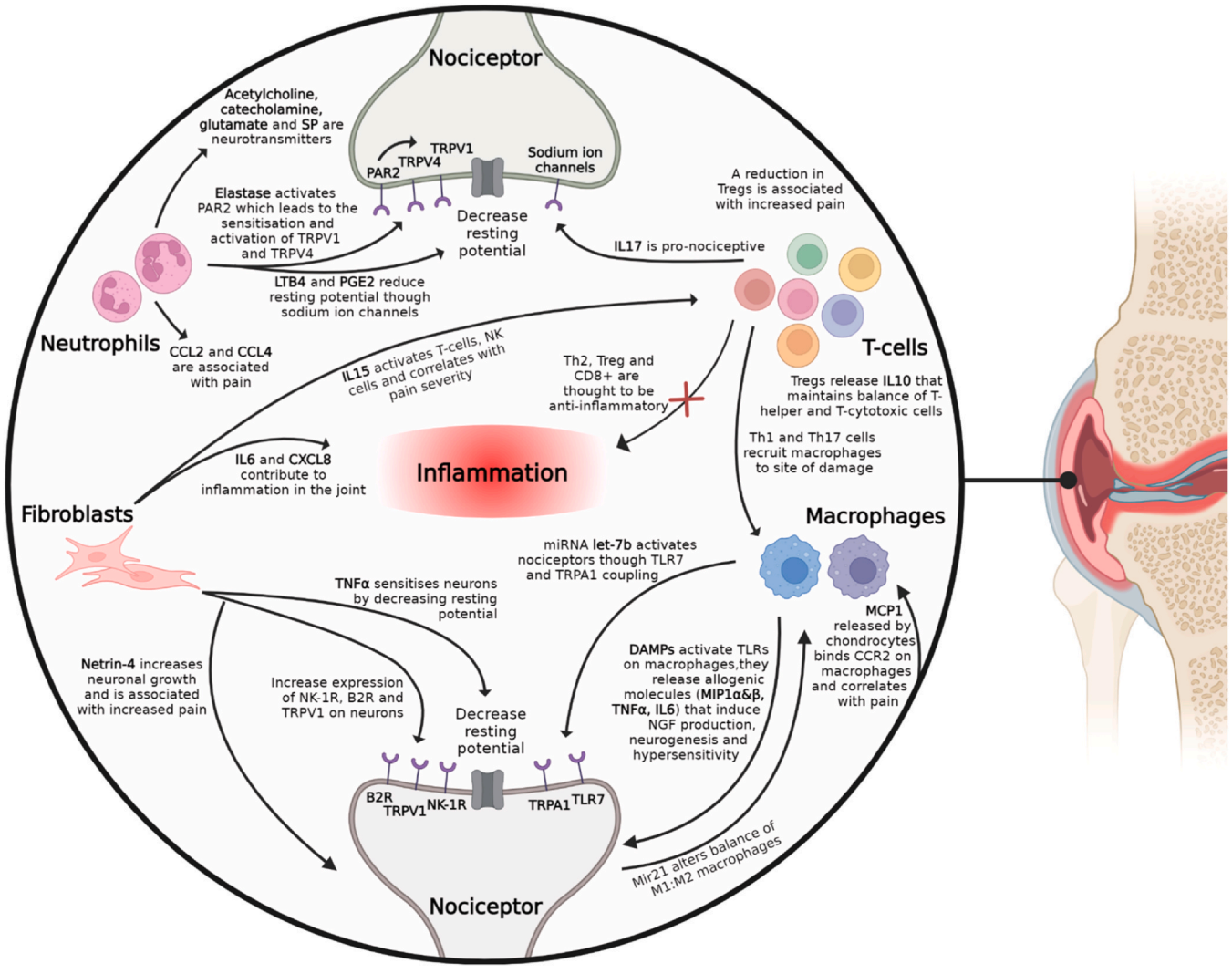

Fig. 1

Osteoarthritis and Cartilage

Schematic of the cellular crosstalk interactions within the inflamed OA synovium that can sensitise and activate sensory joint nociceptors. Fibroblasts, macrophages, T-cells, and neutrophils release a plethora of pro-inflammatory cytokines, chemokines, microRNAs, neurotransmitters and neuromodulatory factors which directly or indirectly modulate pain sensitisation and pain receptors on neuronal membranes. Interestingly, studies report several subpopulations of these cells (not all depicted) which are differentially associated with inflammation and joint pain. Many of those subpopulations are yet to be defined in relation to pain in OA.

into self-renewing F4/80^{high} tissue resident macrophages subpopulations that promote the resolution of RA synovial inflammation.[95] However, reports in OA suggest F4/80^{+ve} macrophages are pro-inflammatory where they accumulate at DRGs, maintaining pain independently of the damaged knee.[45] Whilst CX3CR1^{+ve}, MerTK^{+ve}, HLA-DRA^{+ve} and F4/80^{+ve} macrophage subpopulations are reported in OA,[96] these should be further explored in the context of pain given these subpopulations may influence fundamental analgesic properties. Currently, understanding such diverse and tissue-specific macrophage subsets is very much in its infancy and these are yet to be characterised in the context of nociceptive pain. However, they provide new opportunities for targeted interventions, which is further addressed in a recent review by Wang et al.[97]

T cells

Amongst the mononuclear cells identified within OA synovium and synovial fluid are T lymphocytes (T cells).[98] T cells are a major class of leukocytes, which are a key component of the adaptive arm of the immune system involved in both cell-mediated and humoral immunity. Circulating T cells are also capable of infiltrating vascular tissues, including the synovial membrane, where they form large aggregates surrounding the vasculature.[99] These aggregates express antigens CD69, CD25, CD38 and CD80, representative of activated T-cells.[99,100] Whilst the percentage of peripheral blood T-cells is lower in OA patients compared to RA, these levels are higher than in healthy subjects.[101] Indeed, profiling of joint synovial fluids find

T-cell populations are relatively similar in OA and RA patients, suggesting T-lymphocytes may also contribute to pathogenesis and pain in OA.[102] Interestingly, immunohistochemical characterisation of synovitis in OA found synovium nearest cartilage with higher degrees of synovitis contained more T-lymphocytes.[103] More recently, 22% of immune cell infiltrates within synovium were identified as T cells.[104] Indeed, $CD3^{+ve}$ lymphocytes are reported in 65% of OA patient synovium, particularly within the sub-lining layer.[100,105] Whilst circulating ratios of $CD4^{+ve}$ T-helper and $CD8^{+ve}$ cytotoxic T-cells are similar,[100] infiltrating T-lymphocytes are predominately $CD4^{+ve}$ T-helper cells.[106]

T cells play a role in pain pathology particularly in chronic inflammatory diseases and are implicated in mediating neuropathic pain in models of nerve injury. Nees et al. reported significant correlation between OA-induced disability, knee pain and disease severity with synovium infiltrating T-helper subsets.[107] Although mechanistic studies have shown conflicting results, the discrepancy could be explained by the presence of different T cell subsets at the site of inflammation and/or injury. For example, regulatory T cells (Tregs), Th2 and $CD8^{+ve}$ cells are thought to be beneficial in ameliorating pain in inflammatory models,[108] by promoting an anti-inflammatory microenvironment.[109–111] Baddack-Werncke et al. found depletion of $CD8^{+ve}$ cytotoxic T cells increased arthritis-induced pain hypersensitivity.[111] In an antigen- and collagen-induced arthritis murine model, depletion of $CD8^{+ve}$ T cells resulted in persistent mechanical allodynia and thermal hyperalgesia for more than 2 months after induction of arthritis.[111] Interestingly, $CD8^{+ve}$ T cell depletion did not affect autoantibody formation or severity of joint inflammation, where serum levels of pro-inflammatory cytokines TNFα and IL17 were increased, suggesting a protective role.[111] Conversely, Th1 and Th17 cells facilitate macrophage infiltration in damaged nerve models promoting neuropathic pain,[112,113] which may be of relevance in OA where Th1 and Th17 infiltration of the synovium, correlates with OA-induced disabilities.[107]

Both Th1 and Th17 subpopulations accumulate in OA joint synovial fluid and within OA synovial tissues, with Th1 polarisation specifically reported in end-stage OA knee joint synovial fluid.[101,114,115] Critically, Th17 cells produce the pro-nociceptive cytokine IL17, known to regulate key signalling pathways in the release of nociceptive and inflammatory mediators indirectly influencing neuronal sensitivity.[116] Indeed, IL17 levels were increased in an antigen-induced arthritis mouse model. Furthermore, interarticular injection of IL17-induced hypernociception, which was inhibited on treatment with either infliximab, CXCR1/2 and IL1 receptor agonists, doxycycline, bosentan, indomethacin or guanethidine.[117] Compared to control groups, OA serum levels of IL17 are significantly increased, and positivity correlated with knee pain severity suggesting Th17 cells are a likely contributor of nociceptive pain sensitization in OA.[118] The potential of IL17 as an analgesic target has been addressed by Jiang et al.[116] Interestingly, sympathectomy using 6-hydroxydopamine in a murine antigen-induced arthritic model found both Th1 and Th17 cell responses to be blunted suggesting bidirectional crosstalk between nociceptors and T cells.[119]

Another T-helper subpopulation is the immunoregulatory anti-inflammatory Tregs, which have reduced responses in RA and are reportedly present at similar levels in OA.[101] In OA, immune cell profiling found Tregs were localised to synovial fluid and knee pain was associated with decreased Treg infiltration of synovium,[120] Tregs produce anti-inflammatory IL10 which is important in suppressing the activation of T-helper and cytotoxic T-cells. As such, a reduction in Tregs influences a shift in inflammation from anti-inflammatory to pro-inflammatory.[120] Interestingly, the presence of Tregs was found to be a contributing factor in pain hypersensitivity. Profiling of the immune cell compartment at sites of spinal cord injection of CSF1, identified preferential expansion of Tregs associated with reduced pain behaviours in female mice. Whilst Treg deficient female mice showed increased pain hypersensitivity comparable to male mice,[121] suggesting here pain sensitization induced by CSF1 injection is supressed by Tregs.

Whilst several T cell subsets have been identified within OA synovium and synovial fluid that may directly and indirectly interact with nociceptors at the joint site, determining the cellular mechanisms that mediate OA joint pain will likely require a better understanding of T cell subpopulations in mediating and contributing to pain signalling. These studies should take account of such variables as the pathological nature of pain and inflammation, the presence of particular T cell subsets, and patient characteristics such as age,[122] gender,[121] and BMI,[120] all of which reportedly influence the contribution of T cells to the onset of inflammation and likely resolution of joint pain.

Neutrophils

Produced by the bone marrow at a rate of $1–2\times10^{11}$ per day, neutrophils are the most abundant leukocytes in the blood.[123] As critical effector cells of the innate immune system, they are one of the earliest immune cells recruited to sites of damage or infection.[123] In patients with OA, although the least abundant, neutrophils are thought to be the first type of leukocyte to infiltrate the synovium, reportedly constituting 26% of immune cell populations in knee OA synovial tissue.[53,124] The infiltration and accumulation of neutrophils into the joint is associated with hyperalgesia,[125] where activated neutrophils secrete an abundance of inflammatory mediators, including cytokines, ROS and arachidonic acid derivatives, many of which have been linked to nociceptor activation.[126–129] Genetic ablation of receptors and mediators of neutrophil adhesion and migration, as well as direct depletion of neutrophils, has been shown to reduce mechanical hyperalgesia in mice.[130] Much of this effect is likely driven by the increased release of pro-inflammatory mediators from activated neutrophils. In OA, neutrophil-derived MCP1 and CCL4 are positively associated with OA-induced joint pain.[53] Neutrophils are also a source of neurotransmitters, including acetylcholine, catecholamine, glutamate and SP, further substantiating this crosstalk.[131–133] Notably, leukotrienes and prostaglandins are released by neutrophils in response to inflammatory stimuli, particularly LTB_4 and PGE_2. These act directly on nociceptors causing hypernociception, a reduction in the threshold required for activation by modulating voltage-dependent sodium channels.[126] C5a mediated inflammatory mechanical hypernociception in rats,[127] and the role of LTB_4 in zymosan-induced joint nociception in mice,[127,128] are shown to be neutrophil dependant.

Neutrophils secrete elastase, a known protease-activated receptor-2 (PAR-2) agonist. PAR-2 receptors are activated by proteolytic cleavage and lead to the sensitisation and activation of TRPV1 and TRPV4 ion channels, which are strongly associated with joint nociceptive pain in arthritis.[134,135] Rodent studies have identified a PAR2 receptor activating peptide, SLIGRL-NH(2), which reduces pain thresholds,[136] resulting in increased allodynia and hyperalgesia, whilst neutrophil released elastase caused PAR-2 dependent sensitisation of TRPV4.[137] Hsueh et al. found neutrophils were a major immune population within OA synovium, second only to macrophages, and levels of elastase were significantly associated with radiographic signs of knee OA.[124] Additionally, neutrophil elastases significantly compromised cartilage structure and pain behaviours in Complete Freund's Adjuvant-induced rodent arthritis models.[138] Interestingly, intra-articular injection of a-1-antitrypsin, which has a strong affinity for neutrophil elastase, prevented leukocyte infiltration, improved cartilage integrity, and ameliorated joint pain.[138] Similarly, inhibition of neutrophil elastase with sivelestat and serpinA1 in the early stages of monoiodoacetate-induced OA in mice improved inflammation, pain and nerve damage scores.[139] Sivelestat is an attractive therapeutic given it is a selective inhibitor of neutrophil elastases which can reduces both inflammation and pain through suppression of

TNFα, IL6, nitric oxide, PAR2, p44/42 mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells ((NF-kβ) and as such has scope beyond analgesia.[53,138] Baral et al. reported that TRPV1^{+ve} neuron ablation in mice significantly increased neutrophil migration during lung infection where neuronal calcitonin gene-related peptide secretion inhibited both the recruitment and phagocytic capabilities of neutrophils by inhibiting CXCL1.[140–142] Interestingly, calcitonin gene-related peptide is also produced by synovial macrophages and fibroblasts in OA suggesting there is a complex network of bidirectional, multi-cellular cross-talk which may influence joint inflammation and pain sensitization.[143]

Conclusions

The long-held view of OA as solely a "wear and tear" disease of the cartilage has undoubtedly hampered the development of not only disease-modifying OA drugs but also the development of efficacious OA analgesics. Now, however, with the paradigm shift that OA is a disease of the whole joint encompassing multiple tissues including nerve sensory terminals, there is not only a better understanding of the pathophysiology of OA but also a greater pool of potential targets for the development of new therapeutics. To this end, the relationship between synovitis and OA pain and the understanding that nociceptor activity is influenced by cellular crosstalk within the inflammatory synovium provides new candidate pathways and targets across multiple cell types, including the rationale for targeting specific cellular subsets, which may represent opportunities for the repurposing of existing drugs or the development of new drugs as effective OA analgesics.

Moving forward, the functional validation of these cell subsets and candidate targets in predictive, translatable preclinical models of OA pain will be crucial. To this end, the development of more sophisticated in vitro models, utilising compartmentalised microfluidic-based systems or organoids comprising human cells, will be important in enabling a deeper understanding of the intricate cellular interactions within the inflammatory synovium. *In vivo*, the surgically induced DMM rodent model is considered the gold standard in studying the onset and progression of OA. It is a slow progressing model that replicates many of the pathological features of human OA, resulting in altered pain behaviours including loading-associated pain (weight-bearing) and distal pain (lowered hind paw withdrawal thresholds) from 12 weeks post-surgery.[144] However, the rationale for targeting specific cellular subsets may not be feasible to test in rodent models if significant differences in transcriptomic profiles are found in synovial cell subsets, compared to those reported in the human OA joint. Indeed, Chakrabarti et al. provide a compelling discussion for the use of larger animals in the study of arthritic pain particularly given the biomechanical properties of larger animals are more akin to humans compared to rodent models.[145] Therefore, continued efforts to bridge the gap between animal models and human OA pathology will be pivotal for successful forward and reverse translational studies.

In conclusion, the concept of local environment and cellular crosstalk within the inflammatory synovium as a driver of nociceptive joint pain presents a compelling opportunity for future research and therapeutic advancements. Elucidating the underlying mechanisms and the development of more predictive preclinical models will provide a pathway to translating these discoveries into effective treatments for OA patients, ultimately improving their quality of life and well-being.

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Contributions

SNW, JA, FDB, VC and SWJ are involved in review conceptualisation. SNW, CD and SF wrote the first draft, and CD produced figures. SNW extensively revised the manuscript. SWJ, VC, FDB. JA and ETD revised and approved the final draft.

Declaration of competing interests

The authors report no conflict of interest.

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