Arthritis & Rheumatology
Vol. 77, No. 6, June 2025, pp 664–676
DOI 10.1002/art.43089

American College of Rheumatology
Empowering Rheumatology Professionals

Association of Synovial Innate Immune Exhaustion With Worse Pain in Knee Osteoarthritis

Holly T. Philpott,[1] Trevor B. Birmingham,[1] Garth Blackler,[2] J. Daniel Klapak,[2] Alexander J. Knights,[3] Easton C. Farrell,[3] Benoit Fiset,[4] Logan A. Walsh,[4] J. Robert Giffin,[1] Edward M. Vasarhelyi,[1] Steven J. MacDonald,[1] Brent A. Lanting,[1] Tristan Maerz,[3] and C. Thomas Appleton,[1] on behalf of the WOREO Knee Study Group

Objective. Uncontrolled pain remains a major clinical challenge in the management of knee osteoarthritis (OA), the most common disabling joint disease. Worse pain is associated with synovial innate immune cell infiltration (synovitis), but the role of innate immune-regulatory cells in pain is unknown. Our objective was to identify synovial innate immune cell subsets and pathophysiologic mechanisms associated with worse pain in patients with knee OA.

Methods. Synovial tissue biopsies from 122 patients with mild-to-severe knee OA pain (Knee Injury and OA Outcome Score [KOOS]) were analyzed to identify associations between synovial histopathology and worse pain. We then used spatial transcriptomics and proteomics of synovial tissue microenvironments (n = 32), followed by single-cell RNA sequencing (n = 8), to identify synovial cell composition and cell-cell communication networks in patients with more severe OA pain.

Results. Histopathological signs of synovial microvascular dysfunction and perivascular edema were associated with worse KOOS pain (–10.76; 95% confidence interval [CI] –18.90 to –2.61). Patients with worse pain had fewer immune-regulatory macrophages, expanded fibroblast subsets, and enrichment in neurovascular remodeling pathways. Synovial macrophages from patients with worse pain expressed markers of immune exhaustion and decreased phagocytic function (–19.42%; 95% CI –35.96 to –2.89) and their conditioned media increased neuronal cell stress in dorsal root ganglia.

Conclusion. Although synovitis increases during OA, our findings suggest that exhaustion, dysfunction, and loss of immune-regulatory macrophages is associated with worse pain and may be an important therapeutic target.

INTRODUCTION

Pain is a major clinical challenge in osteoarthritis (OA) and the main driver of disability and poor quality of life. Synovitis is associated with increased pain and OA disease progression.[1–5] Although synovitis is an important and potentially modifiable contributor to knee OA pain and progression, we currently lack sufficient understanding of underlying mechanisms to define effective treatment targets.[1,3,6]

The inflammatory mechanisms associated with OA are clearly different from those driving disease activity and progression in rheumatoid arthritis (RA). Whereas RA is an autoimmune disease with a major component involving the adaptive immune system, inflammation in OA is largely mediated by innate immune cells and mechanisms.[7–11] Understanding the role of innate immune mechanisms in OA may yield important insights related to uncontrolled OA pain.

Supported in part by grants from the Academic Medical Organization of Southwestern Ontario, the Canadian Institutes of Health Research (CIHR), the Canada Research Chairs Program, and Western University's Bone and Joint Institute. Dr Philpott's work was supported by a Frederick Banting and Charles Best Doctoral Award from CIHR. Dr Knights' work was supported by a Pioneer Postdoctoral Fellowship from the University of Michigan and the National Institutes of Health (grant K99-AR-081894).

[1]Holly T. Philpott, PhD, Trevor B. Birmingham, PT, PhD, J. Robert Giffin, MD, MBA, Edward M. Vasarhelyi, MD, MSc, Steven J. MacDonald, MD, Brent A. Lanting, MD, MSc, C. Thomas Appleton, MD, PhD: University of Western Ontario and London Health Sciences Centre-University Hospital, London, Ontario, Canada; [2]Garth Blackler, BAT, J. Daniel Klapak, BAT: University of Western Ontario, London, Ontario, Canada; [3]Alexander J. Knights, PhD, Easton C. Farrell, MSE, Tristan Maerz, PhD: University of Michigan, Ann Arbor; [4]Benoit Fiset, BSc, GrDip Bioinformatics, Logan A. Walsh, PhD: McGill University, Montreal, Quebec, Canada.

Additional supplementary information cited in this article can be found online in the Supporting Information section (https://acrjournals.onlinelibrary.wiley.com/doi/10.1002/art.43089).

Author disclosures and a graphical abstract are available online at https://onlinelibrary.wiley.com/doi/10.1002/art.43089.

Address correspondence via email to C. Thomas Appleton, MD, PhD, at tom.appleton@sjhc.london.on.ca.

Submitted for publication June 13, 2024; accepted in revised form December 10, 2024.

Macrophages dominate the synovial immune cell landscape in OA,[12,13] increase nociception,[14] and orchestrate neurovascular remodeling processes in other diseases.[15–18] However, macrophage ablation does not prevent the development of OA,[19] suggesting that certain populations of immune cells are important for joint homeostasis. Through crosstalk with other immune and nonimmune cells in synovial tissues, proinflammatory and/or regulatory macrophage subsets might be able to modify nociception in complex synovial microenvironments. Although whether synovial macrophage activation, function, and subsets are related to pain severity in OA is not well understood, high-throughput tools including single-cell RNA sequencing are beginning to reveal cellular and molecular profiles in OA synovial tissues,[12,20] allowing us to investigate the relationship between synovial cell subsets and pain. For example, one study found that distinct fibroblast subtypes isolated from painful joint sites were associated with a neuroplastic profile (eg, neuronal cell survival and neurite outgrowth),[21] although synovial immune cells were not studied and detailed molecular data linking synovial immune profiles to clinically relevant pain experiences is lacking.

In this study, we provide the first combined spatial transcriptomic and/or proteomic and single-cell RNA-sequencing analyses of synovial tissues focused on pain in OA. Our objective was to identify synovial macrophage subsets associated with worse pain in patients with knee OA and pathophysiologic mechanisms in synovial lining, sublining, and microvascular microenvironments.

MATERIALS AND METHODS

Study participants and subgroup selection. Participants with symptomatic, radiographic late-stage knee OA (Kellgren-Lawrence [KL] grades 3 or 4),[22] pain (ranging from mild to severe), and compromised function warranting total knee arthroplasty or high tibial osteotomy were included. All patients fulfilled the American College of Rheumatology classification criteria.[23] Patients who received joint injections within six months before surgery were not included. We recruited sequential patients scheduled for surgical intervention, which permitted collection of synchronized demographics, patient-reported measures of pain, and synovial histopathology data in this cross-sectional study. Participants provided written informed consent, and the cohort was approved by Western University's Research Ethics Board for Health Sciences Research Involving Human Subjects (number 109255) and complied with the Declaration of Helsinki. Reporting was aligned with Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[24]

A subgroup of 32 patients (n = 16 more pain; and n = 16 less pain) was selected from the cohort for spatial profiling using GeoMx from NanoString. The subgroup was selected first by splitting the cohort into upper (least pain) and lower (most pain) quartiles of Knee Injury and OA Outcome Score (KOOS) pain subscale score. Based on our regression analysis, we selected patients with more severe pain and worse perivascular edema (more severe disease and tissue damage) and patients with less pain and less perivascular edema. Then, 16 patients with cores that provided adequate tissue to sample three different synovial tissue microenvironments of interest were selected from the upper and lower KOOS pain quartiles. A subgroup of eight patients (n = 4 more pain; n = 4 less pain) analyzed by spatial profiling were selected to also undergo single-cell sequencing.

Patient-reported outcome measures of pain. Participants completed the KOOS Pain subscale (9 items: 0–100), in which lower scores indicate worse pain. The KOOS is valid and reliable for individuals with knee OA.[25,26] A clinically important difference in KOOS is defined as a change in 8.0 to 10.0 points.[27]

Synovial tissue histopathology. Synovial tissue biopsies were collected during surgery from the lateral suprapatellar recess. Serial sections (5-μm thick) spanning a total of 500 μm of tissue were stained with hematoxylin and eosin. Synovial tissue samples were graded, including two to five sections (at least 100 μm apart) per patient, by assessing six features of synovial histopathology as previously described[28]: (1) synovial lining thickness, (2) subsynovial infiltrate, (3) vascularization, (4) surface fibrin deposition, (5) fibrosis, and (6) perivascular edema. The grades for each tissue section per patient were averaged for each individual histopathological feature and then binned into the following categories: category 1 is none or normal (average grade < 0.5); category 2 is mild (average grade between 0.5 to 1.5); and category 3 is moderate or severe (average grade >1.5).

Spatial profiling of gene and protein expression. Three synovial tissue anatomic microenvironment regions of interest (ROIs) were defined as follows: (1) synovial lining, (2) subintima, and (3) microvessel. Therefore, 96 ROIs comprising three different synovial microenvironments from a total of 32 patients were analyzed using both whole-transcriptome spatial profiling and predefined protein panels in parallel. The synovial tissue microenvironment ROIs were selected using known morphologic markers, including nuclei (DAPI), CD68 (macrophages), CD45 (hematopoietic cells), and smooth muscle actin (microvessels). Each ROI included a minimum of 200 cells.

The GeoMX predefined protein profiling consisted of 132 targets across 11 panels: immune activation status, immune cell typing, pan-tumor, immuno-oncology drug target, cell death, MAPK signaling, phosphatidylinositol 3-kinase/protein kinase B signaling, myeloid, autophagy, neural cell profiling, and glial cell subtyping. A full list of protein targets can be found in Supplementary Protein Profiling Targets File. Full details regarding synovial tissue dissociation into single-cell suspension, single-cell RNA library preparation, and single-cell sequencing can be found in Supplementary Materials and Methods.

Analysis of single-cell sequencing data. Alignment was conducted to the GRCh38.p13 (2020-A), reference genome and filtering, barcoding, and unique molecular identifier counting were performed using Cell Ranger version 6.1.1 and the 10x Genomics recommended default parameters. All downstream processing and analysis steps on the feature-barcode matrixes were performed in the R package Seurat version 4.1.0. Full details on single-cell workflow can be found in Supplementary Materials and Methods.

Phagocytosis assay. Phagocytic index was measured in synovial macrophage isolated from a subset of patients reporting more pain (n = 8) and less pain (n = 8). We used the Phagocytosis Assay Kit (number 500290), according to the manufacturer's protocol (Caymen Chemical Company). Phagocytic index was defined as the number of CD68+ macrophages containing one or more phagocytosed fluorescein isothiocyanate+ beads as a proportion of total CD68+ macrophages. Full details can be found in Supplementary Materials and Methods.

Rat dorsal root ganglia explant stimulation. A total of 32 dorsal root ganglia (DRGs) harvested from 18-week-old naive male Sprague Dawley rats were stimulated for 72 hours with one of the following: (1) media conditioned by synovial cells with more pain (n = 8), (2) media conditioned by synovial cells from patients with less pain (n = 8), (3) lipopolysaccharide (LPS) (200 ng/mL; n = 8), or (4) unconditioned complete RPMI media (RPMI, 10% fetal bovine serum, and 1% penicillin/streptomycin; n = 8). LPS is a potent inflammatory mediator and has been used to induce pain sensitivity and neuroinflammation[29–31] and affect neuronal cell morphology in preclinical studies[31,32] and was therefore used as a positive control. DRG cell bodies were immunolabeled for cleaved caspase 3 (CC3), a marker of cell stress, and DRG-supporting cells were labeled with ionized calcium-binding adapter molecule 1 (Iba1), a marker of immune cell activation. Full details can be found in Supplementary Materials and Methods.

Statistical analyses. *Synovial histopathology and pain outcomes.* We fitted a series of multivariate linear regression models to evaluate the associations between features of synovial histopathology and patient-reported outcomes measures of pain (KOOS Pain subscale scores). Results were reported as unstandardized β-coefficients with 95% confidence intervals (CIs). Full details of statistical analyses can be found in Supplementary Materials and Methods.

GeoMX spatial profiling analysis. All analyses were completed in NanoString's GeoMX Analysis Suite (version 2.5.0.145). Differentially expressed genes or proteins were identified in each synovial microenvironment (lining, subintima, and microvessel) using linear mixed modeling while adjusting for patient identifier and body mass index (BMI) and with a Benjamini-Hochberg correction to control for false discovery rate. Full details can be found in Supplementary Material and Methods.

Phagocytic index of synovial macrophages. Phagocytic index of synovial macrophages was compared between groups of patients with more pain (n = 8) and less pain (n = 8) using an unpaired *t*-test. Results are presented as means with 95% CIs.

CC3 and Iba1 quantification in cultured DRGs. Percentage of CC3+ cell bodies and Iba1+ supporting cells were compared between patients reporting more pain (n = 8) and less pain (n = 8) and the unconditioned negative control group (n = 8) or LPS-stimulated positive control group (n = 8) using unpaired one-tailed *t*-tests. Results are presented as mean with 95% CIs.

Data availability. All single-cell RNA-sequencing data and spatial profiling data are publicly available via the NCBI GEO using the accession numbers GSE248453, GSE248454, and GSE248455. Other data and analytic codes are available upon reasonable request.

RESULTS

Out of 125 participants screened for eligibility, synovial biopsy was obtained for 122 participants and included in the primary analysis. Study participant demographics and clinical characteristics are shown in Table 1 for the (1) total cohort, (2) spatial profiling, and (3) single-cell RNA-sequencing subgroups of patients with more versus less pain. Frequency of pain medication prescription is shown in Supplementary Table 1.

Association between worse pain and synovial tissue perivascular edema. Regression analyses were performed while controlling for confounding variables to determine which features of synovial histopathology are associated with worse pain. Perivascular edema was strongly associated with worse KOOS pain that was likely clinically meaningful (−10.76; 95% CI −18.90 to −2.61) (Table 2), suggesting that pain may be related to synovial microvascular dysfunction.

Spatial profiling analysis of synovial microenvironments. We next used spatial profiling of synovial lining, sublining, and microvascular compartments to identify pathophysiologic processes associated with worse OA pain. Subgroups of patients with severe pain or mild-to-moderate KOOS pain and perivascular edema scores were matched on sex, BMI, and radiographic joint damage (KL grade), providing 16 patients from each subgroup for spatial transcriptomic and proteomic analyses. The subgroups had similar demographics to the total cohort (Table 1). As expected, patients with worse pain had higher histopathological scores for perivascular edema, lower histopathological scores for immune infiltrate, and were younger compared with patients with less pain, in keeping with more severe OA disease. The means and distributions of sex,

Table 1. Patient demographics and clinical characteristics for total cohort and digital spatial profiling and single-cell RNA-sequencing subgroups*

Characteristics	Total cohort (n = 122)	Spatial profiling subgroups		scRNAseq subgroups	
		Less pain (n = 16)	More pain (n = 16)	Less pain (n = 4)	More pain (n = 4)
Age, mean ± SD (range), years	67.1 ± 8.5 (41–85)	73.1 ± 5.1 (60–80)	65.4 ± 7.5 (55–79)	74.5 ± 5.5 (67–80)	64.8 ± 4.7 (59–69)
Sex, n (%)					
Women	58 (47.5)	7 (43.8)	6 (37.5)	2 (50.0)	2 (50.0)
Men	64 (52.5)	9 (56.2)	10 (62.5)	2 (50.0)	2 (50.0)
BMI, mean ± SD (range)	32.7 ± 5.5 (21.1–47.2)	33.1 ± 4.9 (24.8–40.3)	33.1 ± 5.1 (25.9–47.2)	30.1 ± 6.8 (24.8–40.0)	29.2 ± 2.9 (25.9–32.7)
KL grade, n (%)					
Grade 3	48 (39.3)	8 (50.0)	8 (50.0)	0 (0.0)	2 (50.0)
Grade 4	74 (60.7)	8 (50.0)	8 (50.0)	4 (100.0)	2 (50.0)
KOOS Pain, mean ± SD (range), 0–100	47.2 ± 15.7 (0–89)	60.6 ± 12.1 (42–89)	37.8 ± 10.0 (19–53)	62.8 ± 5.3 (56–67)	34.5 ± 10.9 (19–44)
Histopathology, mean ± SD (range)					
Lining thickness	0.8 ± 0.8 (0–3)	1.0 ± 0.8 (0–3)	0.9 ± 1.0 (0–3)	0.8 ± 1.0 (0–2)	0.8 ± 1.0 (0–2)
Subsynovial infiltrate	1.1 ± 0.8 (0–3)	1.8 ± 0.9 (0–3)	0.9 ± 0.6 (0–2)	2.3 ± 1.0 (1–3)	1.0 ± 0.0 (1–1)
Vascularization	2.0 ± 0.9 (0–3)	1.7 ± 1.0 (0–3)	2.7 ± 0.5 (2–3)	2.3 ± 1.0 (1–3)	2.3 ± 0.5 (2–3)
Fibrin	0.9 ± 0.4 (0–1)	0.9 ± 0.3 (0–1)	0.9 ± 0.3 (0–1)	1.0 ± 0.0 (1–1)	0.8 ± 0.5 (0–1)
Fibrosis	1.1 ± 0.7 (0–3)	1.2 ± 0.7 (0–3)	1.3 ± 0.5 (0–3)	1.1 ± 0.5 (1–2)	1.3 ± 0.5 (1–2)
Perivascular edema	0.6 ± 0.7 (0–3)	0.0 ± 0.0 (0–0)	1.6 ± 0.6 (0–3)	0.0 ± 0.0 (0–0)	2.3 ± 0.5 (2–3)

* Synovial histopathology scores are reported as the mean of median scores for each feature. Lower KOOS pain scores indicate worse pain. BMI, body mass index; KL, Kellgren-Lawrence; KOOS, Knee Injury and Osteoarthritis Outcome Score; scRNAseq, single-cell RNA sequencing.

KL grade, and BMI were similar among groups, indicating effective matching.

Representative images of the synovial microenvironment ROIs analyzed by spatial profiling as well as differential gene and protein expression of patients with more pain are shown in Figure 1. Genes involved in mitochondrial stress response and regulation of processes such as angiogenesis, vessel maintenance, wound healing, and neurite outgrowth were increased in patients with more pain, whereas genes involved in regulation of innate immune signaling were decreased. Proteins involved in immune exhaustion, cellular response to stress, apoptosis, and wound healing were increased in patients with worse pain, whereas cell surface markers of myeloid and T lymphocytes, immune signaling, and cell growth and survival were decreased, corresponding to diminished innate immune cell content and diversity. A full list of differentially expressed genes from each ROI can be found in Supplementary File - GeoMX DE Lists.

Enrichment of Reactome gene sets in patients with worse pain for each synovial microenvironment are shown in Supplementary Table 2. In the synovial lining, enriched gene sets include those related to neuronal development and maintenance, cell metabolism, and regulation of angiogenesis. In the sublining, enriched gene sets include extracellular matrix (ECM) reorganization and fibrosis, neuronal regulation, immune signaling, and cell metabolism. In the microvascular compartment, enriched gene sets include neuronal regulation, ECM organization, fibrosis, and enrichment of many proinflammatory Toll-like receptor (TLR) cascades. Together, these data show that the synovial microenvironments from patients with more pain express signaling pathways related to neurovascular remodeling, immune signaling, and profibrotic processes.

Table 2. Multivariate linear regression model estimates for KOOS pain and histopathological features of synovial inflammation and damage (n = 122)*

Predictor: histopathology	β-coefficient	SE	95% CI
Lining thickness			
<0.5	Reference	Reference	Reference
0.5–1.5	1.55	3.11	−4.61 to 7.72
>1.5	−4.32	4.28	−12.79 to 4.16
Subsynovial infiltrate			
<0.5	Reference	Reference	Reference
0.5–1.5	−2.45	4.01	−10.39 to 5.50
>1.5	2.30	4.71	−7.03 to 11.63
Vascularization			
<0.5	Reference	Reference	Reference
0.5–1.5	3.05	6.33	−9.49 to 15.60
>1.5	−0.62	5.45	−11.41 to 10.18
Fibrin			
<0.5	Reference	Reference	Reference
0.5–1.5	3.06	5.20	−7.24 to 13.36
>1.5	–	–	–
Fibrosis			
<0.5	Reference	Reference	Reference
0.5–1.5	3.65	3.38	−3.05 to 10.35
>1.5	3.00	4.31	−5.54 to 11.55
Perivascular edema			
<0.5	Reference	Reference	Reference
0.5–1.5	−4.70	2.85	−10.35 to 0.95
>1.5	−10.76	4.11	−18.90 to −2.61

* Adjusting for age, sex, and body mass index. CI, confidence interval; KOOS, Knee Injury and Osteoarthritis Outcome Score.

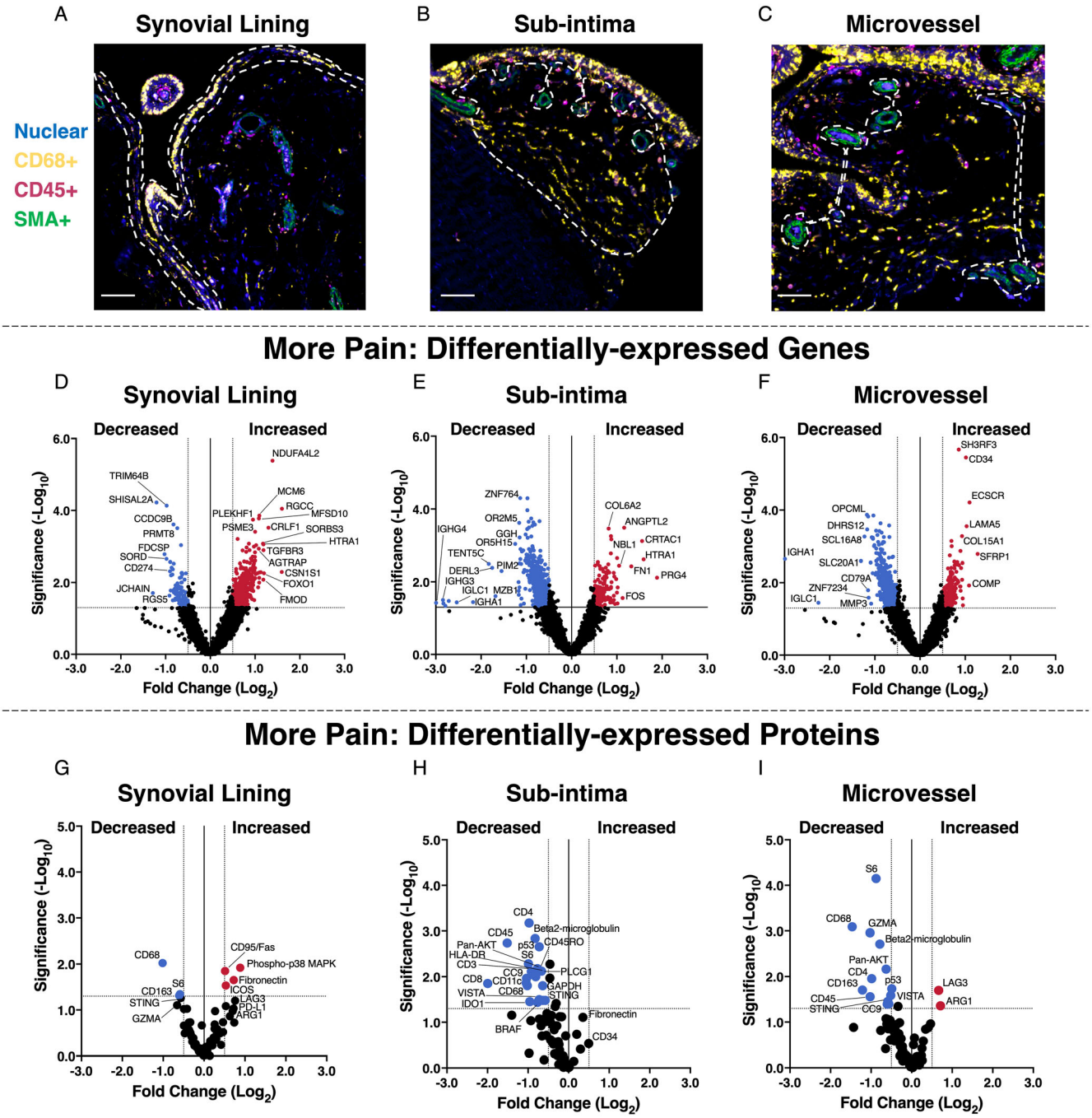

Figure 1. Spatial profiling of synovial lining, subintima, and microvessel microenvironments. (A–C) Representative images of the synovial microenvironment regions of interest. Immunofluorescence images from the spatial profiling analysis with morphology markers for CD68+ macrophages (yellow), CD45+ cells (magenta), SMA+ cells (green), and nuclear stain (blue). Representative synovial microenvironment regions of interest for (A) synovial lining, (B) subintima, and (C) microvessel are outlined with white, dashed lines. Scale bar = 100 μm. Volcano plots displaying the top differentially expressed (increased vs decreased expression) (D–F) genes and (G–I) proteins for each synovial tissue microenvironment: synovial lining, subintima, and microvessel in patients with more pain (relative to less pain). Genes with increased expression in patients with more pain are represented by red dots, and genes with decreased expression in more pain are represented by blue dots. The y-axis represents the log10 *P* value with cut-off set at 1.3 ($P < 0.05$), and the x-axis represents the log2 fold change with cut-off set at 0.5. Differential gene expression (n = 16 per group) was derived by fitting linear mixed models while adjusting for patient ID and BMI and with a Benjamini-Hochberg correction to control for FDR. BMI, body mass index; CD45, hematopoietic cell marker; CD68, macrophage marker; FDR, false discovery rate; ID, identifier; SMA, smooth muscle actin vessel marker.

Cell deconvolution from spatial transcriptomics. Because our gene expression data suggested that worse pain was associated with decreased innate and adaptive immune cells, we used deconvolution algorithms to estimate the proportion of each immune cell type present in each synovial microenvironment (Supplementary Figure 1). Patients with worse pain had a higher proportion of nonconventional and/or intermediate monocytes (mean difference 3.76%; 95% CI 0.53–6.99) in the synovial lining. In the subintima, there were higher proportions of macrophages (mean difference 8.16%; 95% CI 0.16–16.17) and fibroblasts (mean difference 11.2%; 95% CI 3.30–19.07) and lower proportions of plasma cells (mean difference –5.78%; 95% CI –10.52 to –1.04) and naive CD4+ T cells (mean difference –8.05%; 95% CI –13.15 to –2.96) and a similar trend for CD8+ memory T cells (mean difference –1.12%; 95% CI –2.3 to 0.07). In microvessels, there were higher proportions of fibroblasts (mean difference 8.53%; 95% CI 2.86–14.19) and endothelial cells (mean difference 7.73%; 95% CI 1.66–13.80) and lower proportions of CD8+ memory T cells (mean difference –2.32%; 95% CI –3.81 to –0.83) and neutrophils (mean difference –2.84%; 95% CI –4.66 to –1.02) and a similar trend for plasma cells (mean difference –2.79%; 95% CI –5.95 to 0.37). These data suggest that worse pain is associated with decreased immune cell diversity in synovial tissues.

Single-cell RNA-sequencing analysis. To investigate changes in immune cell profiles and crosstalk mechanisms associated with worse pain, we analyzed synovial cell profiles at the cellular level using single-cell RNA sequencing. The pain subgroups demonstrated similar demographics to the total cohort (see Table 1). Patients with more pain had higher histopathological scores for perivascular edema, a lower histopathological score for immune infiltrate, and were younger compared with patients with less pain.

We profiled a total of 14,522 synovial cells, comprising 15 distinct cell populations (Figure 2A), including myeloid and dendritic cells, lining and sublining fibroblasts, T cells, B cells, plasma cells, mast cells, endothelial cells, and mural cells. We observed large differences in the proportions of cell types between patients with worse pain compared with patients reporting less pain, especially in the myeloid and fibroblast subsets (Figure 2C). Patients reporting worse pain had fewer myeloid (–19.5%; 95% CI –32.3 to –6.8) and other immune cells but much higher proportions of lining (19.0%; 95% CI –7.9 to 45.9) and sublining (13.0%; 95% CI –5.3 to 31.4) fibroblasts, endothelial cells, and mural cell clusters (Supplementary Table 3). These data corroborate the spatial profiling findings and suggest that lower immune cell diversity and expansion of fibroblast subsets are important features in synovial tissues from patients experiencing worse pain.

Next, we conducted cell type–specific analyses to identify differences in myeloid cell and fibroblast (Figure 2D–K) subtypes and gene expression between patients reporting more versus less pain. We identified four myeloid subtypes previously described by Huang et al, including transitional macrophages (T-MΦ, expressing both proinflammatory and immune-resolving genes), interferon-stimulated macrophages (IFNS-MΦ, expressing interferon-related genes), S100A8^{hi} macrophages (highly expressing S100A8; S100A8+), and immune-regulatory macrophages (IR-MΦ).[20] We also identified lining macrophages (expressing proteoglycan 4 [*PRG4*], secreted protein acidic and cysteine rich [*SPARC*], chloride intracellular channel 5 [*CLIC5*], and V-set and immunoglobulin containing 4 [*VSIG4*]), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1+) macrophages, dendritic cells, proliferating macrophages, mast cells, and a nonspecific macrophage cluster we were unable to further classify. Strikingly, patients with worse pain had a reduction in all immune cell subtypes except the LYVE1+ macrophages, which were increased, and an almost complete absence of IFNS-MΦ and IR-MΦ (Figure 2E).

Similarly, we identified eight subtypes of fibroblasts, including lining fibroblasts (PRG4, CD55, and collagen type XXII α-1 chain), leucine-rich repeat containing 15 (LRRC15+) lining, senescent lining (calponin-1, cyclin-dependent kinase inhibitor 1A, regulator of G protein signaling 16, and G protein–coupled receptor 1), sublining progenitor (dipeptidylpeptidase 4, peptidase inhibitor 16, and CD34), perivascular sublining (midkine and ANGPT1), HLA-DRA^{hi}, intermediate fibroblasts (PRG4 and Thy-1 cell surface antigen), and a nonspecific fibroblast cluster that did not correspond to any previously reported fibroblast gene signatures (Figure 2F). LRCC15+ lining, senescent lining, and sublining progenitor fibroblasts were increased in patients with worse pain (Figure 2G).

To determine if myeloid and fibroblast subsets demonstrate different physiologic states between patients with worse pain versus less pain, we assessed differentially expressed genes between pain groups and performed gene set enrichment analysis (Figure 2H–K). Numerous processes related to neurovascular signaling and remodeling, proinflammatory innate immune signaling pathways, immune regulation, extracellular matrix remodeling, and cell stress and metabolism were positively enriched in myeloid cells (Figure 2I) and fibroblasts (Figure 2K) from patients with worse pain. Collectively, these results demonstrate that patients with worse pain exhibit striking reductions in immune and regulatory myeloid cell subsets and increases in senescent and sublining fibroblast subsets, in association with increased transcriptional signatures relevant to proalgesic, proinflammatory, neurovascular remodeling, and profibrotic processes.

Immune-stromal crosstalk profiles. Considering that myeloid and stromal cells are known to communicate in wound healing and other disease contexts, we hypothesized that crosstalk between immune and stromal cells may be dysregulated in patients with worse pain. Patients with more pain showed a lack

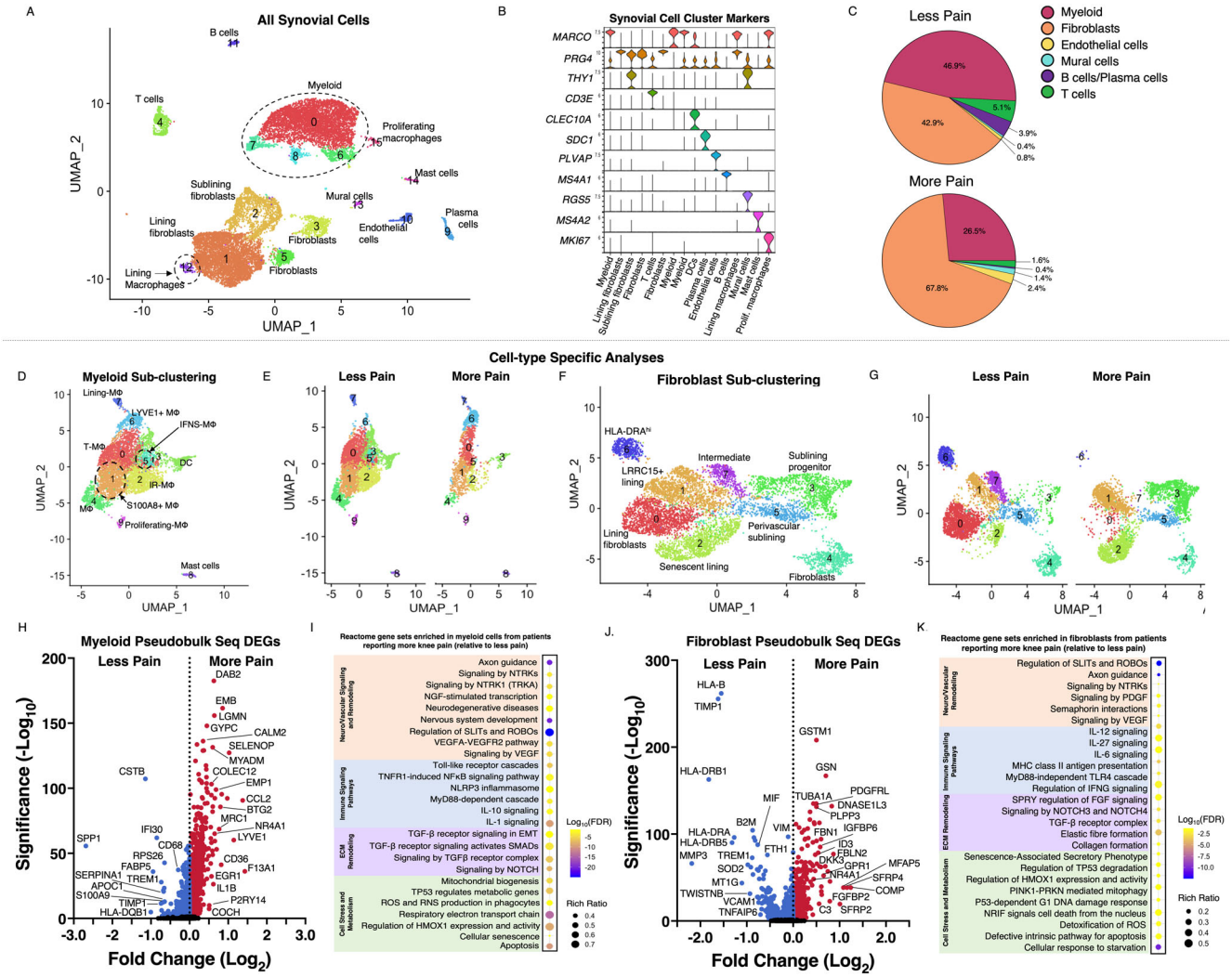

Figure 2. Single-cell transcriptomics of OA synovium from patients with more versus less pain. (A) UMAP plot of synovial cell clusters annotated by cell types. (B) Expression level of the selected marker(s) used for identification of each cluster. (C) Proportion of main cell types in patients reporting "less pain" (n = 4) and patients reporting "more pain" (n = 4). (D–K) Cell type–specific analyses for myeloid cells and fibroblasts. (D) Subclustering of myeloid cells plotted on UMAP and annotated by subtype: T-MΦ, IFNS-MΦ, IR-MΦ, and S100A8+ macrophages (S100A8^{hi}). (E) Myeloid subclustering UMAPs plotted by pain group. (F) Subclustering of fibroblast cells plotted on UMAP and annotated by subtype: lining fibroblasts, LRRC15+ lining, senescent lining, sublining progenitor, fibroblasts, perivascular sublining, HLA-DRA^{hi} fibroblasts, and intermediate fibroblasts. (G) Fibroblast subclutsering UMAPs plotted separated by pain group. (H) Volcano plot of differentially expressed genes from myeloid pseudobulk analysis. (I) Bubble plot of Reactome gene sets enriched by myeloid cells from more pain relative to less pain. (J) Volcano plot of differentially expressed genes from fibroblast pseudobulk analysis. (K) Bubble plot of Reactome gene sets enriched by fibroblasts from more pain relative to less pain. DC, dendritic cell; DEG, differentially expressed gene; EMT, epithelial–mesenchymal transition; IFNS-MΦ, interferon-stimulated macrophage; IL, interleukin; IR-MΦ, immune-regulatory macrophage; LRCC15+, leucine-rich repeat containing 15; MHC, major histocompatibility complex; NGF, nerve growth factor; NTRK, neurotrophic tyrosine receptor kinase; OA, osteoarthritis; PDGF, platelet-derived growth factor; ROBO, roundabout homolog; SLIT, slit guidance ligand; RNS, reactive nitrogen species; ROS, reactive oxygen species; T-MΦ, transitional macrophage; TGF, transforming growth factor; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; TRKA, tropomyosin-related kinase receptor A; UMAP, Uniform Manifold Approximation and Projection for Dimension Reduction; VEGFR, vascular endothelial growth factor receptor. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43089/abstract.

of semaphorin, heparan sulfate, ANGPTL, and vascular endothelial growth factor signaling and instead featured increases in PDGF, CD99, and visfatin crosstalk signaling mechanisms compared with patients with less pain (Supplementary Figure 2). These changes suggest that myeloid-fibroblast crosstalk involving vascular and neural regulatory signaling pathways in patients with lower OA-related pain may be replaced by more pathologic fibrotic and dysregulated neurovascular physiology in patients experiencing worse pain. Although the reason for this change is not clear, it could relate to altered immune-regulatory cell function. Therefore, we further investigated the function of innate immune cells in synovial tissues.

Synovial macrophage function and innate immune exhaustion. The reduction in myeloid cell diversity in single-cell analyses combined with increased expression of immune exhaustion markers from spatial profiling analyses suggested that synovial macrophages may be dysfunctional or exhausted. To investigate whether worse pain is associated with synovial macrophages dysfunction, we measured phagocytic function of CD68+ synovial tissue macrophages in vitro following isolation from synovial tissues and recovery in monolayer culture. Synovial macrophages from patients with worse pain demonstrated a lower phagocytic index (−19.42; 95% CI −35.96 to −2.89) compared with patients with less pain (Figure 3A and B). Functional annotation of the single-cell dataset showed that phagocytosis-related gene sets were expressed primarily by the T-MΦ and the IR-MΦ subsets of macrophages (Figure 3C) and that these subsets were reduced in patients with more pain (Figure 3D and 3E). Loss of phagocytic function in combination with increased expression of markers of exhaustion suggest that innate immune exhaustion in synovial macrophages occurs in patients with worse pain.

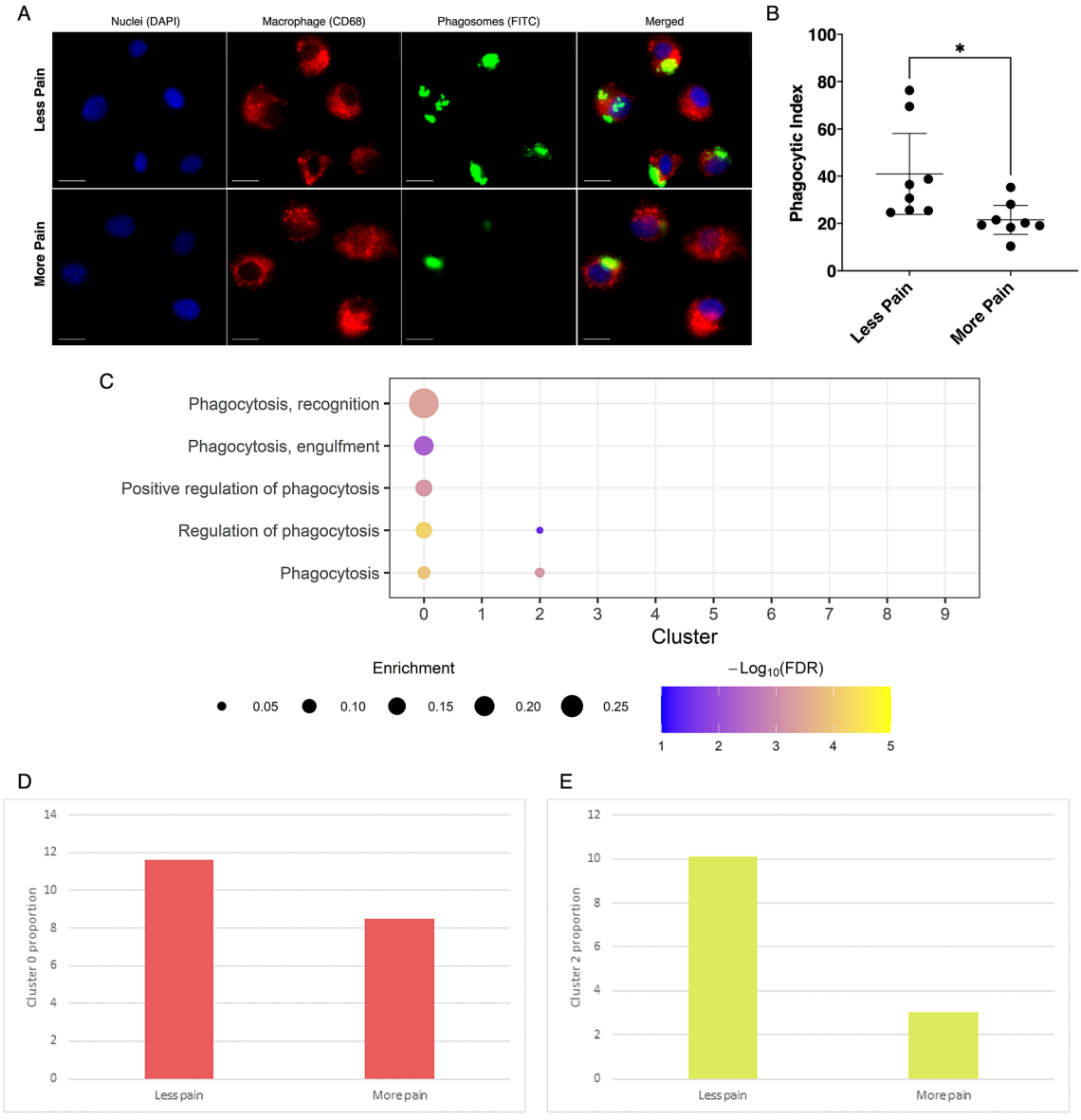

Figure 3. CD68+ macrophage phagocytic index among patients with more versus less pain. (A) Representative immunofluorescence images of CD68+ macrophages (red) and phagocytosing beads (green) from patients with more or less pain. (B) Dot plot displaying the individual measures of and mean ± 95% CI phagocytic index for more pain (n = 8) and less pain (n = 8) groups. Phagocytic index is defined as the number of CD68+ macrophages with one or more phagocytosed beads out of the total number of CD68+ macrophages. An unpaired *t*-test was used to compare phagocytic index between groups. DAPI nuclear stain. Scale bar = 10 μm. *$P < 0.05$. CD68, macrophage marker; CI, confidence interval; FDR, false discovery rate; FITC, fluorescein isothiocyanate latex bead marker. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43089/abstract.

DRG neuronal cell activation. Because synovial tissues from patients with worse pain contained dysfunctional macrophages and enrichment of neurovascular remodeling pathways, we investigated whether secreted factors from synovial cells influenced DRG neuronal cell activation (Figure 4) using organ cultures of rat lumbar DRGs. Media conditioned by synovial cells from patients with worse pain increased cleaved casepase-3 in neuronal cell bodies (13.72%; 95% CI 0.65–28.09) compared with unconditioned media (control), whereas DRGs stimulated with media conditioned by synovial cells from patients with less pain did not (Figure 4A–E). The percentage of Iba1+ supporting cells was similar among conditions (Figure 4A–D and F). Together, these findings suggest that synovial cells from patients with worse pain secrete factors that increase neuronal cell stress signals.

DISCUSSION

Synovitis measured by imaging is strongly associated with increased risk of pain and joint damage in OA. Although it is logical to assume that more inflammation is likely to worsen OA outcomes, we currently lack detailed information about the composition and cell-cell communication networks present in OA-related synovitis that are associated with worse OA pain. In this study, we combined spatial molecular profiling with single-cell RNA-sequencing analyses of synovial tissues to identify immunophenotypic and biologic mechanisms associated with worse pain in knee OA. Two major themes emerged in our findings. First, worse pain was associated with macrophage exhaustion, characterized by increased expression of cell exhaustion and cell stress markers, reduction in innate and adaptive immune cells, and impaired macrophage phagocytic function. Second, worse pain was also associated with increased expression of gene and protein markers and cell-cell communication pathways characteristic of neurovascular signaling and remodeling. This latter finding was strongly supported by a clinically meaningful association of pain with perivascular edema in synovial tissue histopathology. Further suggesting that worse pain outcomes in knee OA are linked to macrophage exhaustion and neurovascular remodeling, our ex vivo organ culture model of nociception in lumbar DRGs demonstrated that cells isolated from synovial tissues containing dysfunctional macrophages secrete factors that increase neuronal cell activation. These experiments therefore uncover an exciting and unexpected link to innate IR-MΦ, which may initially play protective roles but become dysfunctional or lost in patients with worse pain.

All patients included in this study had late-stage knee OA with severe enough symptoms to warrant surgical intervention. Despite this context, there was a very wide range of pain severity. This allowed us to strategically test associations of knee pain with synovial histopathology and identify pathophysiologic mechanisms associated with worse pain while controlling for potential confounders. Synovial perivascular edema was associated with a clinically meaningful decrease (worse pain) in KOOS pain scores. Other histopathological signs of inflammation were not clearly associated with pain, which is likely because inflammation is a common feature among patients with active, symptomatic knee OA. Importantly, in other chronic diseases, perivascular edema indicates the transition from active inflammation to tissue damage.[33,34] Accordingly, reducing or preventing synovial tissue damage might be an important treatment goal in OA. Moreover, these new findings implicate synovial microvascular dysfunction in OA pain experiences, which extends the published literature on angiogenic processes in OA.[35–39]

Because single-cell sequencing and spatial profiling identified decreased innate and adaptive immune cells and suggested that innate immune cells, particularly macrophages, are dysfunctional in patients with more pain, we confirmed that synovial macrophages from patients with more pain have decreased phagocytic capacity. Impaired phagocytic function in synovial tissue macrophages would likely lead to poor clearance of tissue debris and cell turnover products in OA, which in turn could exacerbate proinflammatory and pain signaling through TLR and other DAMP-mediated mechanisms[9,40] and drive synovial cell dysfunction. These results are well-aligned with a recent study in which we identified that synovial macrophages demonstrate impaired efferocytic capability, which can also be induced by exposure to OA synovial fluid from patients with worse pain and rescued by treatment with interleukin-4.[41] Combined with our present findings, these data support the concept that the impaired synovial macrophage function may lead to poor tissue healing and worse pain but may be reversible.

Although innate immune synovial cell dysfunction has not previously been reported in the OA literature, existing knowledge from other disease contexts may help inform the potential role(s) of innate immune-regulatory cells in OA. Interestingly, we observed that a near-total lack of IR-MΦ in patients with worse pain was accompanied by expansion of LRCC15+ lining, senescent lining, and sublining progenitor fibroblasts. In the tumor microenvironment, LRRC15+ fibroblasts suppress tumor-infiltrating immune cells, thereby limiting their effector function.[42] Accordingly, synovial macrophages might play a protective role in regulating and/or suppressing the expansion of pathologic fibroblast subsets in OA, whereas we found that synovial macrophages become exhausted in patients with more painful knee OA. Similarly, in RA, therapeutic enhancement of remission-maintaining (regulatory) macrophages in the synovium of patients with RA can aid in restoring synovial tissue homeostasis.[43] It is therefore possible that enhancing regulatory macrophages in OA might help to restore OA joint homeostasis. Underscoring the importance of macrophages to homeostasis, macrophages are also critically important for mitigating redox stress in tissues,[44] so macrophage exhaustion might also contribute to oxidative damage.

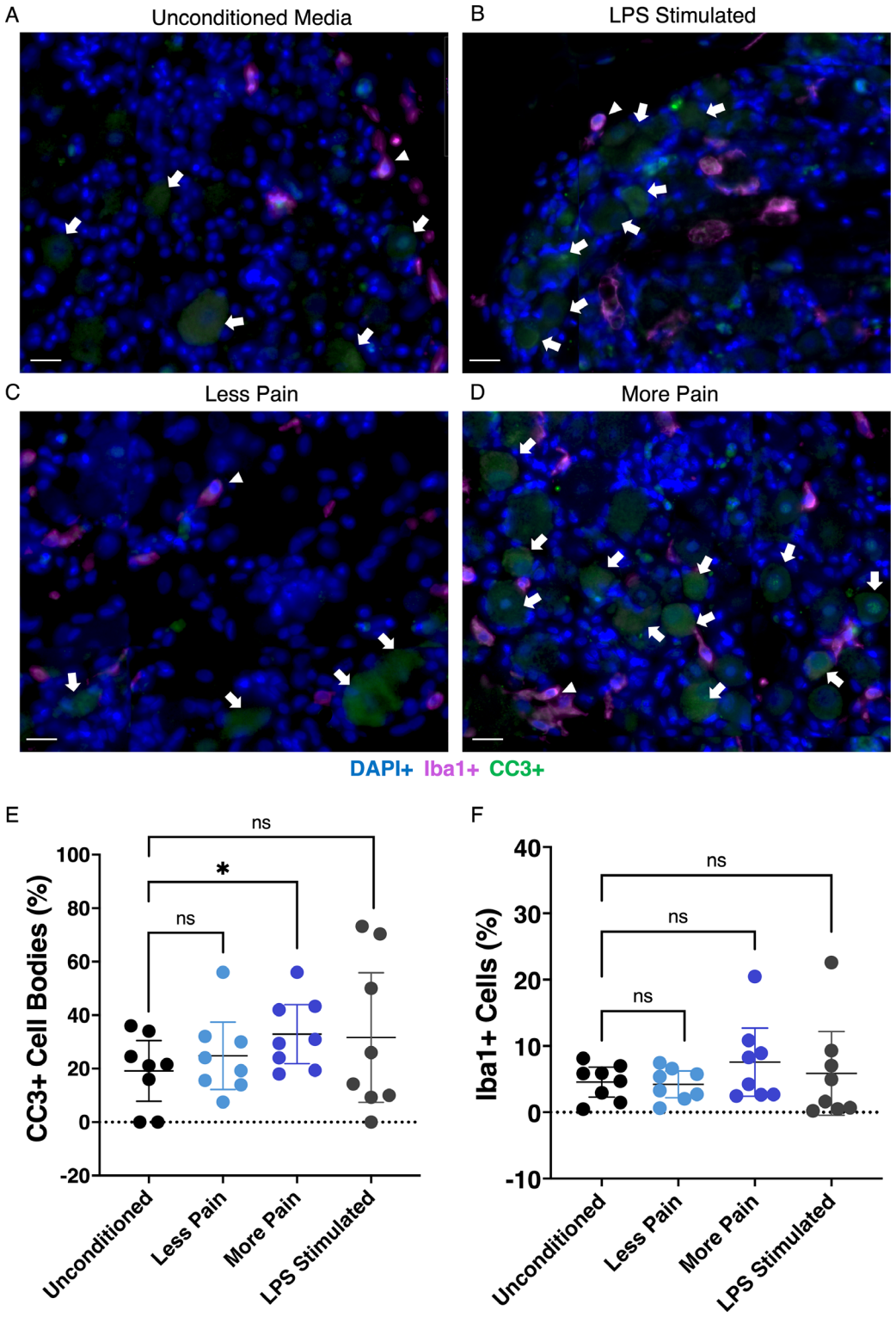

Figure 4. CC3 and Iba1 quantification in rat DRGs after stimulation with media conditioned by synovial cells from patients with more versus less pain. (A–D) Representative immunofluorescence images for each stimulation group (n = 8 per group). Groups are as follows: (A) unconditioned, (B) LPS stimulated, (C) less pain, and (D) more pain. Dot plots displaying the individual measures and mean ± 95% CI of (E) CC3+ cell bodies (green) and (F) Iba1+ cells (magenta) per stimulation group. Unpaired *t*-tests were used to compare the percentage of positive cells between groups. White arrow and arrowhead indicate CC3+ cell bodies and Iba1+ cells, respectively. DAPI nuclear stain. Scale bar = 20 μm. *$P < 0.05$. CC3, cleaved caspase 3; CI, confidence interval; DRG, dorsal root ganglia; Iba1, ionized calcium-binding adapter molecule 1 activated microglia marker; LPS, lipopolysaccharide. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.43089/abstract.

Neuronal and vascular remodeling is essential for joint homeostasis and wound healing.[38,45] However, inappropriate neurovascular sprouting[35,46,47] and microvascular dysfunction (ie, perivascular edema)[28,48] occurs in OA joints, which we found were associated with worse pain. Our findings align with other recent studies that demonstrate neuroplastic transcriptomic profiles in the synovium.[21,49] Nanus et al showed that factors secreted by synovial fibroblasts from patients with painful, early-stage OA joints promoted DRG neuronal cell survival and neurite outgrowth.[21] Our findings support and extend upon this work, suggesting that neuronal plasticity pathways are driven by both fibroblasts and innate immune cells in patients with OA. Further, by demonstrating the ability of synovial cells to alter neuronal cell stress in vitro, these data also suggest that appropriate crosstalk among innate immune cells, fibroblasts, and sensory neurons may be required to prevent OA-related nociception. Understanding both pro- and antinociceptive crosstalk mechanisms should be a major priority for the field.

Our inferred cell-cell communication analysis provides some such insights. For example, myeloid cells participating in semaphorin and angiopoietin-like signaling may play key nociceptive roles in synovial tissues during OA. Semaphorin and angiopoietin-like signaling can regulate appropriate axon guidance, angiogenesis, and modulate immune cell function, inflammation, and metabolism during stressful states.[50,51] Because myeloid and stromal cells are responsible for guiding angiogenic and neurogenic processes,[18,52] loss of synovial myeloid cell diversity may shift the responsibility for carrying out these homeostatic functions to other synovial cell subtypes, contributing to inappropriate or dysfunctional neurovascular changes within the OA joint. Future longitudinal studies should investigate when and how synovial cell subpopulations are shifted or lost and whether restoring innate immune-regulatory function can improve neurovascular derangement and pain in OA. Additionally, immune-stromal crosstalk from patients with more pain was participating in visfatin signaling. Taken together with a recent study from Li and colleagues that identified common progenitors between synovium and fat pad in the joint,[53] this suggests there may be a role in adipose transformation of synovial cells in the more-pain context and should be explored further.

Limitations of our study include the cross-sectional design, precluding us from drawing conclusions about a causal relationship between synovial pathophysiology and the progression of knee OA pain. However, we expect that a causal relationship is likely based on similar findings in preclinical OA models. Furthermore, patients with worse pain in our study were younger with worse tissue damage, implying they had experienced faster disease and pain progression. Although neuronal cells were not represented in our cell-cell communication analysis, our in vitro findings validated that synovial cells from patients with worse pain can transactivate DRGs in culture, as predicted by our single-cell transcriptomic findings.

In conclusion, worse knee pain in OA is associated with macrophage exhaustion and microvascular dysfunction. Our findings suggest that different synovial cell subsets play opposing roles in OA-related pain. In particular, regulatory macrophage dysfunction and expanded senescent lining and sublining fibroblasts are exciting candidate mediators of OA-related pain pathophysiology. Because the synovium is critical for maintaining joint health and homeostasis, innate immune cell exhaustion may be a novel treatment target for preventing the progression of pain and joint failure in OA.

ACKNOWLEDGMENTS

The authors would like to acknowledge all members of the Western Ontario Registry for Early OA (WOREO) Knee Study research team and the participants in the WOREO Knee Study.

AUTHOR CONTRIBUTIONS

All authors contributed to at least one of the following manuscript preparation roles: conceptualization AND/OR methodology, software, investigation, formal analysis, data curation, visualization, and validation AND drafting or reviewing/editing the final draft. As corresponding author, Dr Appleton confirms that all authors have provided the final approval of the version to be published and takes responsibility for the affirmations regarding article submission (eg, not under consideration by another journal), the integrity of the data presented, and the statements regarding compliance with institutional review board/Declaration of Helsinki requirements.

REFERENCES

1. Hill CL, Hunter DJ, Niu J, et al. Synovitis detected on magnetic resonance imaging and its relation to pain and cartilage loss in knee osteoarthritis. Ann Rheum Dis 2007;66(12):1599–1603.
2. Neogi T, Guermazi A, Roemer F, et al. Association of joint inflammation with pain sensitization in knee osteoarthritis: the Multicenter Osteoarthritis Study. Arthritis Rheumatol 2016;68(3):654–661.
3. Baker K, Grainger A, Niu J, et al. Relation of synovitis to knee pain using contrast-enhanced MRIs. Ann Rheum Dis 2010;69(10):1779–1783.
4. Oo WM, Linklater JM, Bennell KL, et al. Are OMERACT knee osteoarthritis ultrasound scores associated with pain severity, other symptoms, and radiographic and magnetic resonance imaging findings? J Rheumatol 2021;48(2):270–278.
5. Sarmanova A, Hall M, Fernandes GS, et al. Association between ultrasound-detected synovitis and knee pain: a population-based case-control study with both cross-sectional and follow-up data. Arthritis Res Ther 2017;19(1):281.
6. Felson DT, Niu J, Neogi T, et al; MOST Investigators Group. Synovitis and the risk of knee osteoarthritis: the MOST Study. Osteoarthritis Cartilage 2016;24(3):458–464.
7. Wang Q, Rozelle AL, Lepus CM, et al. Identification of a central role for complement in osteoarthritis. Nat Med 2011;17(12):1674–1679.
8. Blom AB, van Lent PL, Libregts S, et al. Crucial role of macrophages in matrix metalloproteinase-mediated cartilage destruction during experimental osteoarthritis: involvement of matrix metalloproteinase 3. Arthritis Rheum 2007;56(1):147–157.

9. Lees S, Golub SB, Last K, et al. Bioactivity in an aggrecan 32-mer fragment is mediated via toll-like receptor 2. Arthritis Rheumatol 2015;67(5):1240–1249.
10. Scanzello CRPAC, Plaas A, Crow MK. Innate immune system activation in osteoarthritis: is osteoarthritis a chronic wound? Curr Opin Rheumatol 2008;20(5):565–572.
11. Robinson WH, Lepus CM, Wang Q, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2016;12(10):580–592.
12. Wood MJ, Leckenby A, Reynolds G, et al. Macrophage proliferation distinguishes 2 subgroups of knee osteoarthritis patients. JCI Insight 2019;4(2):e125325.
13. Benito MJ, Veale DJ, FitzGerald O, et al. Synovial tissue inflammation in early and late osteoarthritis. Ann Rheum Dis 2005;64(9): 1263–1267.
14. Geraghty T, Winter DR, Miller RJ, et al. Neuroimmune interactions and osteoarthritis pain: focus on macrophages. Pain Rep 2021;6(1):e892.
15. Haywood L, McWilliams DF, Pearson CI, et al. Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 2003;48(8): 2173–2177.
16. Var SR, Shetty AV, Grande AW, et al. Microglia and macrophages in neuroprotection, neurogenesis, and emerging therapies for stroke. Cells 2021;10(12):3555.
17. Wang R, Liu Y, Ye Q, et al. RNA sequencing reveals novel macrophage transcriptome favoring neurovascular plasticity after ischemic stroke. J Cereb Blood Flow Metab 2020;40(4):720–738.
18. Du Cheyne C, Tay H, De Spiegelaere W. The complex TIE between macrophages and angiogenesis. Anat Histol Embryol 2020;49(5): 585–596.
19. Wu CL, McNeill J, Goon K, et al. Conditional macrophage depletion increases inflammation and does not inhibit the development of osteoarthritis in obese macrophage Fas-induced apoptosis-transgenic mice. Arthritis Rheumatol 2017;69(9):1772–1783.
20. Huang ZY, Luo ZY, Cai YR, et al. Single cell transcriptomics in human osteoarthritis synovium and in silico deconvoluted bulk RNA sequencing. Osteoarthritis Cartilage 2022;30(3):475–480.
21. Nanus DE, Badoume A, Wijesinghe SN, et al. Synovial tissue from sites of joint pain in knee osteoarthritis patients exhibits a differential phenotype with distinct fibroblast subsets. EBioMedicine 2021;72: 103618.
22. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthrosis. Ann Rheum Dis 1957;16(4):494–502.
23. Altman R, Asch E, Bloch D, et al; Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Arthritis Rheum 1986; 29(8):1039–1049.
24. von Elm E, Altman DG, Egger M, et al; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol 2008;61(4):344–349.
25. Ruyssen-Witrand A, Fernandez-Lopez CJ, Gossec L, et al. Psychometric properties of the OARSI/OMERACT osteoarthritis pain and functional impairment scales: ICOAP, KOOS-PS and HOOS-PS. Clin Exp Rheumatol 2011;29(2):231–237.
26. Mehta SP, Sankar A, Venkataramanan V, et al. Cross-cultural validation of the ICOAP and physical function short forms of the HOOS and KOOS in a multi-country study of patients with hip and knee osteoarthritis. Osteoarthritis Cartilage 2016;24(12):2077–2081.
27. Roos EM, Lohmander LS. The Knee Injury and Osteoarthritis Outcome Score (KOOS): from joint injury to osteoarthritis. Health Qual Life Outcomes 2003;1(1):64.
28. Minten MJM, Blom A, Snijders GF, et al. Exploring longitudinal associations of histologically assessed inflammation with symptoms and radiographic damage in knee osteoarthritis: combined results of three prospective cohort studies. Osteoarthritis Cartilage 2019;27: 71–79.
29. Nürnberger F, Ott D, Claßen R, et al. Systemic lipopolysaccharide challenge induces inflammatory changes in rat dorsal root ganglia: an ex vivo study. Int J Mol Sci 2022;23(21):13124.
30. Hsieh C-T, Lee Y-J, Dai X, et al. Systemic lipopolysaccharide-induced pain sensitivity and spinal inflammation were reduced by minocycline in neonatal rats. Int J Mol Sci 2018;19(10):2947.
31. Park S-Y, Han J-S. Neuroprotective effect of Bcl-2 on lipopolysaccharide-induced neuroinflammation in cortical neural stem cells. Int J Mol Sci 2022;23(12):6399.
32. D'Angelo B, Astarita C, Boffo S, et al. LPS-induced inflammatory response triggers cell cycle reactivation in murine neuronal cells through retinoblastoma proteins induction. Cell Cycle 2017;16(24): 2330–2336.
33. Bruni C, Frech T, Manetti M, et al. Vascular leaking, a pivotal and early pathogenetic event in systemic sclerosis: should the door be closed? Front Immunol 2018;9:2045.
34. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities. J Scleroderma Relat Disord 2017;2(3): 137–152.
35. Walsh DA, Bonnet CS, Turner EL, et al. Angiogenesis in the synovium and at the osteochondral junction in osteoarthritis. Osteoarthritis Cartilage 2007;15(7):743–751.
36. Ashraf S, Mapp PI, Walsh DA. Contributions of angiogenesis to inflammation, joint damage, and pain in a rat model of osteoarthritis. Arthritis Rheum 2011;63(9):2700–2710.
37. Ashraf S, Wibberley H, Mapp PI, et al. Increased vascular penetration and nerve growth in the meniscus: a potential source of pain in osteoarthritis. Ann Rheum Dis 2011;70(3):523–529.
38. Mapp PI, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. Nat Rev Rheumatol 2012;8(7): 390–398.
39. Bonnet CS, Walsh DA. Osteoarthritis, angiogenesis and inflammation. Rheumatology (Oxford) 2005;44(1):7–16.
40. Miller RE, Ishihara S, Tran PB, et al. An aggrecan fragment drives osteoarthritis pain through Toll-like receptor 2. JCI Insight 2018;3(6): e95704.
41. Del Sordo L, Blackler GB, Philpott HT, et al. Impaired efferocytosis by synovial macrophages in patients with knee osteoarthritis. Arthritis Rheumatol 2023;75(5):685–696.
42. Krishnamurty AT, Shyer JA, Thai M, et al. LRRC15+ myofibroblasts dictate the stromal setpoint to suppress tumour immunity. Nature 2022;611(7934):148–154.
43. Alivernini S, MacDonald L, Elmesmari A, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020;26(8):1295–1306.
44. Ryan DG, O'Neill LAJ. Krebs cycle reborn in macrophage immunometabolism. Annu Rev Immunol 2020;38(1):289–313.
45. McDougall JJ. Osteoarthritis is a neurological disease - an hypothesis. Osteoarthr Cartil Open 2019;1(1-2):100005.
46. Obeidat AM, Miller RE, Miller RJ, et al. The nociceptive innervation of the normal and osteoarthritic mouse knee. Osteoarthritis Cartilage 2019;27(11):1669–1679.
47. Suri S, Gill SE, Massena de Camin S, et al. Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis. Ann Rheum Dis 2007;66(11):1423–1428.

48. Philpott HT, Carter MM, Birmingham TB, et al. Synovial tissue perivascular edema is associated with altered gait patterns in patients with knee osteoarthritis. Osteoarthritis Cartilage 2022;30(1):42–51.
49. Bratus-Neuenschwander A, Castro-Giner F, Frank-Bertoncelj M, et al. Pain-associated transcriptome changes in synovium of knee osteoarthritis patients. Genes (Basel) 2018;9(7):338.
50. Alto LT, Terman JR. Semaphorin signaling, methods and protocols. In: Terman JR, ed. Semaphorin Signaling. Springer; 2017:1–25.
51. Santulli G. Angiopoietin-like proteins: a comprehensive look. Front Endocrinol (Lausanne) 2014;5:4.
52. Zigmond RE, Echevarria FD. Macrophage biology in the peripheral nervous system after injury. Prog Neurobiol 2019;173:102–121.
53. Li J, Gui T, Yao L, et al. Synovium and infrapatellar fat pad share common mesenchymal progenitors and undergo coordinated changes in osteoarthritis. J Bone Miner Res 2024;39(2):161–176.