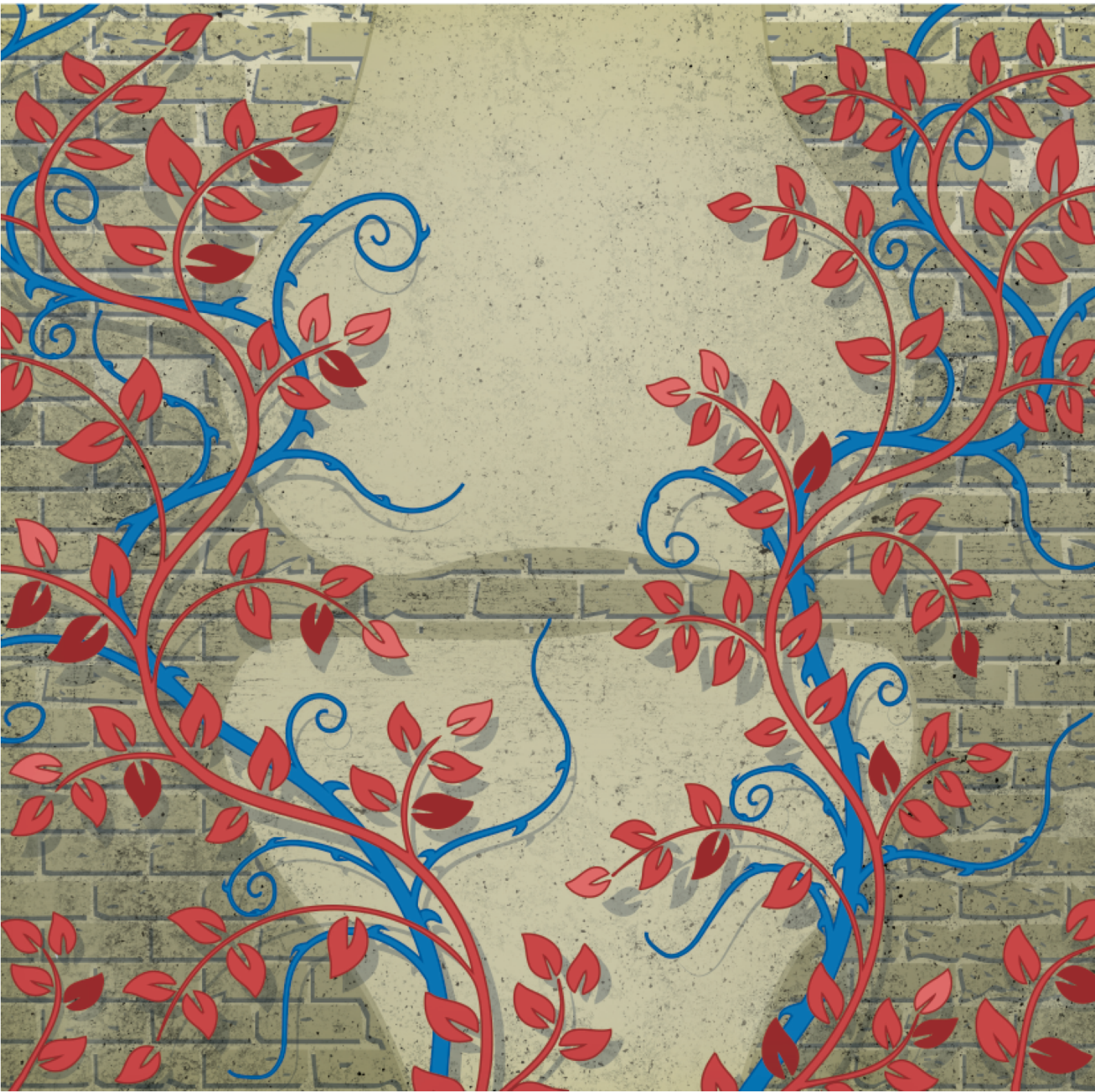
nature reviews
rheumatology

September 2025



https://doi.org/10.1038/s41584-025-01278-x

Location-specific treatment of chronic inflammatory joint disease

Adrian Ciurea & Caroline Ospelt

Over the past decades, considerable progress has been made in the pharmacological treatment of immune-mediated joint diseases such as rheumatoid arthritis and psoriatic arthritis; however, treatment-resistant arthritis remains a major clinical challenge. To tackle insufficient treatment outcomes, further research into the underlying mechanisms and patterns of non-responsiveness is needed.

Randomized controlled trials typically report aggregate joint counts owing to the assumption that all joints respond similarly to a given immunosuppressive agent, but emerging data indicate that treatment response can vary between inflamed joints. A 2025 observational European cohort study of psoriatic arthritis (PsA) investigated response to TNF inhibitors at the level of each individual joint within the 28-joint count[1]. In the upper limbs, the rate of resolution of synovitis was higher in the proximal joints (shoulder and elbow) than in the wrist. Within the hand, the greatest improvement was observed in the ulnar digits (ring and little finger), whereas the joints of the wrist and middle finger showed the poorest response. This pattern of response was confirmed in a follow-up study that involved treating patients from the same cohort with an IL-17 inhibitor. Although this response pattern was observed in both left-sided and right-sided joints, response to TNF and IL-17 inhibitors was lower in right-sided joints than in left-sided joints. Interestingly, the joints that are most frequently affected in PsA, namely the wrist, the knee and the metacarpophalangeal and proximal interphalangeal joints of the index and middle fingers[2,3], also appeared to respond more slowly to treatment. These joints are also among those most commonly involved in rheumatoid arthritis (RA)[4,5], and are prone to recurrent swelling throughout the disease course[5]. These findings suggest that local factors might contribute to the development and persistence of joint inflammation. One potential factor is mechanical stress; biomechanical studies of the hand support the idea that the index finger (digit II) and especially the middle finger (digit III) exert the greatest individual flexion force and grip strength, whereas the ulnar digits (digits IV and V) are less powerful. The carpal bones in the wrist have a crucial role in stabilizing hand motion by forming a proximal transverse arch that supports the longitudinal arches of the fingers, which illustrates the high compressive forces transmitted to the wrist during hand use. In addition, the lever effect created by the length of metacarpals and phalanges amplifies the force at the fingertips by at least tenfold compared with the metacarpal base. Correspondingly, the finger that provides the greatest strength — the middle finger — has evolved to be the longest.

However, the distinct patterns of joint involvement in autoimmune and autoinflammatory arthritis and osteoarthritis of the hand indicate that the interplay between the mechanical forces and pathogenic pathways that lead to joint involvement differ substantially between diseases. Interesting findings in knee osteoarthritis suggest a connection between evolutionary changes in biomechanics owing to upright, bipedal walking and disease susceptibility[6]. Risk variants are enriched in genetic elements that are particularly variable in human knee chondrocytes compared with other species, and these variants show evidence of both positive and deleterious biomechanical effects (antagonistic pleiotropy). The detrimental effect of these variants might have been evolutionarily tolerable but might be aggravated by modern factors such as obesity and longer life expectancy. Similarly, the evolution of the specific morphology and function of human hands and the associated changes in biomechanics and strain might amplify disease-specific pathogenic mechanisms. For example, the tight interconnection of the nail, the interphalangeal extensor tendon and the enthesis in distal interphalangeal joints might favour the development of PsA with increased biomechanical strain in the second and third distal interphalangeal joints leading to disease aggravation.

Signalling pathways that are activated site-specifically in joints during embryonic limb development guide the formation of anatomical structures that have evolved over time to perform species-specific joint functions. Regulation of HOX genes determines the growth and morphology of each digit, a process that has evolved over time to accommodate the specific biomechanical function of each digit. Joint-specific epigenetic and transcription regulation of HOX genes is maintained in adult synovial fibroblasts[7]. In adult tissue, HOX genes continue to regulate signalling pathways that are involved in embryonic limb development, such as Wnt signalling, but are also involved in regulating signalling pathways connected to inflammation, such as IL-6 signalling via the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway[7]. This influence of HOX genes on structural and inflammatory signalling pathways could be relevant for joint-specific characteristics of synovitis; for example, biopsy-obtained synovial samples from the wrists of people with RA have higher synovitis scores and a greater immune cell infiltration than those from the elbows, shoulders or knees[7]. These differences might explain variations in therapeutic responsiveness to specific pathway inhibitors.

Studies have approached this issue in post hoc analyses of randomized controlled trials of agents with a different mode of action. In re-analyses of data from the ORAL Standard and ORAL Strategy trials in RA, patients treated with adalimumab exhibited a more pronounced response in digits IV and V than in the radial-sided fingers, a pattern less evident in those treated with tofacitinib[8]. In data from the ORAL Start trial, joint-specific responses differed between patients receiving tofacitinib versus methotrexate[4]. In contrast to the hands, joint swelling in the feet responded more favourably to methotrexate than to tofacitinib at 3, 6 and 12 months. However, despite better clinical outcomes in the feet,

patients on methotrexate showed greater radiographic progression in foot joints than those treated with tofacitinib. Although foot joints are less frequently affected in RA overall, early erosions commonly appear in the third to fifth metatarsophalangeal joints[6]. Patients with predominant foot involvement but otherwise moderate joint counts might represent a distinct RA subset with a potentially less favourable treatment response, as suggested in a 2024 preprint[9]. This hypothesis is also supported by data showing that 25–36% of patients with RA who are in remission, according to composite measurements of disease activity, still have active foot synovitis[10]. Collectively, these studies indicate that anatomical site-specific response patterns might vary depending on the mechanism of immunosuppression. Investigating this hypothesis further will require sequential biopsy-obtained synovial samples from different sites during treatment to correlate clinical response with joint-specific transcriptomic and epigenomic profiles, ultimately paving the way for precision medicine in this area. The differences in local mechanical load and strain across joints, which might influence the onset and persistence of inflammation, underscore the importance of incorporating individualized, site-specific non-pharmacological therapeutic interventions into the management of difficult-to-treat arthritis.

Current research on joint specificity in treatment response is heavily influenced by the 28-joint count. Further studies are needed to capture the full range of joint involvement patterns, which could provide valuable insights into underlying pathophysiological mechanisms and guide more effective therapeutic strategies. Additional issues for location-specific arthritis research involve underappreciated challenges in clinical assessment of proximal joints, such as the shoulder and the hip. Ideally, studies should incorporate imaging data to confirm both the presence and the severity of synovitis at specific joint sites, thereby increasing inter-observer reliability. Additionally, distinguishing between inflammatory processes and primary and secondary (post-inflammatory) degenerative changes remains a substantial limitation. Translational research faces further obstacles owing to the limited availability of matched synovial tissue samples from different joint locations within the same patient; such samples are crucial for direct comparisons of molecular mechanisms across joints. Without these paired samples, differentiating between intrinsic joint-specific pathology and patient-specific factors that might influence disease progression is difficult.

In summary, the complex interplay between biomechanics, evolutionary forces and the pathogenesis of specific rheumatic diseases highlights joint location as a frequently underestimated factor in assessing treatment outcomes. Advancing the understanding of the molecular underpinnings of this complex interplay is essential for developing site-specific strategies in both pharmacological and non-pharmacological interventions, particularly for treatment-resistant arthritis.

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Published online: 25 June 2025

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Acknowledgements

We would like to thank our teams and collaborators and specifically R. Micheroli and A. Steimer for the ongoing discussions on the site-specificity of treatment responses.

Competing interests

The authors declare no competing interests.

Therapy

iPSC-derived CAR-NK cells for systemic sclerosis

Diffuse cutaneous systemic sclerosis (dcSSc) is a type of systemic sclerosis (SSc) associated with anti-Scl-70 autoantibodies and high mortality. Despite advances in the development of immunosuppressive and cell-based therapies, the management and treatment of dcSSc remains challenging.

Chimeric antigen receptor (CAR) T cells have shown promise for numerous autoimmune diseases, including dcSSc, but are linked to severe adverse events, and their development is costly and time consuming. Similarly to T cells, natural killer (NK) cells can eliminate target cells, but with a lower risk of inducing cytokine-release syndrome and graft-versus-host disease, and can be more easily manufactured to produce off-the-shelf therapeutic options. Now, a study reports the first-in-human use of QN-139b, a CAR-NK cell therapy derived from induced pluripotent stem cells (iPSCs) that targets both CD19 and BCMA.

"The iPSC-based manufacturing process could offer a more scalable, re-producible alternative to the personalized and costly autologous CAR T cell approach, which typically involves weeks of preparation," notes Christian Stockmann, who also studies therapeutic targeting of fibrosis.

A 36-year-old woman with treatment-refractory dcSSc was treated with four weekly infusions of QN-139b with 6 months of follow-up. B cells were depleted after 7 days, and autoantibodies and complement levels were reduced. Interestingly, B cell reconstitution, the timing of which can be associated with successful treatment, occurred after 1 month, and hypogammaglobulinemia, which is typically associated with BCMA targeting, was not observed. Single-cell sequencing analysis showed a loss of pathogenic B cells and expansion of naive B cells. All clinical scores assessed improved over 6 months. The QN-139b therapy seemed to reverse fibrosis and restore the structure of the vasculature and the skin.

Overall, this CAR-NK cell therapy was well tolerated by the patient and there was no evidence of the adverse effects that can be observed with CAR T cell therapy, such as cytokine-release syndrome; however, further studies are needed to confirm the depth of depletion achieved using this construct.

Stockmann summarizes that "This study offers a proof of concept that iPSC-derived, dual-targeting CAR-NK cells can safely induce clinical remission and tissue repair in severe systemic sclerosis. If depletion and efficacy is replicated in larger cohorts, this approach could present a new method for the generation of allogenic cell-based therapies."

Holly Webster

Original article: Wang, X. et al. An iPSC-derived CD19/BCMA CAR-NK therapy in a patient with systemic sclerosis. *Cell* https://doi.org/10.1016/j.cell.2025.05.038 (2025)

Rheumatoid arthritis

CD142$^+$ synovial fibroblasts attack the meniscus in knee RA

Less than 10% of patients with rheumatoid arthritis (RA) develop severe knee damage and undergo total knee arthroplasty (TKA). Whereas both the meniscus and cartilage are known to be damaged in knee RA, the relative contribution of meniscus damage to disease severity has been unclear.

Radiographic assessment of knee joints from patients who required TKA for knee RA or osteoarthritis indicated that the meniscus is more extensively damaged in the former, and this prompted Sun et al. to investigate the mechanisms of meniscus damage in knee RA. The authors used single-cell RNA sequencing to analyze cell populations at the cartilage, synovium and meniscus of knee biopsies from patients with severe knee RA who required TKA. These analyses identified a subpopulation of synovial fibroblasts that expressed the surface marker CD142, had an invasive and proliferative phenotype, and potentially engaged in cell–cell interactions with meniscal cells.

CD142$^+$ synovial fibroblasts were found to populate the sublining layer in joint biopsies from patients with OA, but were enriched in numbers, in both the synovium and synovial fluid, and shifted from the sublining to the lining layer in patients with knee RA. In addition, immunohistochemical staining identified CD142 expression within the menisci of patients with advanced knee RA.

Work in mouse models further indicated that the presence of CD142$^+$ synovial fibroblasts in the knee (as a result of repeated synovial fibroblast injections within the joint) was sufficient to induce meniscus damage. Synovitis was mild in these mice, and cartilage lesions increased only at a late time point in recipients of CD142$^+$ synovial fibroblasts, indicating that cartilage damage in knee RA might be secondary to the destruction of the meniscus.

The pro-inflammatory cytokines IL-1β and TNF promoted the conversion of CD142$^-$ synovial fibroblasts into the CD142$^+$ subset in vitro. Moreover, the invasive phenotype of CD142$^+$ synovial fibroblasts was associated with expression of the ABC transporter family member ABCC4. ABCC4-mediated cAMP efflux lowers PKA signalling. The ABCC4 inhibitor MK571 was able to prevent meniscal invasion by CD142$^+$ synovial fibroblasts and meniscal damage in the above mouse model, and this effect was reversed in the presence of a PKA inhibitor.

Taken together, these results indicate that early differentiation of CD142$^+$ synovial fibroblasts in the presence of pro-inflammatory stimuli might drive damage of the meniscus, and that this damage precedes cartilage destruction in the knee. In accordance with this hypothesis, analysis of 17 patients with early knee RA for a period of approximately 10 years correlated the early presence of CD142$^+$ synovial fibroblasts in the lining layer of the knee joint with a more severe disease outcome in the long term that requires TKA.

Maria Papatriantafyllou

Original article: Sun, H. et al. CD142-positive synovial fibroblasts drive meniscus destruction in rheumatoid arthritis. *Nat. Commun.* https://doi.org/10.1038/s41467-025-61842-7 (2025)

Autoinflammatory diseases

https://doi.org/10.1038/s41584-025-01270-5

Clonal dominance: mutations in VEXAS syndrome take advantage of inflammation

Samuel J. Magaziner & David B. Beck

VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is caused by somatic mutations in *UBA1* arising in hematopoietic stem cells, resulting in systemic autoinflammation and clonal outgrowth of these mutant cells. New research provides insights into the paradoxical mechanism behind this clonal hematopoietic dominance.

Refers to Molteni, R. et al. Mechanisms of hematopoietic clonal dominance in VEXAS syndrome. *Nat. Med.* https://doi.org/10.1038/s41591-025-03623-9 (2025).

Findings from studies on monogenic autoinflammatory diseases have elucidated previously unknown processes in human innate immunity, inflammation and cellular biology by revealing what happens when these pathways malfunction[1]. Unlike adaptive immune disorders, systemic autoinflammatory diseases frequently originate from genetic variants that cause spontaneous and constitutive activation of the innate immune system. VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome is one such disease, and a study by Molteni et al. provides insights into the clonal dominance observed in this disease[2].

First characterized in 2020, VEXAS syndrome is a severe, late-onset autoinflammatory condition that affects 1 in 4,000 men over 50 years of age[3]. VEXAS syndrome is characterized by severe multi-organ inflammation and anaemia, which is driven by a cytokine storm that typically includes type 1 interferon, IL-1β, TNF and IL-6. Thus, VEXAS syndrome is associated with substantial morbidity and mortality, which is partly due to a lack of mechanistic understanding and a reliance on generalized high-dose glucocorticoids as the standard of care. Improvement of care using targeted biologics, cytotoxic agents and transplantation has also been limited by the lack of a tractable organismal-level model and detailed natural history data[4].

VEXAS syndrome is caused by somatic hypomorphic mutations in *UBA1* (*UBA1*^{mutant}), which arise in hematopoietic stem cells (HSCs) and are restricted to the hematopoietic system[5]. Importantly, these mutant HSCs undergo clonal expansion, overtaking normal hematopoiesis (as observed in some leukemic disorders), with disease often leading to cytopenia and myelodysplastic syndrome, in addition to inflammation. *UBA1* encodes the E1 enzyme, which is responsible for initiating the majority of ubiquitylation, a core post-translational modification that regulates diverse cellular functions, including cell cycle progression, DNA repair and immune signaling[6].

Single-cell RNA sequencing (scRNA-seq) and phenotypical analysis of bone marrow mononuclear cells and whole blood immune cells from people with VEXAS syndrome indicate that multiple pro-inflammatory pathways are activated in early progenitor hematopoietic cells, this inflammation is typically associated with *UBA1*^{mutant} cells and this UBA1-dependent inflammation is prominent in the myeloid lineage[7,8]. However, despite these insights, the mechanisms behind the clonal expansion of the *UBA1*^{mutant}, inflammatory HSC population, which drives myeloid skewing and disease, are not well understood. Principally, advances in the underlying aetiology of these seemingly paradoxical outcomes are limited by difficulty in modelling the disease outside of a patient setting.

The study by Molteni et al. reports the first humanized mouse model of VEXAS syndrome and begins to untangle the complexities of clonal dominance[2]. The authors first performed phenotypic analysis of bone marrow aspirates and whole blood from people with VEXAS syndrome. Similar to the results of previous studies, in samples from people with VEXAS syndrome, there was a marked reduction in lymphoid progenitors with distinct myeloid skewing, and increased proportions of circulating CD34^{+} hematopoietic stem and progenitor cells (HSPCs) compared with samples from healthy individuals. However, transplanting these HSPCs into mice was unsuccessful, with poor engraftment suggesting a loss of HSPC stemness, and inherent patient heterogeneity proving intractable for transplantation.

To overcome this substantial limitation, the authors cleverly used base editing in HSPCs obtained from healthy donors. This approach allowed successful editing of the *UBA1* gene, with the modified HSPCs recapitulating core molecular features of the disease, including loss of cytoplasmic UBA1b, decreased polyubiquitylation, vacuolization, endoplasmic reticulum (ER) stress and increased inflammation. Excitingly, engraftment of these edited HSPCs into humanized mice proved viable, with reorganization of the hematopoietic compartment in *UBA1*^{mutant} mice that reflected VEXAS syndrome in humans. Transcriptomic analysis of the human bone marrow cells in these mice using scRNA-seq revealed an increase in inflammatory profiles, as well as profiles associated with senescence.

Fundamentally, *UBA1*^{mutant} clonal dominance could be hypothesized to exist as a function of either an intrinsic enhanced growth of mutant clones or enhanced survival in adverse cellular environments (such as inflammation). To address this question, the authors leveraged competitive mixing experiments of *UBA1*^{WT} HSPCs and *UBA1*^{mutant} HSPCs at various ratios. They observed a non-linear dose–response effect (Fig. 1), with increasing ratios of *UBA1*^{mutant} HSPCs:*UBA1*^{WT} HSPCs leading to increased destabilization of normal hematopoiesis. This observation suggested both a paracrine 'poisoning' effect of *UBA1*^{mutant} HSPCs on *UBA1*^{WT} populations and mutant resistance, possibly conferred by the senescence described previously. Examination of the bone marrow environment revealed increased levels of IL-1β, IL-18, CXCL10 and IL-1RA, which suggests a pro-inflammatory milieu was responsible for the 'poisoning' effect. This 'bystander effect' was also observed in non-human, mouse tissue. Interestingly, when fluorescently labelled

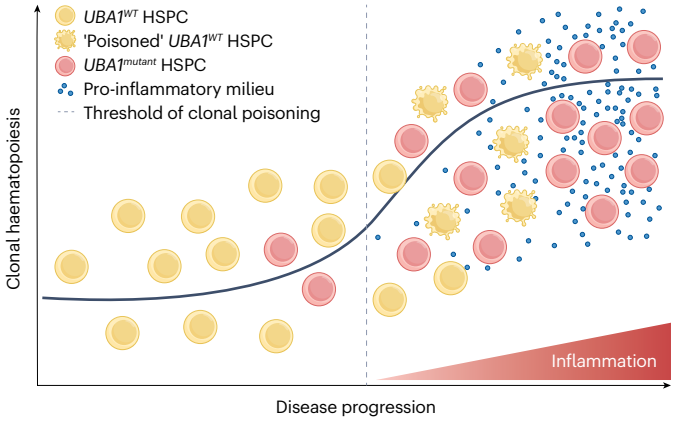

Fig. 1 | Clonal hematopoiesis in VEXAS syndrome. VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome arises from somatic mutations in *UBA1* within hematopoietic stem progenitor cells (HSPCs), which leads to systemic autoinflammation. Clonal dominance of $UBA1^{mutant}$ cells over $UBA1^{WT}$ cells occurs via a 'poisoning' effect within the bone marrow environment. A threshold of $UBA1^{mutant}$ cells is required before 'poisoning' drives clonal suppression of healthy HSPCs and VEXAS disease onset. 'Poisoned' $UBA1^{WT}$ HSPCs exhibit a decreased clonogenic capacity and stemness, which leads to further exponential outgrowth of VEXAS clones. Created with BioRender.com.

$UBA1^{WT}$ HSPCs were removed from the mutant bone marrow environment, they possessed decreased clonogenic capacity ex vivo, suggestive of transcriptional rewiring owing to inflammatory exposure.

This study begins to identify the mechanism of clonal dominance in VEXAS syndrome; however, the precise mechanism and specific molecules that promote clonal 'poisoning' remain unclear. Furthermore, the cell-autonomous and *UBA1*-dependent mechanism that drives inflammation originating in mutant tissue is not well delineated. Edited HSPCs were used in the humanized mouse model and, although these cells produced inflammatory signals on a scRNA-seq basis, they failed to produce the systemic, multi-organ phenotypes observed in patients with VEXAS syndrome and, thus, using this model for anti-inflammatory therapy could be limited. Thus, in the context of therapeutic intervention, future work beyond understanding the molecules responsible for clonal advantage might focus on cell-intrinsic inflammatory mechanisms and delineation of mouse models showing systemic phenotypes.

Correlations between the degree of proteotoxic stress and inflammation have been described in VEXAS syndrome[7,8]. Interestingly, activation of the unfolded protein response (UPR) in mutant HSCs, driven by ER stress, has also been shown to be a therapeutic vulnerability in VEXAS syndrome, with inhibition of the UPR (namely, via PRKR-like ER kinase) leading to synthetic lethality of mutant HSC populations ex vivo in a preprint study[9]. Data from studies on cell-intrinsic inflammation suggest that activation of the UPR is driven by a failure of ER-associated degradation[10]. Thus, there might be a causal link between ER stress and VEXAS syndrome mutant HSC-derived inflammation and senescence-driven resilience, as proposed in the study by Molteni et al.[2]. In such a paradigm, clonal dominance occurs at the cost of organismal inflammation, with activation of the UPR promoting resilience of mutant HSC clones in a pro-inflammatory environment. This mechanism then raises the following unique question: should clinicians be focused on treating clonality or on treating inflammation? Perhaps they are one and the same.

Ultimately, further examination of VEXAS syndrome in these contexts, as pioneered by Molteni et al.[2], could elucidate the role of inflammation in clonal dominance as a fundamental concept that underpins both healthy physiology and disease pathophysiology. In this way, the importance of studying monogenic inflammatory disorders transcends their individual effects by providing critical windows into universal biological mechanisms that would otherwise remain hidden in plain sight.

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Published online: 6 June 2025

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Acknowledgements

We thank J. Hadjadj for critical evaluation of the manuscript.

Competing interests

D.B.B. receives advisory board and consulting fees from Alexion, Novartis, GSK, Sobi and Genesis Therapeutics.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01282-1

Review article

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More than a leaky gut: how gut priming shapes arthritis

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Abstract

The gut microbiome forms an ecosystem that provides the host with numerous benefits such as digestion with nutrient generation, protection from pathogens and immune system maturation. Alterations in the microbial ecosystem associated with rheumatoid arthritis and spondyloarthritis have led to the gut–joint hypothesis, which postulates that these ecological changes cause immune dysfunction that contributes to the development of arthritis. Mechanisms by which dysbiosis might trigger arthritis include molecular mimicry, dysregulation of mucosal immunity, microbial translocation, production of immunomodulatory metabolites and immune cell trafficking. We discuss the data supporting each of these mechanisms, and highlight misconceptions, limitations and gaps in knowledge. In particular, we advise against the term 'leaky-gut' as the mechanisms and effects on the immune system of intestinal permeability and bacterial translocation are distinct. Nevertheless, rheumatoid arthritis and spondyloarthritis possibly result from the convergence of multiple pathways that could be unique to subgroups of individuals within these diseases. To move the field forward, each mechanism needs to be considered through the use of model organisms and interventional trials, individually and in concert.

Sections

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Key points

- Mechanisms by which microbes might cause rheumatoid arthritis (RA) or spondyloarthritis (SpA) include molecular mimicry, microbial translocation, dysregulation of mucosal immunity, production of immunomodulatory metabolites and immune cell trafficking.
- Microbial products and other small molecules transit the epithelium paracellularly, which increases when tight junctions are affected by immune and microbial factors.
- Increased paracellular transport, or epithelial permeability, is associated with RA and SpA, but how this increase contributes to pathophysiology is unclear.
- Bacterial translocation, which occurs via transcellular transport across the intestinal epithelium, is distinct from intestinal permeability and has been observed in both RA and SpA.
- Immunomodulatory products of microbial metabolism that are linked to RA and SpA, such as short-chain fatty acids, tryptophan metabolites and bile acids, can alter immune responses associated with arthritis.
- Lymphocytes and dendritic cells, primed in the gut, traffic to systemic sites, such as the joint and thymus, and alter lymphocyte education and immune responses associated with RA and SpA.

Introduction

Rheumatic and autoimmune diseases are assumed to result from a combination of genetic risk and environmental exposures. In the past decade, the gut microbiome has emerged as a substantial environmental exposure with ~10^{14} microbes colonizing the human host[1]. This microbial ecosystem aids the host by generating nutrients through digestion of dietary products, creating competition and a physical barrier from pathogens and providing key small molecules that mature the immune system. Numerous diseases including rheumatoid arthritis (RA) and spondyloarthritis (SpA) are associated with ecological shifts in the microbiome, often called dysbiosis.

A seminal study in 2013 was the first to associate *Prevotella copri* with new-onset RA[2], which has been replicated in additional studies and identified in populations of individuals at risk of RA[2]. Although direct causality of *P. copri* has not yet been demonstrated, specific strains of *P. copri* isolated from individuals with RA enhanced experimental arthritis in mice compared with those isolated from healthy individuals[3]; however, the mechanistic connection between these bacteria and autoimmunity were not explored. Furthermore, not all studies identified *P. copri* within the gut microbiome of individuals with RA. *Fusobacterium nucleatum*[4], *Eggerthella lenta*[5], *Escherichia coli*[6] and the genera *Collinsella*[5], *Lactobacillus*[7] and *Streptococcus*[6]are a few of the other bacteria that have been linked with RA in human population studies.

Similarly, microbiome associations with SpA vary among studies. In axial SpA (axSpA), *Ruminococcus gnavus*[6,8,9], genus *Dialister*[10], *E. coli*[6] and *Faecalibacterium prausnitzii*[11] are associated with the presence of disease, and in some cases, disease activity. Studies demonstrate that the genera *Ruminococcus* and *Akkermansia* are reduced in individuals with psoriatic arthritis (PsA) compared with healthy individuals and those with psoriasis[12], whereas other studies related specific *E. coli* isolates with inflammatory bowel disease (IBD)-associated SpA (IBD-SpA)[13]; however, the causal mechanism of this finding remains understudied.

Thus, despite this strong associative connection between the microbiome and RA and SpA, the specific mechanisms by which microbes trigger intestinal immune dysregulation and subsequent pathogenic targeting of the joint have not been elucidated. This Review discusses emerging hypotheses that connect microbial and mucosal changes associated with RA and SpA with the development of inflammation in the joint. We use specific examples of bacterial translocation, dysregulation of mucosal immunity, production of immunomodulatory metabolites and immune cell trafficking, and discuss the possible mechanisms by which microbes can trigger arthritis (Fig. 1). As molecular mimicry has been extensively reviewed elsewhere[14], this mechanism is only discussed briefly. Furthermore, this Review will challenge the popular 'leaky gut' hypothesis and propose the concept of mucosal education of systemic immunity, in which cells become licensed through interactions in the intestine and circulate systemically to perform immune surveillance of tissues. Although knowledge of the gut–joint axis (which has been reviewed in RA[15] and SpA[16]) is beginning to advance beyond associative studies, research to elucidate mechanistic links is needed, and we propose ways of addressing this knowledge gap. Finally, we provide perspectives on how the emerging gut–joint pathways might lead to clinical therapeutic applications.

Intestinal barrier permeability and bacterial translocation

The intestinal barrier between the microbiota and host immune system is dynamically regulated at the levels of the mucus, intestinal epithelial cells, tight junctions connecting the epithelial cells and the immune cells residing at the basolateral membrane of the epithelium[17]. Multiple diseases, including RA and SpA, show associations between disruptions in the intestinal epithelial tight junctions, bacterial products in joints and intestinal dysbiosis[18–20]. Taken together, these correlations have led to the 'leaky gut' hypothesis whereby perturbations in the tight junctions between epithelial cells enables the translocation of microbial products from the lumen across the epithelial barrier, which then trigger RA or SpA. However, such simple conclusions should be reconsidered given the complexity of the mechanisms of barrier permeability and bacterial translocation.

Regulation of intestinal permeability

The tight-junction complex includes occludins, claudins and cadherin proteins that connect the epithelial cells extracellularly and are supported intracellularly by actin filaments and the protein ZO-1 (Fig. 2). To enable paracellular transport of small molecules, some tight-junction proteins such as claudin-2 and myosin light chain kinase (MLCK1) increase permeability, whereas others such as occludin, claudin-1 and ZO-1 decrease permeability and increase barrier integrity. In RA studies, immunohistochesmical staining for tight-junction proteins suggests reduced expression of tight-junction proteins. In one study, occludin and claudin-1 were significantly reduced in both new-onset and established RA[18], whereas another study demonstrated that ZO-1 is decreased but occludin and claudin-2 were unaffected[19]. Together, these data suggest increased paracellular permeability of the colon in individuals with RA. Similar conclusions are found in the context of SpA. Using immunohistochemistry for tight-junction proteins in

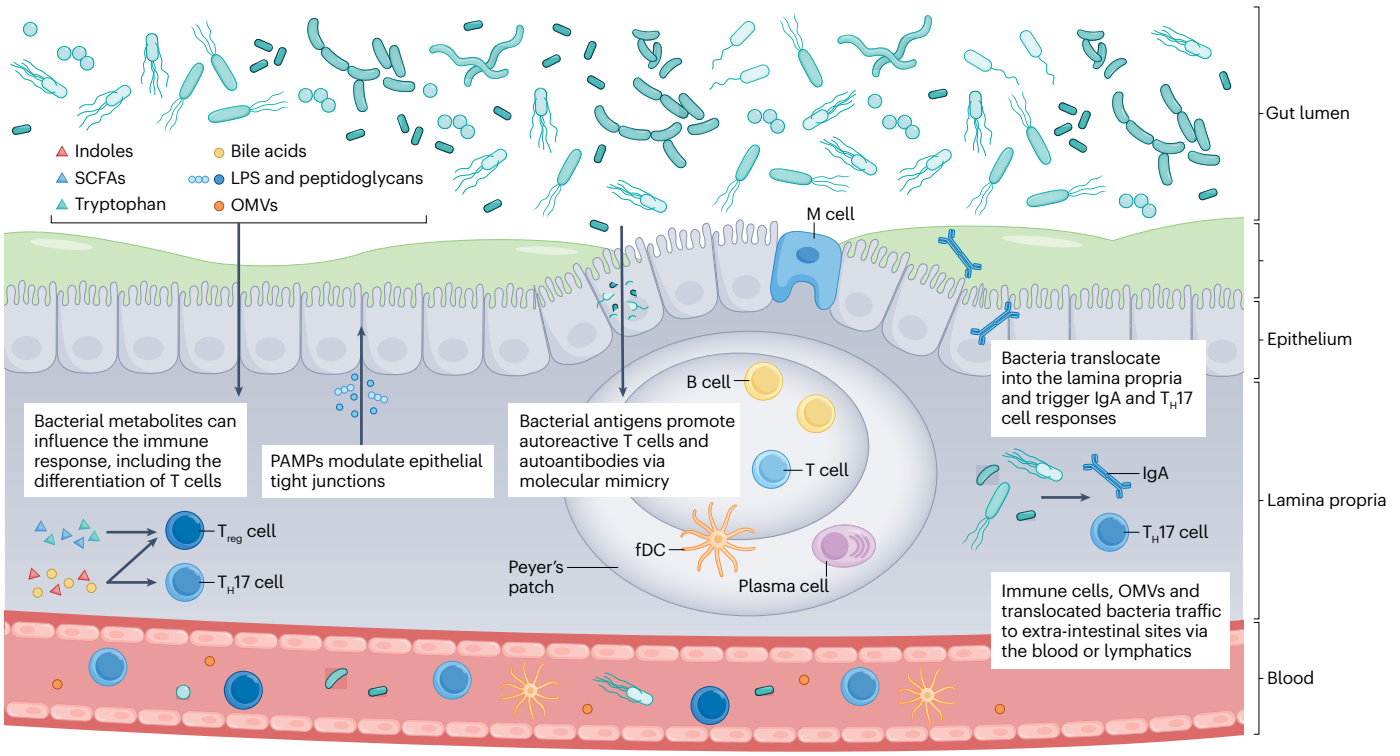

Fig. 1 | Emerging hypotheses that connect microbial and mucosal changes associated with rheumatoid arthritis and spondyloarthritis with the development of inflammation in the joint. Microbes at the intestinal barrier might trigger arthritis through adaptive immune responses that originally target microbial antigens (from bacteria such as *Prevotella copri* and *Subdoligranulum didolesgii*) then cross-react with self, a process known as molecular mimicry. Bacteria such as *S. didolesgii* translocate across the epithelium transcellularly but smaller bacterial products, such as metabolites, translocate paracellularly. Antigen uptake by dendritic cells (DCs) in the lamina propria results in their migration to Peyer's patches where they initiate T cell differentiation (such as T helper 17 (T_H17) cell differentiation), which then leads to B cell production of IgA. Numerous microbial products, such as lipopolysaccharide (LPS) and peptidoglycans, short-chain fatty acids (SCFAs) derived from dietary fibres, bile acid, tryptophan and its metabolites (such as indole) and outer membrane vesicles (OMVs), influence the immune response towards an antigen. In addition, pathogen-associated molecular patterns (PAMPs, such as LPS and peptidoglycans) alter tight junctions. Finally, monocytes and lymphocytes are known to traffic systemically from the gut, affecting immune responses at distal sites such as the joint. fDC, follicular dendritic cells; T_{reg} cell, regulatory T cell.

the ilea from patients with axSpA, claudin-1, occludin and ZO-1 were significantly reduced, even in those patients without underlying intestinal inflammation as assessed by histology[20]. However, the effects of a decrease in tight junctions are unknown, as studies often associate these findings with clinical factors such as disease activity indices but do not directly show causality.

Bacterial regulation of tight junctions. Tight-junction expression is regulated by numerous factors. Bacteria are known to modulate the expression of epithelial tight junctions by producing toxins and pathogen-associated molecular patterns (PAMPs) that affect tight-junction expression and synthesis; for example, host invasive pathogens such as enteropathogenic *E. coli* produce proteins and toxins that disrupt expression of tight-junction proteins in epithelial cells[21,22]. Interestingly, invasive and/or adherent Gram-positive and Gram-negative bacteria were observed in 70% of ilea tissue samples examined from individuals with axSpA, regardless of the presence of intestinal inflammation. Cultivated bacteria obtained from 25% of these ilea included *E. coli* and *Prevotella* species[20]; however, the study did not examine the relationship between the presence of these invasive and/or adherent bacteria and the reduced tight-junction expression observed.

PAMPs detected by pattern recognition receptors, such as Toll-like receptors (TLRs), also modulate tight-junction expression; for example, bacterial signals that activate the MyD88 signalling pathway increase IL-6 expression by intraepithelial lymphocytes, which results in increased claudin-1 expression and intestinal barrier integrity[23]. However, specific pattern recognition receptor signalling can have varying effects on tight-junction expression and barrier permeability; some TLRs will reduce barrier permeability (such as TLR2 signalling[24]) whereas others can increase it (such as TLR4 (ref. 25). Therefore, as microbial dysbiosis occurs in both RA and SpA, the abundance of PAMPs might alter tight-junction expression through these mechanisms, although this concept has not been formally studied in these diseases.

Host regulation of tight junctions. Along with microbiota from the intestinal lumen regulating tight junctions, factors from the basolateral side of the epithelium can also promote or disrupt epithelial permeability. Numerous immune, mesenchymal and epithelial cells contribute to the regulation of tight junctions through the production of cytokines. Several cytokines that are associated with RA and SpA, such as IL-6, IL-17, IL-22, IL-23, IL-10, TGF-β and TNF, are known to contribute to the repair and maintenance of the epithelial barrier[23,26–29], often depending on

context and composition of other cytokines. Patients with axSpA and PsA have increased expression of the cytokines IL-22 and IL-17 and an increased abundance of cells expressing these cytokines within their ilea compared with healthy individuals[30,31], although these studies did not associate barrier permeability with these cytokines.

Rodent models of inflammatory arthritis also associate increased intestinal IL-17, IL-22, IL-23 and TNF with increased barrier permeability or reduced tight-junction expression[20,32,33]; however, thus far, causality between cytokines and tight-junction expression in the setting of inflammatory arthritis has only been shown for IL-10 (ref. 34). In mice with K/BxN autoimmune arthritis or antigen-induced arthritis (AIA), the number of IL-10$^+$ lymphocytes and IL-10 receptor-expressing intestinal epithelial cells are significantly reduced compared with wild type animals[34]. In the AIA model, IL-10 receptor deficiency in epithelial cells led to the loss of ZO-1 expression, increased epithelial permeability, increased intestinal inflammation (determined by histology) and increased knee swelling[34]. These data suggest a model in which IL-10 stabilizes tight-junction proteins in the epithelium as one facet of its many anti-inflammatory mechanisms.

Assessing intestinal permeability

Intestinal permeability, or 'gut leakiness', in RA and SpA can be assessed indirectly by measuring serum levels of proteins that are associated with tight-junction regulation and subsequent paracellular transport (Fig. 2), including fecal calprotectin, the presence of circulating microbial products and functional assessments (such as the lactulose:mannitol ratio[35–37]). Although there is no true gold standard method for the evaluation of intestinal permeability, many of these assays have been used to evaluate intestinal permeability in patients with RA and SpA[18–20,38].

Serum biomarkers such as zonulin, intestinal fatty acid binding protein (iFABP), lipopolysaccharide (LPS), LPS-binding protein (LPS-BP) and soluble CD14 (sCD14) can be measured as surrogate indicators of tight-junction disruption; increases in these biomarkers are thought to reflect increased intestinal permeability[35,36,39]. Zonulin is a protein made by intestinal epithelial cells that degrades ZO-1 and destabilizes tight junctions, which increases permeability[36]. Intestinal epithelial cells also express iFABP, and increased serum levels of this protein are thought to reflect increased tight-junction degradation and epithelial cell disruption[35,37]. LPS is an immunogenic component of the bacterial membrane that is shed from Gram-negative bacteria. LPS shedding triggers the production of LPS-BP and sCD14 to prevent subsequent immune activation. Consequently, the presence of LPS, LPS-BP and sCD14 might indicate increased translocation of bacterial products across tight junctions in the intestinal epithelium. Indeed, increased serum zonulin is observed in patients with axSpA

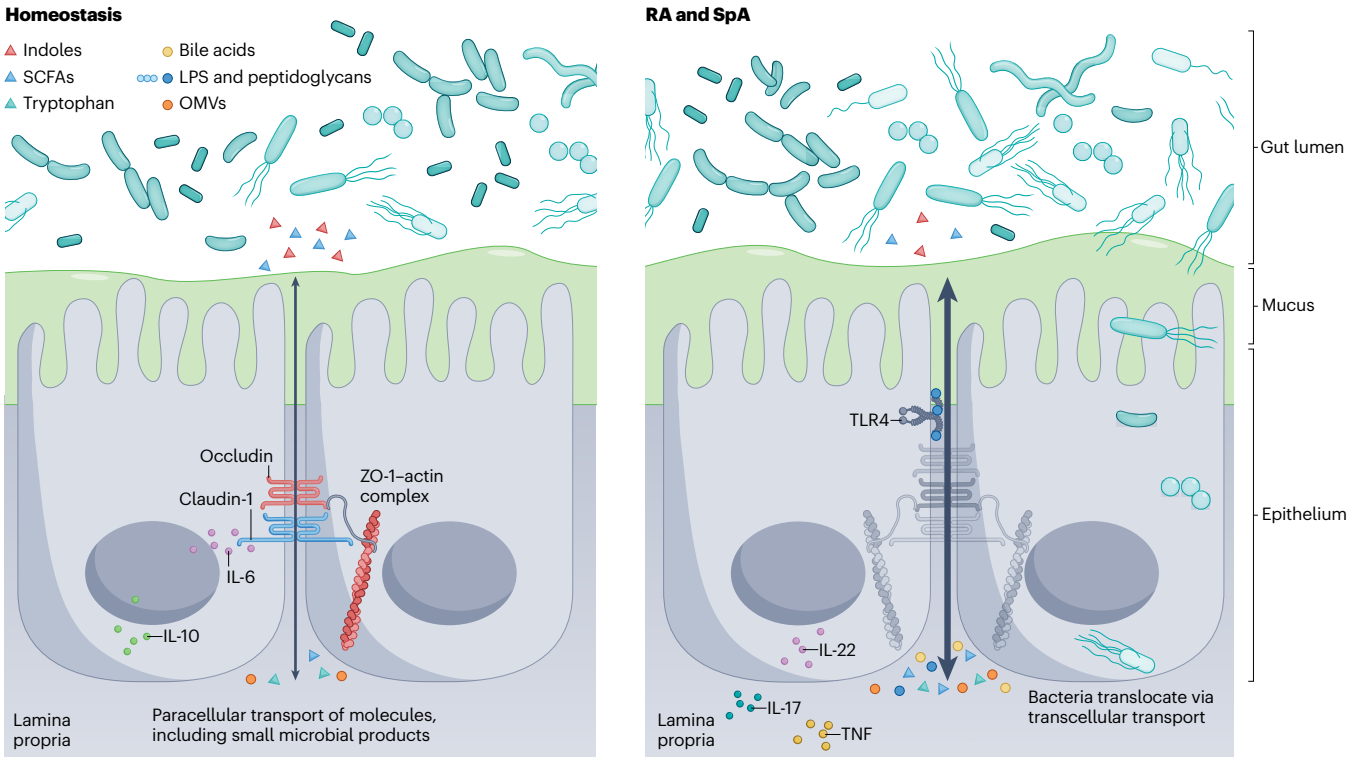

Fig. 2 | Transport of luminal contents across the epithelium at homeostasis and in rheumatoid arthritis and spondyloarthritis. Luminal contents transit the intestinal epithelial barrier through paracellular and transcellular mechanisms. Paracellular mechanisms occur via tight-junction regulation. Tight-junction proteins include occludin, claudin-1 and the ZO-1–actin complex, which includes zonulin. During homeostasis (left panel), cytokines (such as IL-6 and IL-10), microbial metabolites (such as indoles) and short-chain fatty acids (SCFAs) increase tight-junction expression to reduce permeability of small molecules and microbial products (such as lipopolysaccharide (LPS)). In rheumatoid arthritis (RA) and spondyloarthritis (SpA) (right panel), tight-junction expression is reduced, which enables increased epithelial permeability of small molecules. Cytokines, including IL-17, IL-22 and TNF, and pathogen-associated molecular pattern–pattern recognition receptor signalling (such as LPS–Toll-like receptor 4 (TLR4) signalling) are known to decrease tight-junction expression. Bacterial translocation that occurs in RA and SpA does so through transcellular pathways, not tight junctions.

(along with serum LPS, LPS-BP and iFABP)[20] and RA (along with sCD14), including during the preclinical period of RA[18,38], which suggests that intestinal permeability precedes disease onset. Additionally, inhibiting zonulin increases intestinal barrier function and reduces arthritis onset in a mouse model of autoimmune arthritis[18]. However, caution is advised when interpreting studies using these markers; some tests for zonulin cross-react with serum haptoglobin[39] and LPS-BP and sCD14 might be acute phase reactants[40]. As such, better markers of intestinal permeability are needed.

Functional assessment of intestinal permeability via tracing orally administered saccharides, such as sucralose and lactulose, into the urine, is also used to indicate leaks from the intestinal lumen into systemic circulation. Measuring the time it takes for such saccharides to appear in the urine following oral administration can indicate where in the intestine the leak might be located. Saccharides appearing in the urine after 1–2 h indicates the small intestine, whereas if the leak is in the colon it can take 8–24 h for the saccharides to appear[37]. The lactulose:mannitol ratio in the urine of patients with both new-onset and established RA is increased 24 h after consumption compared with healthy individuals[18], suggestive of increased permeability in the colon.

Although these tests are commonly used clinically to indicate increased intestinal permeability, translating the movement of small weight saccharides, which are often 200–300 Da, to much larger components such as live bacteria translocating across tight junctions is difficult. Even the commonly used FITC-dextran assay in rodent models of inflammatory arthritis, which measures orally administered 4 kDa dextran in the serum after 1–4 h, uses a model saccharide 10 times smaller than 40 kDa LPS, which is just one component of live bacteria.

Thus, although disrupted tight junctions and increased leakage of saccharides are observed in RA, SpA and rodent models of inflammatory arthritis, translating these findings to the hypothesis that live bacteria might translocate via leaky tight junctions still remains elusive. Even in the studies in which invasive bacteria were identified by histology of ileal samples from patients with axSpA[20], visualization of live bacteria translocating the epithelium is often associated with transcellular transport of bacteria through cells such as microfold cells or goblet cells[41–43]. In one clear example of bacterial translocation, increased serum FITC-dextran was associated with the presence of culture-recovered *Enterococcus gallinarum* in tissues distal from the gut, such as the liver[44,45]; however, the method of translocation of *E. gallinarum* was shown in vitro to be via transcellular transport rather than via a 'leaky gut'[45]. Additionally, the presence of potential pathobionts[46], such as the fungal species *Candida albicans*, which can invade and destroy enterocytes, enables transcellular bacterial translocation through necrotic epithelial cells[47], although overt epithelial cell death might enable unrestricted paracellular translocation of microbes[17]. Thus, considering the type of barrier permeability, paracellular versus transcellular, is important when interpreting barrier permeability data, and different techniques are needed to measure these different types of permeability. Based on the current knowledge of barrier permeability, we caution against interpreting disrupted tight junctions (such as circulating zonulin) and increased leakage of saccharides as increased bacterial translocation in the setting of RA and SpA. We further caution against the interpretation of increased intestinal permeability measures, as their pathophysiological effects remain unclear for RA and SpA.

Microbial sensing and regulation of immune responses

Paracellular intestinal permeability prevents microbes from passively migrating across the epithelium. Three general mechanisms enable sampling of the luminal environment for education of mucosal immunity: microfold cells, dendritic cells (DCs) and goblet cell-associated passages. Regardless of the sampling method, antigen is processed by DCs that then migrate to the Peyer's patches or mesenteric lymph nodes, where they stimulate adaptive immune responses, such as T helper 17 (T_H17) cells, regulatory T (T_{reg}) cells and B cell production of IgA. IgA coating of bacteria therefore identifies specific microbes that have triggered intestinal immune responses and might have a relevant role in the development of RA or SpA. Indeed, antibacterial IgA in the stool, total IgA in the circulation and synovium and T_H17 cell responses are increased in patients with axSpA compared with healthy individuals[48], possibly owing to the aforementioned mechanisms. IgA responses to fecal microbiota are altered in patients with axSpA compared with healthy individuals[49], but the effects of this finding on disease pathogenesis are unclear. In patients with IBD, IgA coating of a strain of *E. coli* that is analogous to adherent-invasive *E. coli* is higher in people with concomitant SpA than in those without[13]. Intriguingly, the *E. coli* that was targeted by IgA increased the number of mucosal and systemic T_H17 cells and promoted arthritis in mice with K/BxN autoimmune arthritis[13], which supports the hypothesis that IgA-targeted bacteria might have a role in the pathophysiology of SpA.

Bacterial translocation

Much of the literature currently assumes that translocation of intestinal bacteria only occurs in the presence of persistent intestinal or systemic pathology, whereby a substantial mucosal barrier breach occurs. However, live commensal bacteria do not reach internal organs under steady-state conditions[50–54] because two 'firewalls' prevent systemic dissemination of bacteria. The liver represents a 'firewall' after a breach of the intestinal vascular barrier, whereas the mesenteric lymph nodes restrict bacteria that breach the intestinal lymphatic barrier[55,56].

However, several studies now show that bacteria can breach these 'firewalls' during homeostasis as live commensal bacteria have been identified at systemic sites in healthy, conventionally housed mice[57–59]. The conditions under which such a breach occurs remain unknown, but in such cases, the live bacteria in extra-intestinal sites induce systemic IgG as another level of protection to the host[60]. Thus, anti-bacteria IgG might indicate bacterial translocation. In one study, serum IgG was mixed with an autologous fecal sample to identify bacteria that stimulated a systemic IgG response in patients with IBD-SpA[61]; *Mediterraneibacter gnavus* was more consistently coated with serum IgG in individuals with Crohn's disease-associated SpA than in people with Crohn's alone or healthy individuals, and correlated with Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores[61]. Although these findings suggest the translocation of bacteria, it remains unknown if *M. gnavus* circulates or translocates to inflamed joints.

Since 1973 (ref. 62), there have been numerous reports highlighting the existence of bacteria in the synovial fluid of patients with RA. Several studies report the translocation of bacteria, bacterial RNA or bacterial outer membrane vesicles (OMVs, cell membrane 'blebs' of Gram-negative bacteria that can contain proteins and enzymes from the parent bacterium) from the gut to the synovium[63–65]; however, few studies have mechanistically connected bacterial translocation with the onset of inflammation. Many mouse models have been used to examine translocated bacteria associated with human disease.

F. nucleatum is notably more common in the intestinal microbiome of individuals with RA than in that of healthy individuals[4]. This bacterium exacerbated disease in a mouse model of autoimmune arthritis by releasing OMVs. In the case of F. *nucleatum*, OMVs carry the FadA protein, which activates synovial macrophages that promote joint inflammation[4]. These findings highlight that these particles might migrate from the gut to joints, where their presence is associated with progression of RA[4].

Bacterial circulation from the gut to the joints might not be necessary to elicit arthritis. Rather, translocation to lymphoid tissues could be sufficient to stimulate pro-inflammatory pathways that lead to the development of RA and SpA[14,20,34,48,66,67]. For example, in the K/BxN autoimmune arthritis mouse model, bacterial 16S DNA is found in the spleen and lymph nodes and is associated with an increase in intestinal-like T cells and $CD45^+$ cells that express IFNγ, IL-6 and TNF[34]. These data support the hypothesis that bacterial translocation to systemic lymphoid tissues triggers immune responses central to the development of inflammatory arthritis. Alternatively, bacterial translocation across the intestinal epithelium into the lamina propria might be sufficient to stimulate immune responses that lead to arthritis. *Subdoligranulum didolesgii*, which is found in both individuals at risk of RA and in people with RA and is targeted by dual IgA–IgG clonal family plasmablast-derived monoclonal antibodies from individuals at risk of RA, translocates across the intestinal epithelium and triggers T_H17 cell and autoantibody responses that lead to inflammatory arthritis in mice[66]. *P. copri* and *Prevotella intestinalis* might work similarly, with OMVs translocating across the intestinal epithelium and stimulating DCs to produce IL-6 (ref. 68). *E. coli* and *Prevotella* species were identified within the lamina propria of biopsy-obtained ileal samples from patients with ankylosing spondylitis. Antibiotic treatment of transgenic rats that express HLA-B27 and human β_2-microglobulin, which spontaneously develop intestinal inflammation and SpA-like arthritis, increased tight-junction expression, reduced bacterial translocation and reduced intestinal inflammation[20]. However, an ongoing question as to whether bacteria need to persist following translocation and initiation of immune responses remains to be addressed.

Box 1 | Immunomodulatory microbially derived metabolites in rheumatoid arthritis and spondyloarthritis

Short-chain fatty acids
- Increase tight-junction expression and reduce barrier permeability
- Decrease the severity of disease in animal models of inflammatory arthritis
- An increase in dietary fibre increases serum short-chain fatty acids, which reduces the inflammatory immune response in rheumatoid arthritis (RA)

Tryptophan metabolism
- Some indoles decrease barrier permeability
- Decrease kynurenine in the circulation of people with RA
- Indoles are increased in the stool of people with axial spondyloarthritis
- Primary indole increases inflammatory pathways and the severity of disease collagen-induced arthritis in mice

Bile acids
- Have mixed effects on barrier permeability
- Decreased in psoriatic arthritis and people at risk of psoriatic arthritis who progress to disease
- Decreased in the synovial fluid of people with RA
- Decreasing serum bile acids via dietary intervention reduces pain in people with RA

Mucosal education of the systemic immune system

Mucosal education of immune cells by microbes is important for a functional systemic immune system[69]. Here we review the two mechanisms of mucosal education that are associated with RA, SpA or animal models of inflammatory arthritis: immunomodulatory microbial products and trafficking of mucosal cells to extra-intestinal sites.

Immunomodulatory microbial products

As the gut microbiota has emerged as a major modulator of systemic immune responses, metabolites secreted by the microbiota seem to have a crucial role in these processes. As noted previously, several studies show that the composition of gut microbiota is altered in people with RA and SpA. In both diseases, the diversity of the microbiota is reduced; dysbiosis occurs, and is characterized by an increase in pro-inflammatory species such as *P. copri*[2,3] and a decrease in beneficial bacteria such as *Bifidobacterium*[70] and *F. prausnitzii*[11]. It is logical to assume, and has been confirmed by correlative and experimental analyses, that these changes lead to altered production of microbial metabolites that could affect local intestinal-barrier permeability, with consequences for systemic immune responses and inflammatory arthritis.

Microbial metabolites can be distinguished as being synthesized de novo by the microbiome such as short-chain fatty acids (SCFAs) and tryptophan metabolites (such as indole derivatives) or as metabolites that were initially generated by the host and secreted into the lumen where they undergo modifications by gut microbes such as secondary bile acids. We examine the specific role of these three metabolite families (SCFAs, tryptophan metabolites and bile acids) in regulating barrier permeability and immune function, their mechanisms of action and their influence on the pathogenesis of RA and SpA (Box 1).

Short-chain fatty acids. The human diet contains a large amount of dietary fibre and indigestible carbohydrates that escape proximal digestion and are fermented by gut commensals such as *Bifidobacterium* and *Bacteroides* species into SCFAs including butyrate, acetate and propionate[71]. SCFAs, particularly butyrate, enhance the expression and assembly of tight-junction proteins and exert substantial anti-inflammatory effects that protect the intestinal epithelium from immune-mediated damage[72]. Butyrate-producing bacterial species are considerably reduced in the fecal microbiome of people with RA compared with healthy individuals[73], and serum butyrate and propionate are significantly reduced[74]. Interestingly, in a longitudinal study of individuals at risk of RA, defined as those positive for anti-citrullinated protein antibody (ACPA) with musculoskeletal pain but no joint swelling, those who progressed to RA had significantly lower serum butyrate compared with those who did not progress; butyrate concentrations also inversely correlated with ACPA IgA[75]. Similarly, levels of butyrate are significantly decreased in individuals at risk of PsA just prior to

developing disease[76], but are not different when comparing healthy individuals and those with established PsA[12].

Mechanistically, SCFAs reduce disease severity in transgenic rats that express HLA-B27 and human β_2-microglobulin and in mouse models of inflammatory arthritis (including AIA, collagen-induced arthritis (CIA) and the K/BxN autoimmune arthritis model)[73,74,77,78]. In AIA, butyrate altered the microbiome to generate more tryptophan-derived metabolites that increased regulatory B cells and suppressed germinal-centre B cells[74]. In mice with K/BxN autoimmune arthritis and CIA, butyrate increased bone volume and reduced osteoclastogenesis[73,77]. Serum SCFAs could be substantially increased in patients with RA by increasing dietary fibre; increased SCFAs were associated with reduced pro-inflammatory mediators (such as CCL2, IL-18 and IL-33)[79], anti-inflammatory changes to T cells (reduced circulating T_H1 cells and T_H17 cells and increased T_{reg} cells) and a significantly reduced health assessment questionnaire score[80]. Thus, SCFAs are a promising adjunctive therapy for individuals with RA and SpA.

Tryptophan metabolites. A diet rich in meat, fish, egg, cheese, cruciferous vegetables and beans has high levels of the essential amino acid tryptophan. Dietary tryptophan can be metabolized to three major classes: indole, kynurenine and serotonin. Only bacteria express the tryptophanase enzyme that generates the primary indole compound, whereas both the host and bacteria can generate kynurenine and other indole-containing metabolites (indole derivatives such as indole-3-aldehyde, indole-3-pyruvate and indole-3-acetic acid)[81,82]. Primary indole and indole derivatives can reduce barrier permeability and increase expression of tight-junction genes[83–85] in murine models of colitis[84,85].

Although tryptophan metabolism has been long-studied in RA, most work is associated with host-derived metabolism such as kynurenine. In a 2024 study of a large cohort of >500 individuals with RA, serum kynurenine, particularly kynurenic acid and xanthurenic acid, was significantly reduced compared with 98 healthy individuals[86]. However, this pathway of tryptophan metabolism can be performed by either the host (via indolamine 2,3 dioxygenase (also known as IDO), which can be activated by immune signals and microbial products, or tryptophan 2,3 dioxygenase (also known as TDO), in the liver) or by the microbiome (bacteria often use a TDO homologue)[81], and, thus, the contribution of the microbiome is unclear. Microbial products of tryptophan metabolism in RA have not been described, but in SpA, analysis of fecal metabolites from two cohorts of children with enthesitis-related arthritis demonstrated altered tryptophan metabolism[87]. Similarly, in adults with axSpA, fecal metagenomics suggests that tryptophan metabolism favours the generation of indoles, a finding that was confirmed by unbiased metabolomic testing of intestinal biopsies[88].

Mechanistic studies of tryptophan metabolites in models of inflammatory arthritis suggest that there are metabolite-dependent and context-dependent effects. One of the most frequently studied mechanisms through which many, but not all, tryptophan metabolites exert their effects on intestinal-barrier function and immunity is the activation of the aryl hydrocarbon receptor (AhR). AhR activation suppresses the production of pro-inflammatory cytokines and improves tight-junction integrity[89,90]. Conversely, some indoles might have pro-inflammatory properties. Giving mice with CIA a low tryptophan diet, which induces a lack of downstream metabolites, is protective; primary indole promotes CIA by promoting the differentiation of T_H17 cells and the generation of pathogenic autoantibodies[91]. Given the variable effects of tryptophan metabolites in arthritis, more research is needed to clarify the specific metabolites, signalling pathways and disease-specific mechanisms for therapeutic intervention.

Bile acids. Bile acids that are synthesized from cholesterols in liver hepatocytes are referred to as primary bile acids. After generation in the liver, primary bile acids are transported to the gut through the enterohepatic circulation. Primary bile acids are further metabolized by gut bacteria to generate secondary bile acids[92]. Most of the bile acids are then reabsorbed in the terminal ileum and recycled to the liver through enterohepatic circulation. A key feature of bile acids is their dual role in barrier permeability. They can enhance certain protective functions of the intestinal barrier, but excessive or dysregulated bile-acid recycling can contribute to increased barrier permeability and inflammation[92].

Most data in RA and SpA suggest that bile acids are anti-inflammatory. Primary and secondary bile acids were >50% lower in patients with PsA than in individuals at risk of PsA who did not progress during a 4-year monitoring period. In addition, bile acids were low in individuals at risk of PsA who did progress, even before progressing to PsA, and remained low after progression[76]. Bile acids were also reduced in the synovial fluid from patients with RA compared with those with osteoarthritis[93]. In a short 14-day open-label diet-intervention trial promoting 'anti-inflammatory foods', partially based on a Mediterranean diet, individuals with RA who experienced a 50% reduction in pain had significant elevations in their serum bile acids compared with those who did not have pain reduction[94]. As such, modulation of this pathway through diet might serve as a preventative and therapeutic approach to RA and SpA.

Immune-cell trafficking from the gut mucosa to systemic sites

Many studies have indicated active communication between mucosal and systemic sites via the trafficking of immune cells. Studies have shown direct trafficking of monocytes and lymphocytes, with both regulatory and pro-inflammatory properties, from the intestine to the joint in mouse models[18,95,96] (Fig. 3). Some T cells trafficking from the gut also expressed dual T cell receptors (TCRs), with one TCR induced by intestinal bacteria, such as segmented filamentous bacteria (SFB), and the other recognizing self[97]. Such trafficking could be interrupted by treatment with antibiotics or larazotide, a short-term zonulin antagonist, resulting in reduced arthritis severity[18,95].

Further supporting gut–joint lymphocyte trafficking, administration of vedolizumab, an antagonist for the gut homing α4β7 integrin, can lead to manifestations of de novo arthritis in patients with IBD who had previously not displayed arthritis symptoms[98,99]. A potential underlying mechanism is that vedolizumab, by preventing the immune cells from homing back to the gut and thus trapping them in systemic sites, causes an accumulation of inflammation systemically, leading to a flare of SpA. Interestingly, in the K/BxN autoimmune arthritis mouse model, anti-integrin β7 blockade increases arthritis severity by retaining T_H17 cells and T follicular helper (T_{FH}) cells in systemic sites, which supports this hypothesis[100]. In this case, blocking β7 augmented the autoimmune arthritis development, but only when specific gut microbiota, SFB, were present in the host. Combining β7 blockade and SFB colonization was particularly detrimental in this mouse model, as SFB increased the homeostatic level of $\alpha4\beta7^+$ T_{FH} cells and $\alpha4\beta7^+$ T_H17 cells. In addition to the pathogenic T_H17 effector cells located in inflammatory sites, non-pathogenic T_H17 cells and IL-10-expressing T_H17 cells naturally reside in the intestine and function as gatekeepers against microbial infections[101–104]. Overall, as the intestinal environment is known to nurture an immune tolerant environment that must endure

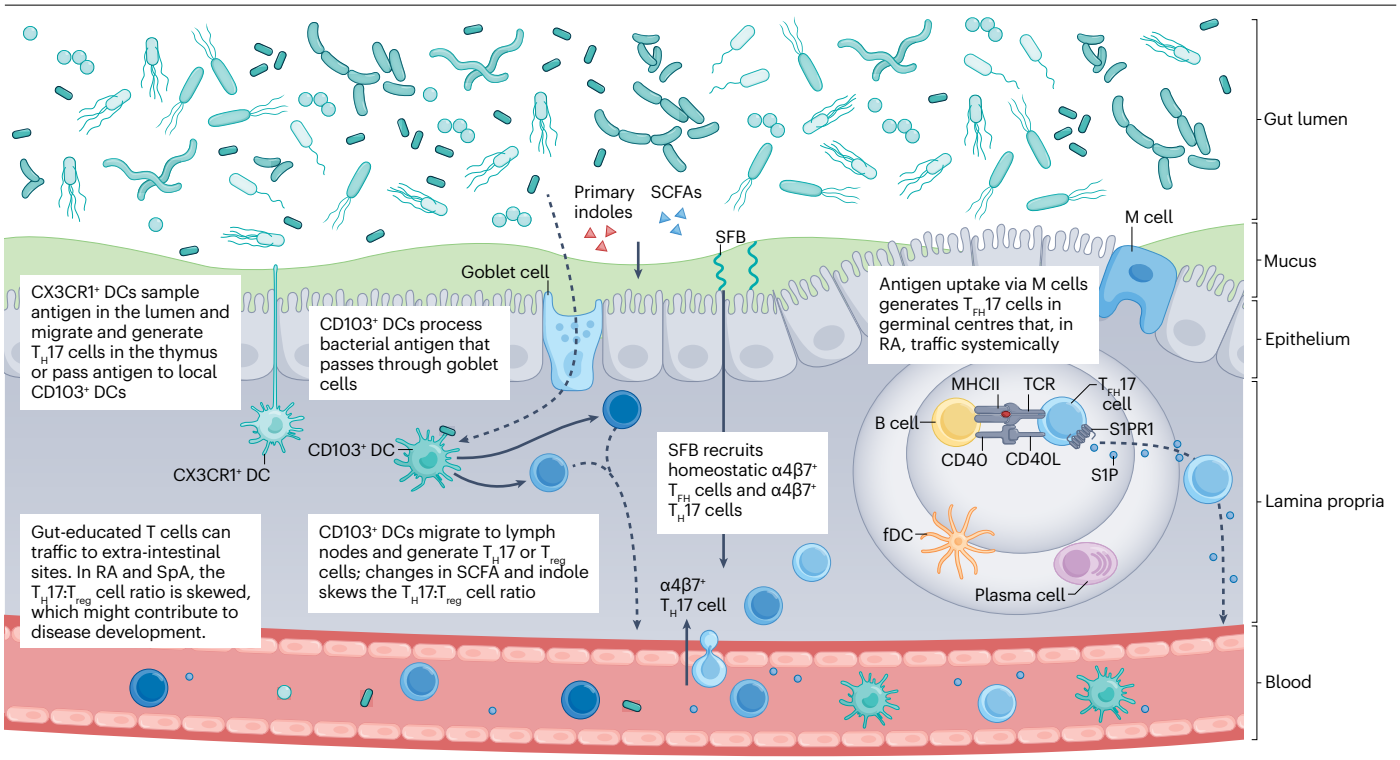

Fig. 3 | Immune-cell trafficking from the intestine. Bacterial antigens are sampled by CD103⁺ dendritic cells (DCs) through goblet-cell-associated passages or by CX3CR1⁺ DCs extending transepithelial dendrites that then pass the antigen to CD103⁺ DCs. The CD103⁺ DCs migrate to lymphoid tissues such as mesenteric lymph nodes or Peyer's patches where they initiate T cell differentiation. T cell differentiation can be influenced by exposure of CD103⁺ DCs to microbial metabolites; for example, DC exposure to indole results in T helper 17 (T_H17) differentiation but exposure to short-chain fatty acids (SCFAs) induces regulatory T (T_{reg}) cells. After antigen and metabolite exposure (DCs) and differentiation (T cells), monocytes and lymphocytes from the gut can traffic to systemic sites including the joint, lung, thymus and other extra-intestinal lymphoid organs. Trafficking of lymphocytes into the intestine is mediated through α4β7 expression by T cells and mucosal addressin-cell adhesion molecule (MAD-CAM) on endothelial cells. From the intestinal lamina propria, sphingosine-1-phosphate (S1P) from the circulation recruits S1P receptor 1 (S1PR1)-expressing T follicular helper 17 ($T_{FH}17$) cells from Peyer's patches. T_H17 cells and T_{reg} cells can migrate from the intestine into joint tissues, although the mechanisms remain unclear. CD40L, CD40 ligand; fDC, follicular DC; M cell, microfold cell; RA, rheumatoid arthritis; SFB, segmented filamentous bacteria; SpA, spondyloarthritis; TCR, T cell receptor.

constant exposure to food and microbial antigens, it is plausible that diverting the inflammatory cells from the gut to the systemic inflammatory sites could lead to systemic autoimmune disease. It is worth mentioning that this cellular trafficking is not limited to the gut–joint axis; gut-derived T cells can migrate and contribute to pathology in the central nervous system[105], eyes[106] and kidneys[107].

Some instances of T cell trafficking from the gut to systemic sites are rather unexpected. The egress of T_{FH} cells from Peyer's patches to systemic sites is essential for the development of autoimmune arthritis[108], which is surprising as T_{FH} cells are a T cell type known to express low levels of the trafficking receptor, S1PR[109]. Further studies indicate that these gut-derived T_{FH} cells in systemic sites are enriched for $T_{FH}17$ cells, a T cell type that has features of both T_{FH} cells and T_H17 cells. Uniquely, these $T_{FH}17$ cells display higher S1PR1 levels than T_{FH} cells, resembling the higher S1PR1 levels observed in T_H17 cells[110]. The chemokine receptor CXCR5 is essential for the retention of T_{FH} cells in the light zone of germinal centres[111] and S1PR1 signals seem to override migration induced by CXCR5 in B cells[112], suggesting a dominant role for S1PR1 compared with CXCR5 in $T_{FH}17$ cell trafficking. Interestingly, in addition to RA, peripheral blood levels of $T_{FH}17$ cells increase in many other autoimmune diseases associated with autoantibodies such as juvenile dermatomyositis, systemic lupus erythematosus, Hashimoto thyroiditis and Guillain–Barré syndrome[113–117]. It would be interesting to examine the mucosal origin of the circulating $T_{FH}17$ cells in patients with these autoimmune diseases.

Another mechanism by which leukocyte migration from the gut might influence inflammatory arthritis is through lymphocyte education. Gut-derived CX3CR1⁺ DCs migrate to the thymus to present microbial antigens, resulting in the generation of microorganism-specific T cells that then differentiate into T_H17 cells in the intestine. This is in contrast to antigens presented by medullary thymic epithelial cells and other thymus-resident antigen-presenting cells that facilitate negative selection[118]. Understanding how these different methods of antigen presentation by CX3CR1⁺ DCs, medullary thymic epithelial cells and other thymic antigen-presenting cells affect T cells might provide new approaches to treating immune disorders that are mediated by gut microbiota.

Challenges and opportunities

Evidence for a gut–joint connection is increasing for both RA and SpA (Table 1); however, further research is needed to mechanistically demonstrate the role of gut microbiota in the development and propagation

of inflammatory arthritis. Advancing understanding of the gut–joint axis presents both challenges and promising opportunities.

In the case of RA, there is accumulating evidence for molecular mimicry between antigens and bacteria such as *P. copri* and *S. didolesgii* and the development of autoantibodies relevant to disease, which has been extensively reviewed elsewhere[14]; however, the local mucosal immune environment also probably supports the evolution of autoimmunity. In the case of *S. didolesgii*, in addition to providing an antigenic stimulus, this pathogenic strain produces indole, which stimulates a local and systemic T_H17 cell response[91]. T_H17 cell responses are necessary for the development of mucosal secretory IgA[119], and the mucosal origins hypothesis is partly based on the development of mucosal IgA[14]. Interestingly, despite the ability of *S. didolesgii* to translocate across the intestinal epithelium into the lamina propria, barrier permeability, as measured by paracellular transport of the indigestible dextran labelled with FITC, is unaffected[66], supporting the transcellular mechanism by which bacteria gain access to the mucosal immune system. Additional studies are required to understand the full mechanism of how *S. didolesgii* as well as other bacteria link to the onset of disease in RA. Questions to be addressed include how microbes are sensed by the mucosal immune system, how innate and adaptive immune responses are triggered and how the response becomes systemic, targeting joints.

As noted previously, T_H17-derived cytokines such as IL-21 are influential in mediating the IgA immune response to microbes. Additional influences on the type of mucosal immune response come from the type of antigen-presenting cell that activates T cells. Resident $CX3CR1^+$ DCs that extend cellular protrusions into the lumen are known to produce IL-10 and promote the differentiation of T_{reg} cells[120]. Conversely, many $CD11c^+$ $MHCII^{hi}$ DCs that sample antigen from goblet-cell-associated passages stimulate effector T cell responses in the draining mesenteric lymph nodes. Furthermore, intestinal epithelial cells are also capable of antigen presentation via MHCII, which favours T_{reg} cells over T_H17 cells[121,122]. Thus, not only are the environmental signals such as metabolites influential but also the method of antigen acquisition and type of antigen-presenting cell are important mediators of the immune responses that might lead to inflammatory arthritis.

Despite these limitations, advances in the understanding of mucosal immunity have enabled the development of methods in which immune responses can be used to aid the identification of relevant microbes that might influence the development of inflammatory arthritis; for example, IgA-sequencing is a method used to identify immunologically relevant bacteria in the intestine[123]. As mucosal IgA autoantibodies and increased circulating IgA plasmablasts have been observed in those at risk of RA[124,125], researchers have adapted this concept to determine if autoantibodies can target intestinal bacteria, which would suggest that such bacteria could trigger initial adaptive immunity, leading to autoimmunity[66]. Other studies suggest that circulating anti-bacteria IgG is indicative of translocated bacteria that have breached the mucosal 'firewall'[60], and thus, these antibodies might help to identify relevant microbes, as has been done in IBD-SpA[61]. Finally, using defined bacteria to stimulate T cells from humans and measuring activation-induced markers on these T cells can identify microorganism-reactive T cells, although this method is limited to a prior suspicion of a specific bacteria hypothesized to trigger such T cells.

Another outstanding question is whether microbes act locally at the mucosa or translocate to the joint, and if so, are specific parts or persistence of the microorganism sufficient or necessary, respectively, for arthritis? Thus far, microbial products (such as nucleic acids, proteins and OMVs) have been identified in joint fluid and synovium,

Table 1 | Evidence for the gut–joint axis in rheumatoid arthritis and spondyloarthritis

Observation	RA	SpA
Intestinal bacterial dysbiosis	*Prevotella copri*[2], *Fusobacterium nucleatum*[4], *Eggerthella lenta*[5], *Collinsella*[5], *Lactobacillus*[7], *Streptococcus* species[6], *Escherichia coli*[6] and *Subdoligranulum didolesgii*[66], among others	Several candidates including *Ruminococcus gnavus*[6,8,9], *Dialister*[10], *E. coli*[5] and *Faecalibacterium prausnitzii*[11], among others
Impaired intestinal barrier	Increased serum zonulin[18], LPS-BP and soluble CD14 (ref. 38) in people at risk of and with RA Decreased expression of ZO-1 protein in the colon of people with RA[19]	Increased serum zonulin, LPS, LPS-BP and iFABP in people with axSpA[20] Decreased tight-junction expression as determined by immunohistochemistry in people with axSpA[20] Increased bacterial translocation into the lamina propria in people with axSpA[20]
Mucosal immune perturbations	Not reported	Expanded IL-17-producing immune cells in people with axSpA[30] Reduced ileal and colon IEL subsets in people with SpA[126,127] Increased IL-9 and T_H9, T_H17, and T_H22 cell expansion in PsA[31]
Bacterial translocation	Bacterial RNA and OMVs[63–65] in circulation and joints in people with RA	*E. coli* and *Prevotella* species in the intestinal lamina propria of people with axSpA[20]
Microbial metabolites	Decreased SCFAs in people with RA[128] but increased SCFAs in individuals at risk of RA who do not progress to disease compared with those who do develop RA[75] Altered tryptophan metabolism in people with RA[86] Altered bile acids in people with RA[94]	Altered tryptophan metabolism in people with axSpA[88] Decreased SCFAs and altered bile acids in people with PsA[76] Increased 3-hydroxypropionic acid in people with axSpA[129]
Cellular trafficking	Impaired function and altered migratory behaviour of MAIT cells in people with RA[130–132] Enhanced expression of β7 in unswitched memory B cells that correlates with RF titres in people with RA[133]	Increased $\alpha4\beta7^+$ T cells and MAIT cells in the synovium, and increased circulating IL-17 producing cells in people with axSpA[30,134]

AS, ankylosing spondylitis; axSpA, axial spondyloarthritis; IEL, intraepithelial lymphocyte; iFABP, intestinal fatty acid binding protein; LPS, lipopolysaccharide; LPS-BP, LPS binding protein; MAIT, mucosa-associated invariant T cell; OMV, outer membrane vesicle; RA, rheumatoid arthritis; RF, rheumatoid factor; SCFA, short-chain fatty acid; SpA, spondyloarthritis; ZO-1, zonulin-1.

Glossary

Commensal
Microbe that lives in a host without causing harm or receiving benefit.

Dysbiosis
Changes in the microbial community composition as compared with a defined control population.

Indole
A chemical structure including a benzine ring attached to a pyrrole ring forming an aromatic heterocycle; additional indole derivatives form when one of the hydrogen atoms of either ring is replaced with other atoms or side chains.

Leaky gut
Perturbations in the tight junctions between epithelial cells that enable the translocation of microbial products, but not whole bacteria, from the intestinal lumen across the epithelial barrier into the lamina propria.

Paracellular transport
Passive movement of molecules and microbial products between epithelial cells.

Potential pathobionts
Microorganisms capable of pathology when specific conditions are present in the host, but causality and mechanistic understanding have yet to be demonstrated.

Transcellular transport
Active shuttling of molecules and microbes through an epithelial cell.

yet other studies identify bacteria in lymphoid tissues or the intestinal lamina propria. Bacteria in these tissue sites other than the joint can probably trigger immune responses relevant to the development of arthritis. Additionally, the presence of bacteria in host tissues has been examined at single timepoints rather than evaluated at multiple timepoints to indicate persistence. Whether these bacteria only need to be present at a single time point or if they need to persist in the tissue to cause immune responses relevant to the development of arthritis remains to be determined. However, detecting potential pathobionts in tissues remains challenging owing to low abundance and lack of adequate environmental controls.

Nevertheless, we argue that increased intestinal permeability, as measured by paracellular transport of molecules that indicate passage through tight junctions, aka 'leaky gut', does not suggest bacterial translocation. Rather, increased exposure of microbial products that transit paracellularly have independent mechanisms for influencing mucosal immune responses that might be relevant to the development of RA and SpA. However, identifying the microbial products (such as metabolites) that are generated in the gut remains challenging. Although measurements of microbial products in the serum and fecal material are often used, we again caution interpretation as many bacterial metabolites that are absorbed in intestinal tissues are further metabolized locally or modified by liver metabolism, which could affect serum measurements. Measurements of bacterial products in the fecal material are limited to what has not been absorbed by the host. Sampling of intestinal tissue provides the most direct measurement of absorbed bacterial products, but obtaining samples requires invasive procedures such as biopsy during endoscopy.

Reproducibility of microbiome findings, barrier permeability and bacterial translocation remain a considerable challenge, possibly owing to the large heterogeneity in RA and SpA study populations. Factors such as disease features, biomarkers, disease duration, treatments and comorbidities across study populations can confound study results. Additionally, treatments such as NSAIDs and DMARDs are known to alter the gut microbiome and intestinal permeability. Mediation analyses, standardizing cohorts, controlling for treatment and natural history studies will improve the rigor and reproducibility of findings.

Finally, a challenge for the gut–joint connection is demonstrating causality; most causal studies are limited to animal models, and some studies, such as cellular trafficking, do not have clear paths for investigation in humans. Therefore, current knowledge is limited to associative studies in patients with disease, although natural-history studies are increasingly enabling better associative investigation. In addition, interventional studies might also facilitate understanding of causality in patients. Potential interventions include selective depletion of microbes through phage, nanoligomers, prebiotics, other microbiome manipulations or even metabolite supplements to enhance anti-inflammatory or block pro-inflammatory metabolic products. Preclinical studies also suggest a role for antagonism of zonulin in reducing arthritis severity[18]. As blocking the α4β7 integrin potentially increases arthritis, other targets of lymphocyte trafficking, such as S1P inhibitors, might be beneficial in stopping pro-inflammatory lymphocytes from migrating to the joint. Such interventional studies will further understanding of the gut–joint axis in individuals with RA and SpA.

Conclusions

Understanding of the gut–joint axis continues to evolve beyond associations of bacterial dysbiosis with RA and SpA. Many mechanisms link changes in the microbiome to the immune dysfunction of these diseases, including paracellular and transcellular passage of microbes and their products, immune interactions with such microbes and their products and the migration of lymphocytes from the gut to extra-intestinal sites. To claim that RA and SpA, or other autoimmune diseases, is a result of a 'leaky gut' is an oversimplification and misleading, rather, these diseases probably occur because of the convergence of multiple mechanisms, and the specific mechanisms and combinations unique to subgroups of individuals with diseases such as RA and SpA. Natural history and interventional studies should be intentionally designed to address current limitations of confounding variables and investigate the specific mechanisms outlined herein.

Published online: 31 July 2025

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Acknowledgements

K.A.K. is supported by R01 AR075033, T32 AR07534, P30 AR079369, Spondyloarthritis Association of America and the Shear Family Foundation. K.Y. is supported by K08DK128544. K.K. is supported by R01 DK134366. H.-J.J.W. is supported by grants R01 AI107117 and R01 HL148347. M.M.Z. is supported by grants from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) via projects DFG499424281, DFG-RU2886-Project A01, DFG-CRC1181-Project-No. B07 and the Versus Arthritis Foundation, London.

Author contributions

K.A.K., K.Y., K.K., H.-J.J.W. and M.M.Z. researched data for the article. K.A.K., H.-J.J.W. and M.M.Z. contributed substantially to discussion of the content. K.A.K., K.Y., K.K., H.-J.J.W. and M.M.Z. wrote the article. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Rheumatology* thanks Axel Finckh, who co-reviewed with Benoit Gilbert, Rebecca Blank and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01280-3

Review article

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Peripheral neuronal sensitization and neurovascular remodelling in osteoarthritis pain

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Abstract

Pain is the primary complaint in individuals with osteoarthritis (OA) and changes as the disease progresses. Anatomical changes in several joint structures potentially contribute to pain, including the increased innervation of the periosteum, synovium and subchondral bone, and the pathological innervation of articular cartilage, which is aneural under physiological conditions. Research has focused on molecules that sensitize afferent neurons, such as neuropeptides, neurotrophins, pro-inflammatory cytokines and ion channels. The neurotrophin nerve growth factor (NGF) is the best validated target in OA pain, with proven analgesic effects in preclinical and clinical studies, although the development of NGF-targeted therapeutics has been hampered by serious side effects. One relatively neglected area of research is the contribution to OA pain of the molecular pathways that mediate remodelling of nerves in disease. Remodelling requires coordination between the nerve and the associated vasculature, along with signals that are received from the surrounding parenchyma. Key cell guidance molecules, including angiogenic factors, ephrins, semaphorins and SLIT proteins are involved in nerve growth during development, and their expression is increased in osteoarthritic joints.

Sections

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Key points

- There is increasing evidence to suggest that remodelling of blood vessels and nerves occurs in OA, including in the normally non-innervated articular cartilage, and improved microscopy methods have enabled characterization of nerve and blood-vessel remodelling during the course of experimental OA.
- Molecular drivers in disease may control neoinnervation and neovascularisation of joint tissues through neurotrophic factors, axon guidance molecules and angiogenic factors.
- Increased expression of nociceptive molecules within the joint tissues, and of ion channels along the sensory nerves innervating the joint, is associated with peripheral sensitization of nerves and to OA-related pain.
- Therapeutic targeting of several neurotrophins is showing promise in clinical trials targeting OA pain.

Introduction

Osteoarthritis (OA) is the most prevalent form of arthritis, affecting approximately 500 million people worldwide, and is a leading cause of disability globally[1,2], primarily owing to pain. The pain experience in OA evolves over time, and although it is typically localized to the affected joint, pain may radiate beyond[3,4]. In early-stage disease, pain is usually mechanical in nature, often triggered by joint use on weight bearing[5,6]. This type of pain is typically intermittent and can be alleviated by rest or reduced activity. However, as the disease progresses, pain becomes more persistent, with a high proportion of patients experiencing pain at rest, possibly reflecting an evolving pain disorder phenotype leading to the amplification of pain signals within the central nervous system[7]. The epidemiology of OA pain has revealed complex associations between structural features of disease and patient-reported joint pain[8,9]. Radiographic associations with knee OA pain include the occurrence of bone-marrow lesions, cartilage loss and increased synovial-tissue volume[10–13]. Although structural damage is probably the primary driver of nociceptive OA pain, differences in pain perception are also controlled by other factors. For example, central sensitization has been shown to exacerbate pain independently of the degree of joint damage[14–16]. Nevertheless, the transition from mechanically induced pain to continuous pain is usually associated with worsening structural disease and reflects the complexity of the pain experience[17]. Advances in our understanding of the mechanisms of OA pain[18] has continued to highlight complex and multifactorial aetiological factors, involving both intra-articular and extra-articular mechanisms[19]. Additionally, psychological factors such as mood disorders, pain catastrophizing[20,21], learned helplessness and genetic predisposition[22–24] contribute to the variability in pain experience. The heterogeneity of pain behaviour in OA has been associated with clinical phenotypes of the disease, including 'OA with chronic pain' and 'inflammatory OA'[5,25]. The ability to identify OA phenotypes reliably and link them to specific biological mechanisms ('endotypes') might enable targeted and effective treatment strategies for OA-related pain.

Pain is encoded by nociceptive sensory neurons (nociceptors) that have cell bodies located in the dorsal root ganglia (DRG) adjacent to the spinal column[26]. These neurons are pseudo-unipolar — they have two projections from the cell body that are fused initially but then branch and extend in opposite directions. One projection is towards the spinal cord and central nervous system, synapsing onto the dorsal horn; and the other projection is a long axon projecting towards the target tissues, such as the joint[27]. Noxious stimuli are initially detected by the peripheral sensory terminals in the target tissue. Nociceptors express specialized receptors that detect various stimuli, such as high or low temperatures, mechanical load and chemical signals[28–30]. Information from the receptive fields is then relayed along the peripheral axon and central projection to the dorsal horn of the spinal cord. Pain signals are then relayed along the ascending pathways from the spinal cord towards the brain, ultimately resulting in the perception of pain[31]. Subtypes of sensory nerves can be further distinguished based on myelination status and axon diameter; in bone only medium-diameter Aδ fibres and small-diameter C-fibres are detected[32]. Neuronal classes are also distinguished based on action potential conduction velocity. Small unmyelinated C-fibre nerves are the slowest conducting and are associated with dull pain, whereas medium conducting myelinated Aδ fibres are typically associated with fast and sharp pain[33,34]. The functional classifications have been complemented by detailed molecular classification through cross-species single-cell sequencing of DRG[35].

The anatomical distribution of nerves within healthy and diseased joints is likely to help us to understand the drivers and evolution of pain in diseases such as OA. In healthy joints, the periosteum, synovium and bone marrow are the most highly innervated tissues[36–39]. These tissues have been associated with OA pain, although increased innervation, including neoinnervation and neovascularization, of previously non-innervated tissues such as the articular cartilage, also occur in OA and might additionally contribute to OA pain[40–42]. Neurovascular coupling in OA is not a new concept, yet advances in this have been slow owing to ongoing imaging challenges, even with modern microscopic techniques. The high metabolic demands of nerves are supported by their co-localization or close proximity to blood vessels throughout the body[43]. During development, both structures require molecular cues to support directional growth (tropism) and differentiation and survival (trophism)[43,44]. These molecular cues are also detected postnatally and are increased in diseased tissue, including in OA. Evidence from developmental studies and models of bone pain demonstrate that changes in neuron and vessel anatomy occur in parallel, in a symbiotic relationship[43]. Taken together, there is still much to learn about the mechanisms of neurovascular remodelling in disease.

This Review focuses on the peripheral drivers of pain in OA, highlighting not only the molecules that we know sensitize nerves in OA but also those that might drive neurovascular remodelling and could be targeted for therapeutic benefit. After reviewing the anatomy of joint neurovascular structures in health and OA, we focus on axon and angiogenic guidance molecules, as well as neuropeptides, neurotrophic factors, pro-inflammatory molecules and ion channels that sensitize pain fibres in disease.

Anatomy of neurovascular structures in the joint

Neurovascular architecture in the healthy joint

Of the joint tissues, bone has been one of the best characterized with regard to innervation as well as vascularization (Fig. 1a). Early mapping of neuronal subtypes using immunohistochemistry revealed that the synovium and periosteum are two of the most densely innervated tissues of the joint, containing both sympathetic[37,45,46] and sensory innervation[38,39,45]. These are followed by the bone marrow and cortical bone[47] (Fig. 1a). Recent advancements in microscopy techniques have

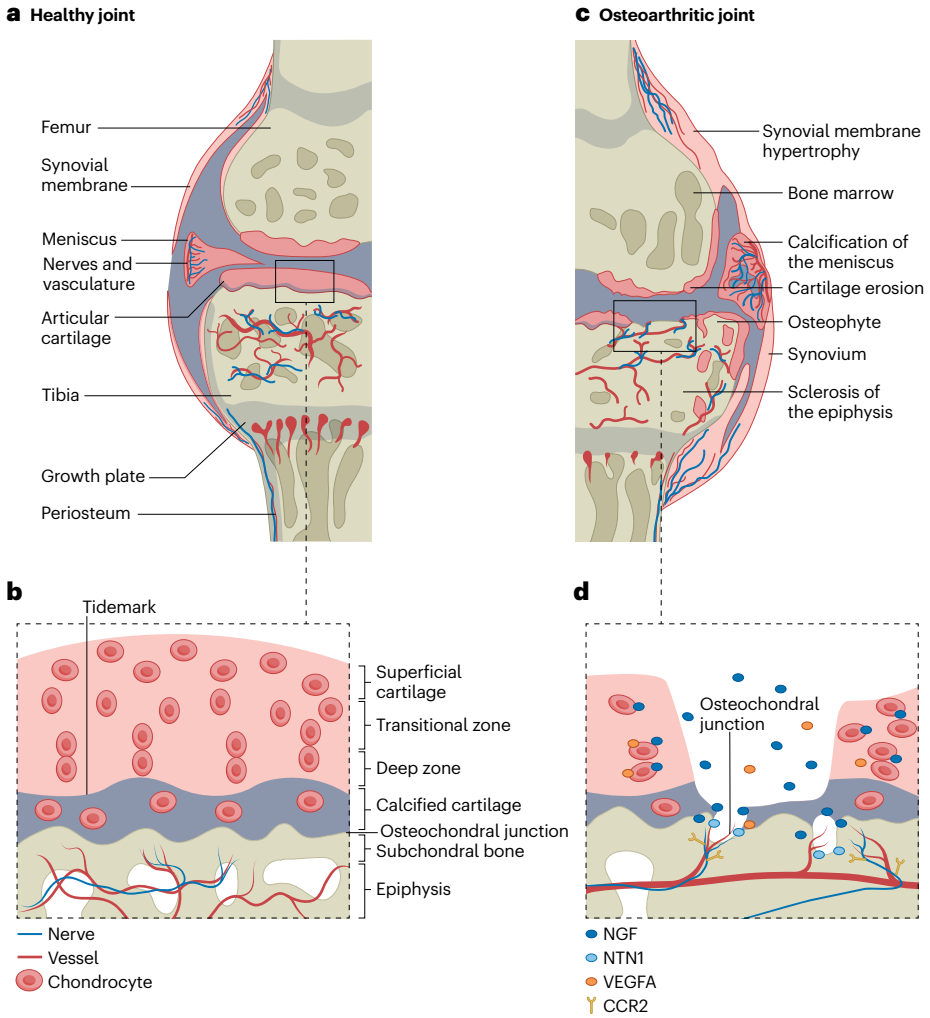

Fig. 1 | The neurovascular architecture of the synovial joint in health and osteoarthritis. a, Synovial joints are encased within a synovial membrane, which is responsible for nutrient supply to joint tissues, clearance of waste products and production of molecules that contribute to joint lubrication such as hyaluronan and lubricin. The menisci and ligaments help to stabilize the joint, thus providing important mechanoprotective mechanisms. Articular cartilage both protects the subchondral bone and mechanoadapts to mitigate mechanical stress. The synovium, meniscus and ligaments (particularly insertion sites) are well vascularized and innervated. The periosteum is the most innervated region of the joint, with nerves associated with blood vessels. In the epiphysis, nerves and blood vessels are mostly present within the bone marrow. They form a continuous network with branch points extending towards the subchondral bone. **b**, The healthy articular cartilage is avascular and aneural. **c**, The anatomical hallmarks of osteoarthritis are synovial membrane hypertrophy, cartilage erosion in the load-bearing region of the joint, calcification of the meniscus, sclerosis of the epiphysis and the formation of new bone (osteophyte). In disease, new vessels are reported within the epiphysis, particularly in the osteophyte, which also contains sensory innervation. Blood vessels and nerve density are increased in the synovium and meniscus. **d**, At regions of cartilage damage, chondrocytes and bone cells produce neurotrophic factors (such as nerve growth factor (NGF)), cell-guidance molecules (such as netrin-1 (NTN1)) and angiogenic factors (such as NGF, NTN1, and vascular endothelial growth factor-A (VEGFA)). Additionally, sensory neurons demonstrate increased expression of receptors (such as C-C chemokine receptor type 2 (CCR2)) that alter their excitability and CCR2 response to inflammatory mediators such as monocyte chemoattractant protein-1 (CCL2). This is associated with the appearance of osteochondral channels containing neurovascular bundles that penetrate the calcified cartilage from subchondral bone.

enabled further characterization of the nerves in bone. In long bones, sensory and sympathetic axons enter the bone marrow cavity through the nutrient foramen travelling alongside or wrapped around vessels, respectively[48–50]. In the epiphysis, axons appear to enter through the epiphyseal foramen and run predominantly within bone marrow and terminate at the epiphyseal growth plate[48,49]. Within the cortical bone, sensory axons, such as calcitonin gene-related peptide (CGRP)-positive axons, and sympathetic axons, such as tyrosine hydroxylase (TH)-positive axons, are present within Haversian canals and often run alongside or wrap around vessels[51,52]. The presence of nerves in the subchondral cortical bone is low[47], but could be visualized in single, 60-µm-thick healthy murine joint sections[51].

High-resolution three-dimensional imaging of the long bone demonstrates a complex network of arterial and venous vessels that enter the bone marrow at multiple sites along its length[53]. Other tissues of the joint are also well innervated and vascularized including

the synovium, meniscus and cruciate ligaments, particularly bony insertions[54–56] (Fig. 1a). Additionally, the joint capsule is highly innervated, with the main source of innervation originating from the sciatic, fibular and tibial nerves[57,58]. The joint capsule is densely populated with sensory neurons, including those expressing mechanoreceptors[59]. The synovium comprises the lining layer, the lining–sublining interface and the sublining region. High-resolution imaging of mouse synovial tissue has demonstrated crosstalk between nociceptors, capillaries and macrophages in these three layers of the synovium[60,61]. In the sublining layer, arterioles are present with sympathetic, TH-positive, neurons wrapped around them and sensory, CGRP-positive, neurons running in parallel. The main arterial source for the knee is the genicular arteries[62,63]. In the healthy synovial joint, articular cartilage and the inner region of the menisci are avascular and aneural, relying on adjacent structures for nutrient transfer[55,64–66]. In healthy joints, insensitivity of articular cartilage to needle injection has been previously reported, supporting evidence that articular cartilage is both aneural and avascular[67] (Fig. 1b).

Neurovascular architecture in the osteoarthritic joint

Although the characterization of vasculature and innervation in healthy bone is well documented, only a few anatomical descriptions exist in OA (Fig. 1c), and these use varied methods to quantify innervation and vasculature. These include segmentation analysis[68], tracing innervation to calculate the density within regions of interest[42,69], manual scoring of sensory neuron-positive 'channels'[70] and measuring the fluorescence intensity of immunohistochemistry-stained sections[40] or signal emitted from fluorescent protein tagged transgenic animal tissues[71]. All quantification methods rely on high image resolution and a good signal-to-noise ratio in order to segment fine structures such as sensory nerves from background fluorescence. Collectively, these studies, which are largely performed in animal models, have corroborated the anatomical changes seen in individuals with OA, including cartilage damage, subchondral bone sclerosis[72] and osteophyte formation[73]. In OA, blood-vessel density increases in the synovium, osteophytes, subchondral bone plate and menisci[74–77]. This altered vessel organization might contribute to ossification of osteophytes and of the deep layers of articular cartilage[54]. Furthermore, lymphatic vessel volume and drainage function have been shown to decrease with disease in mouse models[78,79].

Evidence of neoinnervation of cartilage in OA emerged in 2007 (ref. 41). Histology from late-stage human osteoarthritic joint sections showed evidence of 'osteochondral channels', containing vasculature and sensory, CGRP-positive, nerves invading the cartilage and osteophyte[41]. Subsequently, sections from symptomatic OA joints showed evidence of osteochondral channels invading the calcified cartilage[80] (Fig. 1d). Another study in early, surgically-induced, OA in mice demonstrated that vessels were increased in the epiphysis preceding cartilage degradation and bone remodelling[40]. Furthermore, using an antibody to detect PGP9.5, a protein expressed in most neurons and neuroendocrine cells, increased fluorescence intensity was detected within the epiphysis in mice with OA, compared with sham-operated controls[40,81,82]. Few other studies have looked at epiphyseal vasculature and innervation concurrently, with studies in rodent models of OA predominantly examining sensory neuron remodelling alone. These studies typically utilized sections from the damaged load-bearing region of the joint to map innervation. For example, confocal images of knee sections from a nociceptor-fluorescent reporter mouse, showed signal consistent with nociceptor remodelling in the subchondral bone; whereby high tdTomato-positive structures (presumed neurovascular) appeared to branch towards the damaged region of cartilage[71]. The density of innervation was also increased in the meniscus and synovium of mice with OA compared with controls[71]. In addition, sensory nerve density, measured by confocal images of CGRP immunoreactivity, was increased in the subchondral bone at the onset of pain behaviour in a rat model of OA[69,80]. Inhibiting tropomyosin receptor kinase A (TRKA), the high-affinity receptor for nerve growth factor (NGF), reduced pain behaviour along with CGRP-positive sensory nerves in osteochondral channels within the subchondral bone but not the subchondral bone marrow, suggesting that NGF is involved in pathological sensory neuron remodelling[80]. Further studies are needed to map neurovascular coupling in OA and to generate quantitative measures that would enable validation of putative molecular drivers of neurovascular remodelling.

Peripheral neuronal sensitization in osteoarthritis

Nociceptors are equipped with a variety of receptors that respond to harmful stimuli, such as mechanical stress, temperature and chemical irritants. These receptors include mechanosensitive ion channels, transient receptor channels (TRPs)[83,84], purinergic receptors such as P2X[85], G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs)[86,87]. The activation of these receptors leads to the initiation of pain signals by direct opening, or controlling the threshold for opening, of gated ion channels that depolarize the neuron or control the amplitude and frequency of the action potential, as measured by electrophysiology techniques[29].

Peripheral sensory neuron sensitization emerges as a key process in the development of OA pain. Nociceptor sensitization is described as reduced thresholds for eliciting an action potential or as an increased rate of action potential firing[29]. In general, sensitization follows aberrant stimulation of a nociceptor through a soluble neuronal sensitizer, such as an inflammatory mediator, or a noxious stimulus. Some nociceptors that are termed 'silent nociceptors' exhibit mechanical sensing properties but only respond to stimuli following priming with NGF in models of inflammation[88]. Sensitization can be attributed to altered expression or activity of receptors and ion channels on nociceptors that reduce thresholds for eliciting an action potential. In OA, several candidate pain sensitizers have been proposed that can induce these changes, including neuropeptides, neurotrophins, inflammatory molecules and others.

Ion channels in peripheral sensitization

Ligand-gated ion channels are amongst the most important structures for detecting various noxious stimuli[89,90] (Table 1). The non-selective cation channel transient receptor potential vanilloid-1 (TRPV1), is expressed on a substantial subset of nociceptors and responds to heat, capsaicin or protons by transporting sodium and calcium ions into the nerve terminal. This depolarizes the sensory neuron to elicit action potentials resulting in pain perception (Supplementary Fig. 1).

Peripheral sensitization in OA involves an upregulation of ion channels and receptors that are integral to nociception[91]. This ultimately reduces the threshold required to depolarize and activate sensory nerves. In rat models of OA, joint afferent neurons exhibit altered mechanical sensitivity, along with increased expression of receptors and ion channels in the peripheral afferent terminals[92]. In mouse models of OA, expression of voltage gated sodium channels, including $Na_v1.8$ (encoded by *Scn10a*), is increased at both the DRG[93] and the peripheral joint[71,94,95]. The increased expression of $Na_v1.8$ in the peripheral joint

Table 1 | Key receptors and ion channels expressed on sensory neurons

Receptor category	Gene	Mechanism of activation or action
Transient receptor potential cation channel family (TRP)	*TRPA1* *TRPC3* *TRPC4* *TRPC5* *TRPV1* *TRPV3* *TRPV4* *TRPM2* *TRPM8*	Sensation of heat and pain Directly activated by capsaicin
Acid-sensing ion channels (ASICs)	*ASIC1, ASIC2, ASIC3, ASIC4, ASIC5*	Sensation of altered pH
Voltage-gated ion channels (sodium, potassium and calcium ion channels)	$Na_v1.7$ (*SCN9A*) $Na_v1.8$ (*SCN10A*) $K_v1.4$ (*KCNA4*) $K_v1.1$ (*KCNA1*) $K_v1.2$ (*KCNA2*) $K_v9.1$ (*KCNS1*) $Ca_v3.1$ (*CACNA1G*) $Ca_v3.2$ (*CACNA1H*) $Ca_v3.3$ (*CACNA1I*)	Generating action potentials from transporting ions, which enables modulation of neuron excitability
Mechanically activated ion channels	*PIEZO1* *PIEZO2*	Activated upon mechanical stimulation to modulate neuron excitability
Purinergic receptors	P2X receptors (*P2RX1* to *P2RX7*) P2Y receptors (*P2RY1, P2RY2, P2RY4, P2RY6, P2RY11, P2RY12, P2RY13* and *P2RY14*)	Detection of ATP following noxious mechanical stimuli
Tropomyosin receptor kinase A (TRKA)	*NTRK1*	High-affinity receptor for NGF, which is expressed on most sensory neurons and modulates neuron excitability

$Ca_v3.1$, calcium channel, voltage-dependent, T type, alpha 1G subunit; $Ca_v3.2$, calcium channel, voltage-dependent, T type, alpha 1H subunit; $Ca_v3.3$, calcium voltage-gated channel subunit alpha1 I; $K_v1.4$, potassium voltage-gated channel subfamily A member 4; $K_v1.1$, potassium voltage-gated channel subfamily A member 1; $K_v1.2$, potassium voltage-gated channel subfamily A member 2; $K_v9.1$, potassium voltage-gated channel subfamily S member 1; $Na_v1.7$, sodium voltage-gated channel alpha subunit 9; $Na_v1.8$, sodium voltage-gated channel alpha subunit 10; P2X; ATP-gated P2X receptor cation channel family; P2Y, purinergic receptor.

tissues correlates with heightened pain sensitivity in OA. Upregulation of TRPV1 is also observed in mice with OA and TRPV1 has been investigated as a potential analgesic therapeutic target[30,96–98]. The mechanically activated cation channels, PIEZO1 and PIEZO2, are also implicated in peripheral sensitization[99]. Work in various mouse models of OA has indicated that PIEZO2, expressed on $Na_v1.8$-positive sensory neurons, might be the primary mediator of mechanical pain associated with OA[94]. These neurons are mainly localized to the subchondral bone during late-stage OA, rather than the joint capsule[100]. Further work is needed to understand the tissue-specific regulation of different ion channels in OA, and their contribution to sensitization of the sensory nerves supplying the joint.

Other pain-sensitizing molecules

Other pain sensitizers are broadly classified into neuropeptides (Box 1); neurotrophins (see also next section); pro-inflammatory cytokines or chemokines (Box 2); and other signalling molecules such as angiotensin II (Box 2). Neuropeptides, neurotrophic factors and cytokines or chemokines have increased expression during inflammation, and act directly on sensory neurons that express their cognate receptors[29]. Inflammation seems to involve bidirectional activation of immune cells and neurons[101]. For example, within the synovium of mice following a systemic immune challenge, sensory neurons of the interface layer interact with capillaries, through CGRP, to modulate circulating levels of defensive macrophages. Upon inflammation, macrophages respond locally and activate nociceptors to secrete CGRP and further enhance the macrophage response[60]. Inflammation is also induced by mechanical injury, as occurs in OA, through 'mechanoflammation'[102,103]. Many pain-sensitizing molecules, such as NGF and inflammatory cytokines are upregulated in osteoarthritic knee joints (as detected in synovial fluid, cartilage, synovium or whole-joint profiling) (Box 1). After injury, the cartilage, despite being aneural, has been shown in preclinical models of OA to become an important source of nociceptive molecules including NGF, bradykinins and tachykinins[104–106], some of which have therapeutic implications (see below). Whether the same sensitizers act both peripherally and centrally in OA pain remains to be determined. Central sensitization is common in individuals with advanced OA, and is associated with poorly localized pain[107].

Neuropeptides are released at the synaptic terminal by exocytosis upon neuron activation[108]. Upon release, neuropeptides act directly on neighbouring synapses to activate GPCRs, which initiate secondary neuron excitability[109] (Table 2). Neuropeptides are also released from peripheral terminals, leading to neurogenic inflammation[110]. As these neuropeptides are expressed in various subsets of neurons, they are useful targets for imaging sympathetic and sensory neurons. Interestingly, the expression of neuropeptide receptors is not exclusive to neurons. For example, several neuropeptide receptors are expressed on endothelial cells[111], adipocytes[112], synovial fibroblasts[113], chondrocytes[114] and immune cells[115,116]. As a result, neuropeptide signalling modulates multiple systems, including the vasculature, where neuropeptides promote vasodilation. In symptomatic OA, several neuropeptides are increased in the affected joint. Neuropeptide Y (NPY) levels are increased in the osteoarthritic synovial fluid of individuals with OA compared with healthy donors[117], and the NPY receptor Y_1 (encoded by *NPY1R*) and receptor Y_2 (*NPY2R*) are expressed by chondrocytes and might have a role in cartilage homeostasis[114]. Substance P, a member of the tachykinin family, is detected in the synovial fluid of rheumatoid arthritis and osteoarthritic joints[118]. Additionally, polymorphisms in the gene for tachykinin receptor 1 (*TACR1*; also known as neurokinin-1 (NK_1) receptor) have reported associations with symptomatic OA[119].

Nociceptor sensitizers might also be secreted by resident or recruited immune cells in OA[95]. The contribution of pro-inflammatory cytokines, including interleukin-1β (IL-1β), to OA joint degradation remains unclear[120]. However, IL-1β[121], as well as IL-6 (ref. 122) and tumour necrosis factor (TNF)[123] have been implicated in OA-associated pain. Chemokines are also implicated in OA pain. For example, monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) signals through C-C chemokine receptor 2 (CCR2), and CCR2 deletion in mice with

surgically induced OA has been associated with reduced macrophage numbers in the DRG and delayed pain behaviour[124,125].

Neurotrophic factors in osteoarthritis pain

During development, axon guidance molecules (including angiogenic factors) and neurotrophic factors orchestrate innervation and vessel patterning across tissues[126–128]. These pathways are well described in development but have not been explored in depth in the context of disease. Whether molecules that act as neurotrophins in development can assume the same functions in disease remains unknown. In light of the above-discussed anatomical changes to vasculature and sensory neurons in OA, this is probably an omission. Understanding the role of neurotrophic factors in the postnatal joint might open up potential therapeutic avenues in OA pain. In the next section, candidate molecules are described based on their role in neuronal development and OA pain, where this has been studied.

Neurotrophic factors have many described functions during development, including supporting neuronal survival, development and differentiation[129]. The neurotrophic factors fall into two categories: neurotrophins and the glial cell line-derived neurotrophic factor (GDNF) family of ligands[130]. In mammals, neurotrophins include NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3; encoded by *NTF3*) and neurotrophin-4 (NT-4; encoded by *NTF4*, also known as *NT-4/5*)[128]. They bind to high-affinity tyrosine receptor kinases, TRKA, TRKB and TRKC, and to p75, at low affinity. NGF binds with high affinity to TRKA, BDNF and NT-4 bind to TRKB, and NT-3 binds to TRKC[87]. Since their discovery, additional functions of these 'trophic' factors have been identified, including in neuron sensitization (Fig. 2).

Nerve growth factor

NGF was the first identified developmental neurotrophic factor[131] and remains one of the best studied members of the neurotrophin family. The discovery that endogenous NGF is also a potent nociceptor stimulator revealed its potential role in pain[132,133]. ProNGF, the precursor to mature NGF, binds preferentially to p75. ProNGF is cleaved by the convertase enzyme furin to create the mature neurotrophin[134]. NGF is thought to induce pain predominantly through its high-affinity receptor, TRKA[135]. The low-affinity receptor, p75, also has multiple NGF-dependent actions including enabling high-affinity binding to TRKA by acting as a co-receptor[136], binding to neurotrophins alone with low affinity[137], and binding to proNGF with high affinity to induce apoptosis[138]. NGF signalling regulates cell survival and apoptosis in both sympathetic and sensory neurons[139,140]. Mature NGF and proNGF bind to their receptors directly at the afferent terminals, and this leads to retrograde transport of the TRKA–NGF and TRKA–proNGF complexes to the DRG[141]. At the DRG, these complexes induce long-term transcriptional changes, including the upregulation of nociceptive neuropeptides such as CGRP and ion channels such as $Na_v1.8$ (ref. 142). NGF also acts directly within the joint to sensitize neurons by stimulating the release of mediators from inflammatory cells, by turning on previously silent mechanoreceptors, and by increasing the activity of other receptors and ion channels, including TRPV1 and Ca^{2+} channels[143,144].

Several cell types besides neurons produce NGF and contribute to NGF–TRKA signalling responses. Endothelial cells[145] and skin vascular smooth muscle cells secrete NGF to coordinate neuronal migration and sympathetic innervation during embryogenesis[146]. NGF signalling has a role in coordinating axon development and survival in the embryo. It has also been implicated in bone physiology during development and postnatally. For example, skeletal disorders have been associated with loss-of-function *NTRK1* mutations in humans[147,148]. During primary ossification, osteochondral progenitors secrete NGF, which directs innervation, vascularization and subsequent ossification of the joint in a TRKA-dependent manner[149]. Additionally, osteoblast-derived NGF drives changes in bone density following mechanical force[150]. In rodent models of OA, confocal imaging using immunohistochemistry and gene expression analyses demonstrated increased NGF expression in chondrocytes, osteochondral channels and the bone marrow of late-stage OA joints[69,104]. p75 is expressed in osteochondral cells in vivo, and knocking out p75 in murine OA reduces subchondral bone and osteophyte volume[151].

Studies using exogenous NGF in the context of pain have associated it with nerve fibre sprouting and sensitization[152]. The nociceptive

Box 1 | Other neuropeptides and neurotransmitters implicated in peripheral pain sensitization

Opioid peptides

Proenkephalin is the precursor for enkephalins, which are endogenous opioid peptides and regulate physiological processes such as stress, behaviour, pain and anxiety. Opioids are a potent and addictive analgesic drug class, whereby overprescription has caused an 'opioid crisis' in the USA and Canada. The mRNA transcript expression of μ-opioid receptor (*MOR*) is increased in the dorsal root ganglia at the time of pain behaviour, and opioid antagonists accelerate the development of pain in a meniscectomy model of mouse osteoarthritis (OA). However, a clinical trial has reported that the efficacy of opioids is no greater than that of non-opioid medications. Opioids are thus not a recommended treatment option for OA, especially considering the side effects of continual opioid use[332–334].

Neuropeptide Y

Neuropeptide Y (NPY) is abundantly expressed in the nervous system and has an important role in autonomic function. NPY is often released alongside noradrenaline from sympathetic neurons and is an inhibitor of noradrenaline. NPY is expressed in the bone, including in the periosteal sympathetic axons and osteocytes. NPY signalling has also been implicated in acute and chronic pain, and depending on the signalling mechanism the effects of NPY have been shown to be either pro-algesic or analgesic. NPY is increased in synovial fluid, and both NPY and the NPY receptors Y_1 and Y_2 are increased in cartilage from individuals with knee OA. In mice, intra-articular injections of NPY following induction of OA (destabilization surgery model) increased cartilage degradation and chondrocyte hypertrophy[114,117,335–338].

Calcitonin gene-related peptide

Calcitonin gene-related peptide (CGRP) is released by sensory afferents, thereby encoding pain signalling. CGRP also acts as a vasodilator and increases blood flow. In addition, CGRP has been implicated in other cell types within the joint. For example, synovial fibroblasts from symptomatic OA biopsies express CGRP. Intra-articular injections of CGRP in the knee of healthy rats or rats with monoiodoacetate-induced OA led to mechanical hypersensitivity, which was reversed by a CGRP antagonist[339–341].

properties of NGF have been demonstrated in rats, with intra-articular injections of NGF leading to reduced weight bearing on the injected side within 15 min, and pain recovery occurring within 30 min[144]. TRKA blockade with a soluble receptor was analgesic in late-stage, surgically induced mouse OA, a time at which *Ngf* was upregulated in the joint[153]. Although inhibitors of TRKA were ineffective at reducing pain in human OA studies[154], neutralizing antibodies against NGF have shown efficacy. In mouse and rabbit models of surgically induced OA, NGF sequestering by specific antibodies was analgesic[155,156], albeit with associated increased bone volume and trabecular thickness[155]. NGF monoclonal antibodies have also shown effective analgesia in patients with OA[157]. Despite several successful phase II and III clinical trials, monoclonal antibodies that target NGF were not recommended for licencing by the FDA and EMA as the results from clinical trials revealed rapidly progressive OA in around 8% of treated participants[158,159]. Whether this side effect resulted from removal of a protective pain response, or from other biological actions of NGF within the joint given the role of NGF in bone remodelling in development, is unknown. These data suggest that beyond encoding pain in OA, NGF signalling might have an underappreciated and complex interaction with tissue homeostasis and disease.

Brain-derived neurotrophic factor

Similar to NGF, BDNF has a role in neuronal development, survival and growth[160]. The immature proneurotrophin isoform of BDNF is proBDNF, which, like proNGF, binds preferentially to p75. Similar to

Box 2 | Other inflammatory mediators implicated in peripheral pain sensitization

Bradykinin

Bradykinin mediates inflammatory pain signalling and symptoms associated with inflammation such as increasing vasodilation. Bradykinin receptors B_1 and B_2 are expressed on nociceptors and on chondrocytes. Bradykinin also has the ability to mediate pain signalling by transactivating other receptors, such as the ion channel TRPV1, which is highly expressed on knee nociceptors, to decrease nociceptor activation thresholds. Bradykinin levels are detectable in synovial fluid from individuals with osteoarthritis (OA) or rheumatoid arthritis (RA), and particularly increased in RA and inflammatory OA[342–344].

Interleukin-1β

Interleukin-1β (IL-1β) has been implicated in the pathogenesis of OA as a potent inducer of cartilage degradation. IL-1β directly acts on nociceptors to increase pain sensitivity. In rodent models of inflammatory arthritis, nociceptors express increased levels of IL-1 receptor (IL-1R), and activation of IL-1R signalling increases TRPV1 expression, thereby reducing thermal sensitivity and sensory neuron activation thresholds. Deletion of IL-1β in TRPV1-positive nociceptors prevents allodynia without effecting joint damage in an inflammatory model of arthritis. Altogether, evidence suggests that IL-1β is involved in pain associated with inflammation[345,346].

Tumour necrosis factor

The levels of tumour necrosis factor (TNF) are increased during injury and inflammation and act on nociceptors to signal for pain. Additionally, TNF signalling induces the release of other nociceptive factors, such as NGF and IL-1β. In the joint, TNF drives the acute pain phase that follows after destabilization, but chronic pain in OA cannot be supressed by anti-TNF treatment, demonstrating that chronic pain is not dependent entirely on inflammatory signalling[153,347,348].

Interleukin-6

Interleukin-6 (IL-6) levels are increased during inflammation and in chronic conditions such as RA. IL-6 mediates pain associated with inflammation, as it reinforces IL-1β and TNF production and acts directly on nociceptors to increase TRPV1 expression. IL-6 levels are positively associated with bradykinin levels in synovial fluid from individuals with OA. IL-6 expression is also increased in the dorsal horn in a rat model of OA. Furthermore, blocking IL-6 signalling reduced pain as measured from weight-bearing asymmetry. In a mouse model of post-traumatic OA, IL-6 signalling contributed to joint damage and pain in a sex-dependent manner[343,349–352].

Monocyte chemoattractant protein-1

Monocyte chemoattractant protein-1 (MCP-1) regulates the migration and infiltration of inflammatory cells, acting through the high-affinity receptor CCR2, which is expressed on various cell types, including sensory neuron presynaptic terminals. In mouse models of chronic low-grade osteoarthritic inflammation, macrophages were seen to infiltrate the dorsal root ganglia in addition to an upregulation of the chemokine CCL2 and its receptor at the onset of painful behaviours. Additionally, CCR2 protein expression was upregulated on sensory neurons in the joint 8 weeks post-destabilization surgery in mice[70,125,353].

Prostaglandins

Prostaglandin E_2 (PGE_2) is produced by the cyclooxygenases PTGS1 and PTGS2 during inflammation and acts directly on nociceptors. The PGE_2 pathway is implicated in inflammatory and neuropathic pain. PGE_2 is also upregulated in the subchondral bone in OA, and PTGS1 and PTGS2 are up- and downregulated, respectively, in synovial biopsies from individuals with symptomatic OA. Cyclooxygenase inhibitors such as NSAIDs are one of the most common treatments for pain but are ineffective in the long term for treating OA pain and can lead to persistent side effects[354–357].

Angiotensin II

Angiotensin II (Ang II) binds with high affinity to the angiotensin receptors AT_1 and AT_2 in the classical renin–angiotensin system. Blockade of Ang II signalling has been effective in models of pain, including a trial in patients with post-herpetic neuralgia and in mouse models of cancer-induced bone pain. AT_2 receptor signalling overlaps with the NGF pathway, whereby blockage of the AT_2 receptor inhibits NGF-induced neurite outgrowth. AT_2 receptor signalling might be involved in OA pain considering both the crosstalk with NGF signalling and the increased expression of renin–angiotensin system enzymes (such as the angiotensin-converting enzyme) in biopsies from individuals with OA[357–360].

proNGF, proBDNF is cleaved enzymatically, resulting in mature BDNF, and both BDNF isoforms can be secreted extracellularly[161]. The levels of proBDNF and p75 are high early in development and decrease as levels of mature BDNF increase with age[162]. ProBDNF is biologically functional and acts through p75 to drive apoptosis and negatively regulate neuronal complexity and density[163,164]. By contrast, the functions of mature BDNF, which signals through TRKB, have been associated with neuroprotection and synaptic plasticity[165,166].

Postnatally, increases in the levels of BDNF in the central nervous system are associated with neuroprotection and enhanced cognition after exercise[167,168]. Voluntary aerobic exercise was associated with improved cognitive function and increased serum levels of BDNF in humans, as well as increased levels of *Bdnf* mRNA in the spinal cord and muscle of rodents. Following noxious capsaicin stimuli or NGF exposure, BDNF is anterogradely transported to primary afferent terminals in the dorsal horn to increase central hyperexcitability[169]. Additionally, BDNF–TRKB signalling modulates central excitability by suppressing inhibitory synapses[170], potentially through the downregulation of the K-Cl cotransporter 2 (KCC2)[171]. In the joint, TRKB is expressed primarily on myelinated neurons, which are also TRKA positive, and in a small subset of non-peptidergic neurons[172]. Sustained BDNF signalling, in response to a noxious stimulus, might contribute to neuron sensitization. Indeed, deletion of *Bdnf* in sensory neurons protected mice from developing mechanical hyperalgesia[173]. Increased levels of BDNF have been reported in chronic pain (including in OA) and neuropathic pain[174,175], but the exact mechanism of BDNF-dependent pain sensitization remains unclear. Intra-articular injections of BDNF into healthy rodent joints did not elicit a pain response, as assessed by weight bearing[176], unlike the effects observed with intra-articular NGF[94,177]. However, injections of BDNF into an OA joint in a monoiodoacetate-induced OA model led to increased pain-like behaviour as inferred from reduced weight bearing on the affected paw[176]. Moreover, BDNF sensitized medium-diameter Aδ fibres during acute and late pain phases in rats with monoiodoacetate-induced OA[172]. In this model, regulation of proBDNF changed over the course of disease, whereby proBDNF protein levels increased transiently in the synovium during the acute pain phase and in the subchondral bone only later, during the chronic pain phase. Future work is needed to understand how BDNF signalling interacts with NGF-driven sensitization of TRKA-positive neurons[144] and the potential role of BDNF in neuron remodelling.

Neurotrophin-3 and neurotrophin-4

NT-4 has been shown to act as a chemoattractant supporting peripheral axon elongation in development[178]. In a model of peripheral axon regeneration, using grafts from either *Bdnf*- or *Ntf4*-knockout mice, a reduction in regenerated lengths was observed with *Ntf4*-knockout grafts compared with wild type or *Bdnf*-knockout grafts[179]. Furthermore, exogenous treatment with either NT-4 or BDNF reduced this effect. NT-4 also supports morphine-mediated analgesia, and *Ntf4*-knockout mice showed reduced analgesic responses to morphine that are thought to be driven by TRKB in the brainstem[180].

During embryonic development, NT-3 supports innervation of the peripheral nervous system[181] and, similar to NGF, the development of sensory neurons by acting as a chemoattractant[182,183]. In neonatal skin, hyperinnervation of sensory neuron afferents after injury is driven in an NT-3-dependent manner[184]. Taken together, the evidence lays a foundation for a role for NT-3 in sensory neuron growth during development and following injury. Whether this signalling cue drives pain as a result of neoinnervation of the injured tissue is unknown. Both analgesic and pro-algesic roles have been described for NT-3 postnatally. Specifically, in a model of diabetic neuropathy, which is characterized by demyelination and pain, administration of NT-3 improved pain behaviour and suppressed motor and nerve conduction velocities[185]. Systemic administration of NT-3 supressed NGF-induced mechanical hyperalgesia in rats[186]. By contrast, in a model of nerve injury by sciatic nerve ligation, NT-3 was involved in injury-associated hyperalgesia and sprouting[187].

Few studies have directly investigated NT-3 or NT-4 expression or activity within joints in OA. Both *NTF3* and *NTF4* mRNA are expressed within the joint, and seem to be further upregulated in late-stage OA potentially in a sex-dependent manner[188]. Moreover, administration of a TRK inhibitor that blocks TRKA, TRKB and TRKC activity had analgesic effects in bone-fracture pain[189]. Recent clinical studies using NT-3 inhibition in OA have shown clinical efficacy in an interim analysis of a phase II clinical trial (Clinical Trial ID: NCT05618782)[190,191]. Thus, the relatively neglected neurotrophins NT-3 and NT-4 deserve further attention.

Table 2 | Neuropeptides and neuropeptide receptors potentially involved in peripheral pain in osteoarthritis

Neuropeptide ligand	Receptor(s)	Key function
Vasoactive intestinal peptide (VIP)	VIP receptor 1 (VPAC1) and VPAC2	Vasodilation
Neuropeptide Y (NPY)	NPY receptors (five types; Y_1–Y_5)	Vasoconstrictor
Neurotensin	Neurotensin receptor 1 (NTS_1 receptor) and NTS_2 receptor	Regulation of dopamine pathways, vasodilation, analgesia
Substance P	Neurokinin-1 (NK_1) receptor	Vasodilation, inflammation, pain
Enkephalin	δ-opioid and μ-opioid receptors	Regulating nociception
Calcitonin gene-related peptide (CGRP)	Calcitonin receptor-like receptor (CRLR) and receptor activity-modifying protein (RAMP)	Pain, vasodilation

Glial cell line-derived family of ligands

The GDNF family of ligands (GFLs) is distantly related to the transforming growth factor-β (TGFβ) family[192] and includes four proteins: GDNF, neurturin, persephin and artemin[193–196]. These ligands signal through four cell-surface-linked GDNF family receptor alpha (GFRα) receptors, GFRα1–GFRα4, which are bound to glycosyl phosphatidylinositol (GPI) anchors and activate transmembrane receptor tyrosine kinase (RET) to form complexes[197–201]. GFRα receptors bind their specific ligands with high affinity: GFR-α1 for GDNF, GFR-α2 for neurturin, GFR-α3 for artemin and GFR-α4 for persephin, although low-affinity interactions between ligands and alternative GFRs also occur[202].

GFLs, like other neurotrophic factors, display pleiotropic functions that might differ across development, adulthood and in response to disease or injury[203]. GDNF was identified in 1993 as a survival factor in embryonic midbrain cultures from rats; cultures treated with recombinant human GDNF maintained the number of dopaminergic and total neurons compared with control cultures after 21 days. Moreover, GDNF has a crucial role in supporting the survival of various neuronal populations of the central nervous system (CNS) including motor neurons[204,205]. In adults, GDNF has been detected by

immunohistochemistry in the human cortex, cerebellum, hippocampus and the occipital lobe[206]. Neurturin, which shares structural and functional similarities with GDNF, promotes the survival of sensory neurons in the nodose ganglia in cranial nerves, DRG, and the survival of sympathetic neurons in the superior cervical ganglia, as described in rats using neuronal-survival assays[194]. The widespread expression of neurturin in various tissues, including the brain and blood, suggests potential roles in both CNS development and regulation of non-neuronal tissues[194,207].

GFLs enhance sympathetic axon growth. Neurturin, artemin and GDNF, but not persephin, have been shown to promote axonal initiation in cultured DRG neurons from young adult mice[208]. Similarly, artemin has been shown to support sympathetic nerves by enhancing axon growth along blood vessels[209,210]. How GDNF guides axon growth is still debated, although it appears to work together with the A-class ephrins to facilitate axon turning. Specifically, GDNF acts as a chemoattractant whilst also reducing ephrinA-induced neuronal cone collapse and retraction[211,212] (see also the next section).

Although GFLs have a well-documented role in both the CNS and peripheral nervous system, their involvement in musculoskeletal pain and joint signalling remains relatively underexplored[130]. There is accumulating evidence, however, for a role of GFL signalling in pain development in inflammatory and neuropathic pain models[203,213,214]. For example, hind-paw injections of artemin or neurturin produced significant acute hyperalgesia in mice, as measured by the latency withdrawal from a noxious thermal stimulus compared with naive or contralateral paw[215]. Interestingly, in the same study, subsets of TRPV1-expressing nociceptors colocalized by immunohistochemistry with GFRα2 and GFRα3, thereby suggesting that TRPV1-positive nociceptors might respond to artemin or neurturin signalling in vivo. Other studies in rats have shown, using in vivo electrophysiology, that GDNF and neurturin, when applied to the bone-marrow cavity, increase whole-nerve activity by bone afferent neurons[200]. Moreover, GDNF was shown to reduce sensory neuron discharge after nerve injury in a model of neuropathic pain[216]. Although research on GFLs suggests positive effects on recovery from nerve injury, additional research is required to explore their potential pro-algesic effects in OA pain.

Neurovascular remodelling factors in osteoarthritis

During development, migrating neurons and endothelial cells explore their surroundings through growth cones, which respond to attractive and repulsive cues that guide their migration. These cues fall into a family of molecules termed guidance molecules. Guidance molecules often signal to both endothelial and neuronal cells, which means that they move together, ensuring oxygen delivery to the migrating nerve. Therefore, there are similarities between how both cell types migrate, arborize and sprout towards their target tissues during development (reviewed in Carmeliet and Tessier-Lavigne[43]). A large number of cell guidance molecules have been described in development and increasingly these are being implicated in postnatal remodelling processes and disease. Here, we discuss cell guidance molecules and angiogenic factors in the context of development and in postnatal studies investigating OA pain pathogenesis.

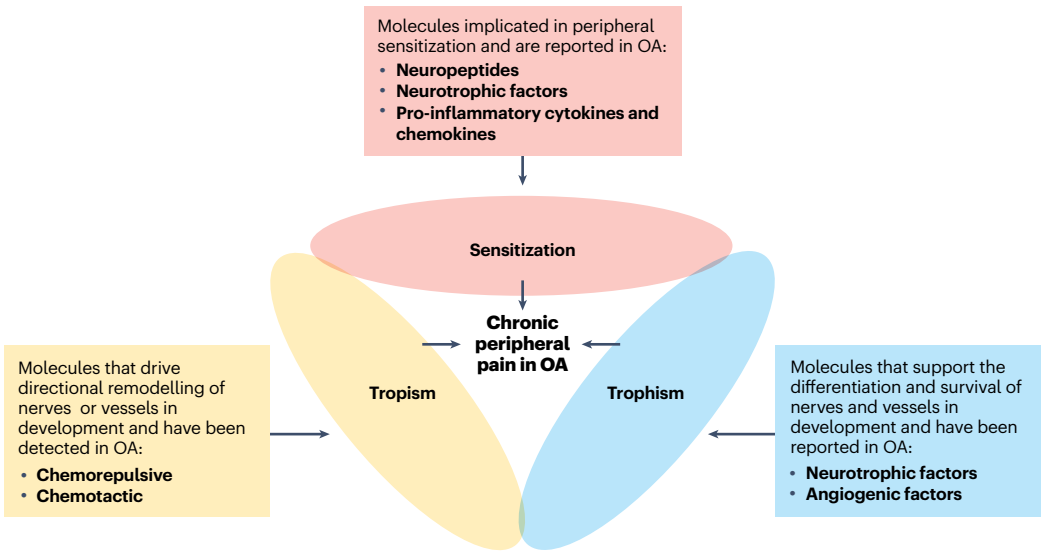

Fig. 2 | Molecular components driving chronic peripheral pain in osteoarthritis. An array of nociceptive molecules have been associated with painful osteoarthritis (OA) in humans and in murine models of OA. The function of the molecules involved in the development of chronic peripheral osteoarthritic pain fall into at least one of three categories: neuronal sensitization; neuronal tropism; and trophism. Molecules that support tropism are reported to drive directional remodelling of nerves or vessels during development and can be classified as chemorepulsive or chemotactic. Trophism refers to the support of differentiation and survival of nerves and vessels. Neurotrophic factors include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and glial cell line-derived neurotrophic factor (GDNF), and angiogenic factors include transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and prostaglandin E_2 (PGE_2). Neuropeptides (such as calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), tachykinins and enkephalins), neurotrophic factors, and pro-inflammatory cytokines and chemokines (such as interleukin-1β (IL-1β), IL-6, tumour necrosis factor (TNF) and monocyte chemoattractant molecule-1 (MCP-1)), or prostaglandin, angiotensin II and bradykinin sensitize neurons by reducing the threshold of action potentials in nociceptors and are implicated in peripheral sensitization. Sensitization is also mediated by ion channels on the neuronal surface including PIEZO1 and PIEZO2, sodium voltage-gated channel 1.7 ($Na_v1.7$) and $Na_v1.8$, and transient receptor potential vanilloid-1 (TRPV1) channel. Further details on sensitizing molecules are provided in Boxes 1 and 2. Some molecules, such as NGF, fall into more than one functional category.

Netrins

Netrins are extracellular signalling proteins that act as guidance molecules during development[217]. Within this family of proteins are netrin-1 (NTN1), netrin-2, netrin-3, netrin-G and netrin-4, of which NTN1 is the most widely studied. Netrins were first identified as guidance molecules expressed in the midline during development[218]. Multiple receptors mediate the functions of netrins, including 'deleted in colorectal cancer' (DCC) and UNC5. NTN1 binding to DCC on a target tissue leads to growth cone attraction, and inhibition of DCC signalling results in a loss of neuron outgrowth[219]. Conversely, NTN1 signalling through the UNC5 receptors results in repulsion, and deletion of UNC5 receptors leads to excessive extension and branching of neurons and vessels[220,221]. These cues act in a similar way in vascular development[222].

NTN1 pathways seem to be involved in the neurovascular remodelling that occurs in some chronic pain conditions. For example, in a rat model of lower back pain, *Ntn1* expression was increased and correlated with nerve volume, and high levels of NTN1 were associated with induction of vascular endothelial cell migration in vitro[223]. In synovial fluid from human temporomandibular OA joints, an increase in NTN1 protein was observed relative to samples from healthy individuals[224]. Following induction of OA in rats by anterior cruciate ligament transection, DCC was upregulated and UNC5 was downregulated in the DRG[225]. This shift in the ratio of UNC5 to DCC receptors might be important in OA pain, given the opposite direction of action of these receptors upon NTN1 binding. In a mouse model of OA, osteoclast-secreted NTN1 facilitated sensory neuron growth within the subchondral bone in a DCC receptor-dependent manner[42]. This study directly implicated neuronal guidance molecules in the development of OA pain and provided a molecular mechanism by which bisphosphonates, through suppression of osteoclastic activity, might potentially reduce OA-related pain[226], although bisphosphonates do not seem to be therapeutically useful in patients with OA[227].

Ephrins

Ephrin signalling involves the binding of membrane-bound ligands, the ephrins, to their receptors (Eph receptors), the largest described family of tyrosine kinases. This signalling pathway has been implicated in axon guidance and synapse formation[228–231]. All ephrin family ligands are membrane attached, either through transmembrane proteins or bound to the surface by a glycosylphosphatidylinositol (GPI) linkage protein (Fig. 3). Ephrins have the ability to bind multiple receptors with similar affinities.

Ephrin ligands are divided into two major classes, ephrinAs and ephrinBs, based on whether their attachment to the cell is through a GPI-linked membrane anchor or a transmembrane insertion, respectively[232,233]. Ephrin receptors have been divided into EphA and EphB classes depending on whether they bind ephrinA or ephrinB. In the human genome, nine EphAs have been identified, which bind to five GPI-linked ephrinAs, and five EphB receptors that bind to three transmembrane ephrinB ligands[228] (Fig. 3). The interaction between ligand and receptor activates both forward and reverse cell signalling, influencing processes such as cell mobility and cytoskeleton reorganization[233–235]. Ephrin ligands can become cleaved to create soluble ligands that are also active.

Ephrins, particularly ephrinBs, have been implicated in neuronal development and pain modulation[236]. In the spinal cord, ephrinB–EphB signalling controls synaptic plasticity, a mechanism crucial for chronic pain development[237]. The interaction between EphB receptors and *N*-methyl-D-aspartate receptors (NMDARs), modulated by calcium influx[238,239], might lead to an increase in nociceptor activity[236]. A recent study reported that mice injected with ephrinB2 into the hind paw developed acute mechanical hypersensitivity compared with vehicle-injected animals[240]. A complementary study showed that mice injected with an EphB1 extracellular domain-Fc fusion protein (ephrinB1-Fc) had a dose-dependent decrease in thermal and mechanical nociceptive thresholds detectable 30 min after injection, compared with saline[241]. Moreover, mice with conditional deletion of ephrinB2 in $Na_v1.8$-expressing nociceptors, showed less mechanical allodynia and attenuated thermal hyperalgesia than control littermates following partial sciatic nerve ligation[242]. EphB receptor blockade by intrathecal injection suppressed pain behaviours, including nerve injury-induced thermal hyperalgesia and mechanical allodynia in rats[237]. Moreover, ephrinB2 expression was significantly increased in DRG and spinal-cord neurons in a model of neuropathic pain, and siRNA-mediated knockdown of ephrinB was shown to attenuate mechanical allodynia[243]. Targeting Eph–ephrin signalling, particularly EphB–ephrinB interactions, has emerged as a potential therapeutic approach to treating chronic and neuropathic pain[244,245] but its role in OA pain has yet to be explored. In the context of OA, there are some data showing that EphB4 receptor expression is significantly increased in human OA chondrocytes from total knee replacement specimens compared with controls from cadaver specimens, yet ephrinB2 expression was similar across groups[246].

Semaphorins

Semaphorins (SEMAs) constitute a large family of more than 30 members that include secreted, cell-surface and membrane-bound proteins that have been categorized into eight classes, of which classes 3 to 7 are found in vertebrates[247–249]. SEMAs are characterized by the presence of a single cysteine-rich extracellular sema domain[249] that facilitates dimerization between SEMAs, and is required for their function[249,250]. Although most of the SEMAs are membrane bound, SEMA3s can be cleaved to form soluble dimers[251]. SEMA signalling predominantly occurs through nine plexin receptors (PLXs), either directly or through additional interactions with the co-receptors neuropilin 1 (NRP1) and NRP2 (ref. 252) (Fig. 3). Some SEMAs can bind and activate PLXs only when the plexin is bound with an NRP1 or NRP2 co-receptor[252]. For example, class A plexins are activated by membrane-bound class 5 and 6 SEMAs directly, but by secreted class 3 SEMAs only when bound to neuropilin[252].

SEMA signalling causes axons to collapse or repels axons as they migrate to their targets in the developing nervous system[247,253]. SEMAs also have a key role in bone and cartilage development. In vitro differentiation of osteoblasts and chondrocytes is associated with expression of SEMA3A family members[254], and *Sema3a*$^{-/-}$ mice show low bone mass[255], although mice with an osteoblast-specific *Sema3a* deletion had normal bone mass. Interestingly, mice with a neuron-specific deletion had low bone mass, similar to global *Sema3a*$^{-/-}$ mice, suggesting that neuron-derived SEMA3A is an important factor in bone remodelling[255]. SEMA3A is one of the best characterized members of class 3 SEMAs (one of seven members, A–G[256]) and has been shown to induce growth cone collapse and axon repulsion of several neuronal populations[257–261]. As members of the SEMA family have been shown to support the development of normal sensory innervation, there is interest in whether SEMA-specific inhibitors can be used to promote nerve regeneration. In vivo studies of SEMA3A inhibitors have shown promise, with nerve regeneration; prevention of growth-cone collapse and nerve preservation were observed across several studies[262–264].

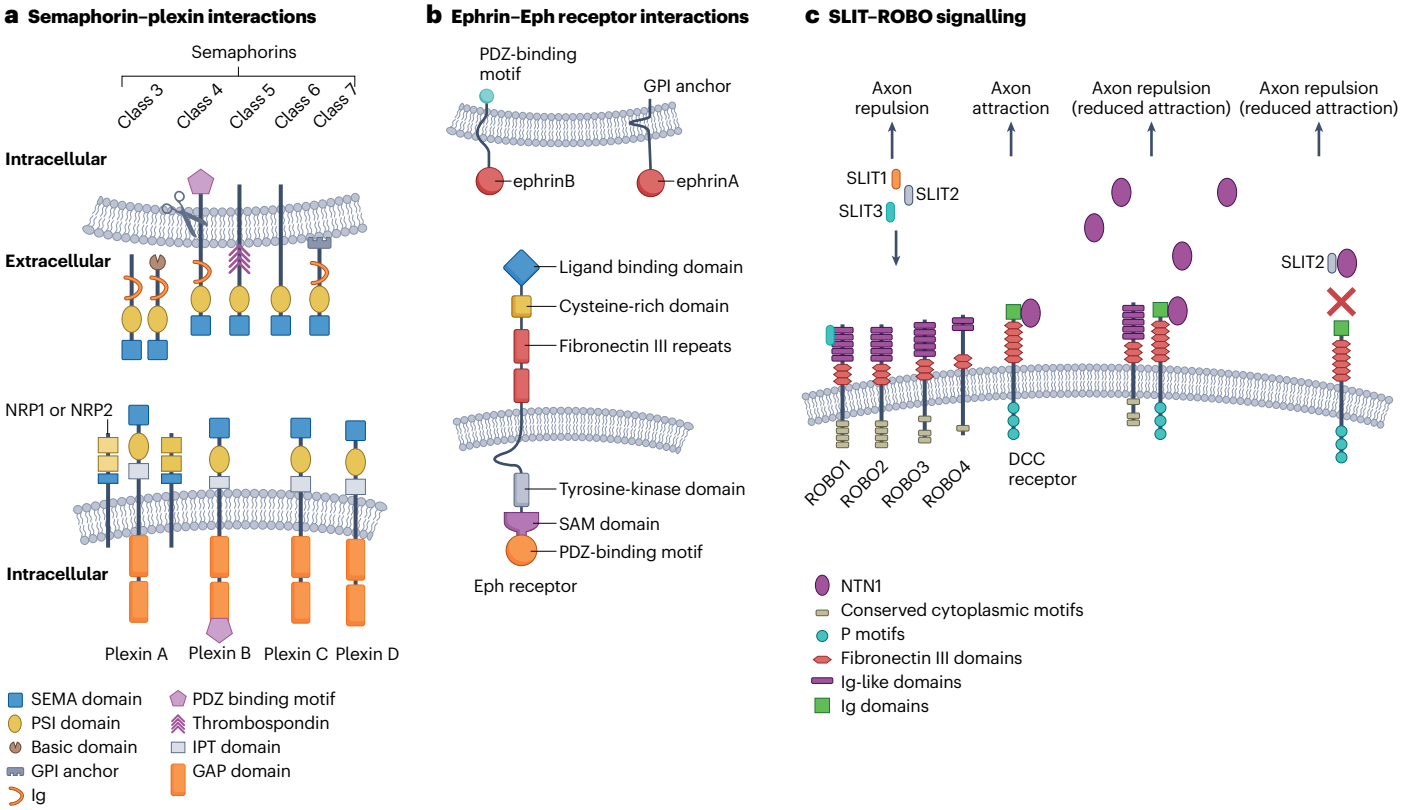

Fig. 3 | Ligand–receptor interactions of semaphorins, ephrins and SLIT proteins. a, Five classes of semaphorins (classes 3–7) have been characterized in mammals. Semaphorins are membrane linked, although class 3 semaphorins undergo proteolytic cleavage to generate soluble, dimeric proteins. Semaphorins bind to plexin receptors, which are classified into four classes (plexin A–D), with or without a neuropilin 1 (NRP1) or NRP2 co-receptor. **b**, Transmembrane ephrin–ephrin receptor (Eph) interactions give rise to bidirectional signalling. Five ephrinA ligands interact with nine EphA receptors, and three ephrinB ligands interact with five EphB receptors. **c**, SLIT proteins are soluble ligands (SLIT1, SLIT2 and SLIT3) that are known to interact with four classes of ROBO receptors to predominantly mediate axon repulsion. The ROBO receptors have been shown to interact with other membrane receptors, including the netrin-1 (NTN1) receptor deleted in colorectal cancer (DCC). The intracellular region of ROBO receptors also binds to and interacts with DCC through cytoplasmic domains, which inhibits cellular attraction downstream of the chemoattractant NTN1. SLIT2 can also bind NTN1, preventing binding to DCC, thereby silencing NTN1-induced axonal attraction. NTN1 disassociates from DCC on the cell membrane, leading to cessation of downstream signalling.

In OA, increased expression of SEMA3A has been described[265,266]. In a mouse model of OA caused by mechanical joint loading, the expression of *Sema3a* and *Plxna1* (encoding plexin A1) was increased in joint-relevant DRG neurons compared with healthy controls, and this increase was suppressed by zoledronate, which is an inhibitor of bone resorption and bone pain[267]. Sprouting of CGRP-positive axons caused by injection of NGF into the spinal cord, was significantly inhibited when SEMA3A was co-injected (SEMA3A:NGF ratio, 1:0.04) using a viral vector[258]. In addition, paw injections of Complete Freund's adjuvant (CFA) in mice led to increased *Sema4C* expression in adult DRG neurons, and *Sema4C*$^{-/-}$ mice developed inflammatory mechanical hypersensitivity to a lesser extent than controls[268]. In healthy mice, SEMA3A is expressed by most tissues in the joint, with expression increasing upon joint overloading[267]. In the same study, circulating levels of SEMA3A were increased 6 weeks after induction of OA, compared with control mice. A variable response was seen in the joint tissues[267]. Whether and how SEMAs affect nerve sprouting, nerve sensitization and subsequent pain generation in OA remains unclear and further investigation is warranted.

SLIT–ROBO signalling

The SLIT protein family comprises predominantly secreted ligands of the ROBO receptor[269]. Three SLIT proteins (SLIT1–SLIT3) and four ROBO proteins (ROBO1, ROBO2, ROBO3 (also known as RIG1) and ROBO4) have been identified in mammals[270,271]. ROBO1–ROBO3 receptors are expressed and function in the nervous system, whereas ROBO4 expression is exclusive to vasculature during embryonic development[272].

SLIT–ROBO signalling regulates axon extension in the developing nervous system and guides the positioning of glial populations along the midline[273,274]. Mutations in ROBO have been associated with misrouting of some growth cone subpopulations across the midline[275] and with increased axon crossing and re-crossing at the midline[276,277]. SLIT is secreted by glial cells along the midline[278], and ROBO receptors are highly expressed on axon growth cones[276]. SLIT acts as a chemorepellent in axon guidance[279–281], although SLIT proteins have also been shown to promote elongation and branch extension[282]. Beyond the CNS, SLIT–ROBO signalling has also been implicated in other physiological or pathological processes, including angiogenesis, cancer development, cellular proliferation and cell migration[283,284].

The capacity of SLIT to act as both a repellent and attractant has also been reported in tumour angiogenesis[285]. Given the role of SLIT–ROBO signalling in axon extension regulation, it might have a potential role in the regulation of pain.

SLIT–ROBO signalling is also involved in bone metabolism. Mice deficient in *Slit3* or *Robo1* develop an osteopenic phenotype with a decrease in bone formation[286] and decreased cranial neural crest-cell production[287]. Moreover, human SLIT2 inhibited osteoclastogenesis by suppressing in vitro differentiation of primary mouse bone marrow-derived macrophages in a dose-dependent manner[288]. In rheumatoid arthritis, synovial fibroblasts have increased ROBO3 expression compared with synovial fibroblasts from healthy donors[289]. Moreover, the levels of SLIT3 are increased in diseased subchondral bone compared with healthy subchondral bone from individuals with OA[290], and the supernatant of SLIT3-overexpressing osteoblasts was able to induce H-type vessel formation in endothelial progenitor cells. Together with the other cell-guidance molecules, these data justify exploring whether SLIT–ROBO signalling has a role in painful musculoskeletal disease.

Angiogenic factors in osteoarthritis pain

Angiogenesis and innervation are processes that function in parallel given the high metabolic demands of nerves, but whether the neuron or endothelial cell takes the lead in migration is unclear. Evidence from developmental studies and models of bone pain demonstrate that changes in neuron and vessel anatomy occur in a coordinated manner[146,291]. As described throughout this review, neurovascular coupling in musculoskeletal tissues is not a new concept, but remains challenging to study owing to difficulty in nerve visualization in calcified tissues. These challenges have impeded development of this area in OA research. Vascular imaging provides a more sensitive measure of neurovascular remodelling in disease and might help to validate neuronal staining patterns.

During skeletal development, long bones are formed through endochondral ossification, a process by which hyaline cartilage is replaced by bone to form the growing skeleton. Endochondral ossification is highly dependent on angiogenesis and innervation, and involves the formation of a cartilage template that is primarily ossified in the metaphysis, followed by secondary ossification of the epiphysis[149,292]. Structural remodelling in OA shares some common features with endochondral ossification[293], including angiogenesis, cartilage matrix degradation and formation of new bone (osteophyte)[294]. In development, the angiogenic class of capillaries that drive primary ossification are termed type-H vessels and are characterized by high expression of endomucin and platelet endothelial cell adhesion molecule-1 (PECAM-1, also known as CD31)[295]. These vessels are directed towards the hypertrophic chondrocytes in the growth plate that express vascular endothelial growth factor-A (VEGFA)[296]. VEGFA binds to the receptors VEGFR1 and VEGFR2, as well as to the SEMA co-receptors NRP1 and NRP2. VEGFA is part of a larger VEGF family that also includes VEGFB, placenta growth factor (PIGF), VEGFC and VEGFD (reviewed in refs. 297,298). Lymphangiogenesis is strongly regulated by both VEGFC and VEGFD signalling through VEGFR3 receptor (reviewed in ref. 299). In the healthy joint, cartilage expresses anti-angiogenic factors such as dentin matrix protein 1 (DMP1)[300,301], endostatin[302] and chondromodulin-I[303], and the withdrawal of these factors coupled with increased VEGFA expression helps to direct the temporal invasion of angiogenic vessels during long-bone development. Towards the end of adolescence, mechanical forces suppress VEGFA expression, thereby switching off angiogenesis and osteogenesis[304]. At this stage blood vessels express low levels of endomucin and CD31 and are called type-L vessels. As discussed above, NGF–TRKA signalling is required for the initial invasion of nerves and vessels into the developing bone[149]. Blood flow is another important factor associated with angiogenesis. Impaired blood flow reduces vessel extension, anastomoses and coupled osteogenesis, processes that decrease with age[305,306].

During OA, the articular cartilage produces angiogenic factors including VEGFA, TGFβ and platelet-derived growth factor (PDGF)[76,307]. OA is also associated with a decrease in VEGFC and VEGFR3 in the synovium, and this decrease is associated with reduced lymphatic vessel drainage function[79]. Other vascular features, such as bone-marrow lesions ('bone bruises'), are common in individuals with OA[308]. Additionally, there is some evidence to suggest that blood flow and vessel branching around the knee joint is increased in early OA in humans[309]. Thus, vessel morphology and function might change in a tissue- and time-specific manner within the OA joint. Further studies are required to understand the distribution and function of vessels in disease, as well as how this distribution might drive structural changes and contribute to pain by supporting neoinnervation. Animal and human studies indicate that inflammatory, nociceptive and angiogenic factors are co-regulated in OA (Fig. 2) and could potentially be targeted for therapeutic pain benefit. The FDA-approved anti-VEGF antibody bevacizumab[310] was analgesic in late OA induced by destabilization surgery in rabbits[311], when administered intra-articularly rather than intravenously. Knockdown of *Vegf* in chondrocytes at 2 weeks of age, attenuated adult OA after surgical joint destabilization in mice[296]. Intra-articular injection of an anti-VEGFA antibody or a VEGFR2 kinase inhibitor also reduced disease severity in this model. By contrast, conditional knockdown of *Vegf* in chondrocytes at 5 weeks of age, or knockdown of *Vegf* in endothelial cells, had no effect on disease severity. That said, most studies of VEGF in OA have focused on structural disease modification rather than pain, although blocking the interaction of VEGF with neuropilin-1 suppressed neuronal sensitization by VEGF in a neuropathic pain model[312].

VEGF signalling might directly regulate NGF-dependent activity in OA, as exogenous VEGF both induces an OA disease-like phenotype[313] and upregulates NGF in the cartilage and synovium in mice[296]. In a reciprocal fashion, NGF is also involved in promoting angiogenesis and tissue repair following injury[291]. Complete resection of the Achilles tendon increased expression of NGF by perivascular cells and drove re-innervation and vascularization of the tissue in a TRKA-dependent manner[314,315]. More research is warranted to understand the molecular mechanisms and overlap in neurovascular signalling in painful OA.

Identifying effective targets for osteoarthritis pain

OA pain is a complex trait that evolves over time and has peripheral and central drivers. Peripheral drivers include those soluble factors that drive neuronal sensitization, regulation of ion channels and molecules that drive neurovascular remodelling. Some molecules seem to be involved in both processes and their targeting is predicted to be analgesic in the short term and to affect evolution of pain in the longer term. NGF is the best recognized molecule in this group, albeit not suitable for patient targeting because of associated structural joint damage. Other neurotrophins are showing promise, including NT-3. A phase II clinical trial in knee OA using the p75 fusion protein (p75NTR-Fc; termed LEVI-04), which reported that this inhibitor of NT-3 was analgesic in patients with moderate to severe OA, without apparently causing worsening structural disease[190,191]. We await further details on the specificity and mechanism of action of LEVI-04.

Table 3 | Emerging targets tested in clinical trials for treating osteoarthritis pain

Target	Drug or intervention	Trial status
Voltage-gated ion channels on sensory neurons		
TRPV1	TRPV1 agonist resiniferatoxin	Completed: NCT03542838 NCT04885972 Phase III clinical trial (withdrawn): NCT04044742
TRPV1	TRPV1 agonist CNTX-4975-05	Completed: NCT03660943 NCT03429049 NCT03661996
$Na_v1.8$	Selective $Na_v1.8$ inhibitor VX-548 (Vertex)	Completed: NCT06420765 NCT04977336 NCT05034952
$Na_v1.8$	Selective $Na_v1.8$ inhibitor VX-150 (Vertex)	Completed: NCT02660424
$Na_v1.7$	Selective blocker of $Na_v1.7$ (iN1011-N17)	Completed: NCT05496205 Recruiting: NCT06218784
Peripheral sensitizers/trophic factors		
NGF and TNF	Monoclonal antibody targeting TNF and NGF: MEDI7352	Completed: NCT02508155 NCT04675034
NGF	Monoclonal antibody targeting NGF: tanezumab	Completed: NCT00863304 NCT00733902 NCT00744471 Terminated: NCT00864097 NCT00809354
NT-3	p75 fusion protein: LEVI-04	Active, not recruiting: NCT05618782
Angiogenesis		
VEGF	Monoclonal antibodies targeting VEGF (such as bevacizumab)	No trial ongoing
Embolization of new vessels associated with OA	Genicular artery embolization	Recruiting: NCT04456569 NCT05818150
Central nervous system targets		
Serotonin reuptake	Serotonin reuptake inhibitor: duloxetine	Completed: NCT01510457 NCT01558700 NCT02248480 NCT01931475 NCT04532684
Targeting aberrant structural remodelling-associated pain		
Inhibiting osteoclastic bone resorption	Osteoclast inhibitor: zoledronic acid	Recruiting: NCT06051344 NCT04303026
Targeting aberrant structural remodelling-associated pain (continued)		
Targeting joint erosion	Inhibitor of receptor activator of nuclear factor-κB ligand (RANKL): denosumab	Completed: NCT02771860
FGF18 to target cartilage repair	Recombinant human FGF18: sprifermin	Completed: NCT01919164 NCT01033994 NCT00911469

FGF18, fibroblast growth factor 18; $Na_v1.7$, sodium voltage-gated channel alpha subunit 9; $Na_v1.8$, sodium voltage-gated channel alpha subunit 10; NGF, nerve growth factor; NT-3, neurotrophin-3; TRPV1, transient receptor potential vanilloid 1; VEGF, vascular endothelial growth factor.

Other peripheral sensitization-targeting strategies are shown in Table 3, including the inhibition of the voltage gated channels TRPV1 and $Na_v1.8$. Phase I clinical trials of VX-150, a highly selective $Na_v1.8$ inhibitor, showed an analgesic effect in healthy adult men after pain was induced by a variety of stimuli such as a cold pressor and heat[316]. In addition, two phase II clinical trials of VX-548, an oral, selective inhibitor of $Na_v1.8$, in participants with acute pain after abdominoplasty or bunionectomy, showed reduced pain sustained over a period of 48 h (pre-publication press release)[317]. Results are awaited for additional clinical trials focusing on OA and deliberations by the FDA.

There are currently no clinical studies specifically targeting neurovascular uncoupling, although suppression of neurotrophic factors potentially works in part through this mechanism. Direct targeting of axon guidance molecules offers an alternative avenue for future OA pain research through the modulation of either neurovascular remodelling or their direct sensitizing actions. Molecules that target remodelling of the bone in OA using bisphosphonates have been tested in multiple preclinical and clinical studies with variable success, although no overall benefit is observed by meta-analysis[227,318]. Most recently, denosumab, an inhibitor of receptor activator of nuclear factor-κB (RANK) ligand failed to meet its primary pain end point after 48 weeks of treatment, although denosumab did show benefit in a subset subsequently followed until week 96 (ref. 319). The mechanism behind this might be associated with the inhibition of osteoclast-derived netrin, as reported in mouse OA[42].

Future directions

Research into neurovascular remodelling in knee OA pain is an emerging and promising area, with potential for novel therapeutic discovery and clinical application. Future studies should focus on several key areas to increase understanding of pain mechanisms in OA while also increasing the chances of developing successful pain management strategies. First, the potential role of SEMAs and SLITs in pain modulation, specifically through their effects on neurovascular coupling and sensory nerve sensitization, warrants further investigation. Future research could explore the timely use of SEMA, SLIT and ROBO inhibitors as possible therapeutic agents, particularly for their capacity to regulate nerve sprouting and prevent unregulated neuronal activation in OA.

Second, despite historical evidence supporting the analgesic effect of VEGF therapies[320], there are no ongoing clinical trials investigating the efficacy of anti-angiogenic compounds in OA (Table 3). Angiogenic and neurotrophic factors that regulate both vasculature and sensory nerves in the joint might be valuable targets for pain

management. Understanding how these factors co-regulate vascular and neural remodelling, and the optimal time window at which these should be targeted, will be crucial for developing interventions that modulate both components simultaneously. Clinical studies targeting neurovascular coupling in OA might reveal novel analgesic strategies that work in early disease prior to neoinnervation of joint tissues.

Third, as highlighted by ongoing clinical trials targeting $Na_v1.8$ and TRPV1 channels, the peripheral sensitization of neurons is likely to be key to the development of therapies for OA pain. Detailed investigations into how these channels interact with neurotrophic and angiogenic factors might uncover new ways of preventing or reversing neurovascular remodelling in the joint and peripheral sensitization.

Lessons learnt from anti-NGF clinical trials suggest that targeting pain without protecting the joint from further structural damage might be challenging. At the very least we need to understand whether pathways involved in neurovascular remodelling are also linked to trophic support of the surrounding connective tissue — for example, because they are potential contributors to normal tissue turnover and the repair process. Alternatively, increased joint damage might simply be a feature of a mechanically over-loaded, now analgesed, joint. Intuitively, targeting both pain and joint structure might be the answer. Targeting angiogenesis has been proposed previously[321,322] with some success[323,324], yet caution is also warranted as these pathways are likely to be involved in many other extra-articular processes in the body and could have an impact on wound repair, tissue homeostasis and cancer progression.

An argument remains, whether structural repair of the damaged joint tissues will also ultimately result in reduced pain. This is suggested when the remodelling of bone in hand OA is blocked with denosumab (post hoc analysis)[319], and also by cartilage regrowth studies using sprifermin, in which sprifermin was associated with reduced pain in a subset of patients from the original FORWARD trial (post hoc study)[325,326]. Finally, there are very few drugs that impact central sensitization in OA pain. Duloxetine, a selective serotonin reuptake inhibitor, has shown some success in clinical trials but effect sizes are modest[327]. New drugs targeting the CNS would potentially provide additional options, particularly for those hard-to-treat patients.

One area that is presently under-explored is the difference in structural and symptomatic disease between biological sexes. Typically, pain studies[328] and OA models are performed in male animals, as these develop robust structural changes that are easily measurable over time. Female animals are somewhat protected from this degree of structural damage; a feature that has been recognized across several strains and models[188,329]. The extent to which this observation can be attributed to hormones is yet to be validated, with conflicting results in rodents following ovariectomy[330]. Curiously, pain behaviour is evident in female mice with relatively low levels of joint damage, suggesting distinct pain mechanisms or different sensitivity. Some specific nerve-sensitizing factors have been suggested to account for sex differences, including at the level of the whole joint[188], the synovium[95] and the DRG[331], but these are yet to be validated. The relative role of neurovascular remodelling in male and female joints is likewise under-explored.

Given the variability in OA progression and pain perception between individuals, including biological sex, personalized medicine approaches should be considered. The availability of large genome-wide association study data, combined with powerful omics tools and detailed phenotyping of patient-reported pain, might enable identification of biomarkers that predict treatment response. Such biomarker candidates would include the aforementioned neuropeptides and neurotrophic factors, along with molecules implicated in neurovascular remodelling.

Glossary

Action potential
The propagation of rapid charges in voltage across the cellular membrane. Eliciting an action potential is the main way in which neurons send their signals.

Action potential conduction velocity
The speed at which electrical signals travel along an axon.

Neuropeptides
Peptides that are primarily made and released by nerve cells. They are produced within the cell body of neurons and are packaged in vesicles for transport along axons.

Neurotrophins
Trophic factors that support neuronal survival, growth and function.

Conclusions

Pain in OA is partly driven by sensitization of sensory neurons within the joint, and the associated increase in innervation and remodelling of neurovascular structures, even within the normally avascular and aneural articular cartilage. Many cell types contribute to the production of pain sensitizers and several sensitizers also control the direction of new neurovascular structures as the disease progresses. The interplay of angiogenesis and sensory nerve growth with each other and with the other joint tissues involves complex interactions and underscores the potential contribution of these processes to both structural and pain outcomes in OA[54]. This has become particularly apparent from clinical studies targeting NGF[157,159]. Whether targeting the classical axon guidance molecules including SEMAs, SLITs and ephrins will affect pain remains unclear from the small number of studies published thus far.

Published online: 12 August 2025

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

T.L.V. has received research support for STEpUP OA from Biosplice, Fidia, Galapagos, GSK, Novartis, Pfizer and UCB, and has performed ad hoc consultancy for Zoetis 2024. M.Z.C. is Founder and Director of Oxford StemTech Ltd, Human-Cetric DD Ltd, has performed Consultancy and received Honoraria from AbbVie, Brandon Capital, Pfizer, TEVA, has received research support from GSK; and public–private partnership funding for the IM2PACT project. V.B. and T.A.P. declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41584-025-01280-3.

Peer review information *Nature Reviews Rheumatology* thanks Susanne Grässel, Alfredo Ribeiro-da-Silva, who co-reviewed with Thomas Deluc, and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01279-w

Review article

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Dynamic macrophage phenotypes in autoimmune and inflammatory rheumatic diseases

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Abstract

Macrophages regulate inflammatory and fibrotic processes in several autoimmune and inflammatory rheumatic diseases. They are highly plastic cells, shifting within a range of pro-inflammatory and anti-inflammatory or pro-fibrotic phenotypes in response to dynamic interactions with other cells, environmental factors and cytokine signatures. The terms 'M1 macrophages', or classically activated, and 'M2 macrophages', or alternatively activated, were previously used to denote pro- and anti-inflammatory macrophage subsets, respectively, but this classification system has been outdated by in vivo evidence of a continuum of macrophage phenotypes that includes M1-like, M2-like and hybrid phenotypes. Deciphering the specific mechanisms that drive macrophage plasticity and function during the progression of rheumatoid arthritis, psoriatic arthritis, systemic sclerosis, systemic lupus erythematosus, and in synovitis and large vessel vasculitis in polymyalgia rheumatica and giant-cell arteritis, can improve our understanding of disease pathophysiology. Macrophage plasticity is enhanced in synovial tissue in rheumatoid arthritis, fibrotic skin and lung in systemic sclerosis, damaged kidney in systemic lupus erythematosus, and the bursal tissues or large vessels in polymyalgia rheumatica and giant-cell arteritis. Sophisticated transcriptomic analyses have revealed various phenotypic clusters of macrophages in biopsies of affected organs. Moreover, macrophage plasticity seems to be targeted by some standardized drugs used to treat the aforementioned conditions.

Sections

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Key points

- Macrophage plasticity across pro-inflammatory and anti-inflammatory phenotypes has a central role in the pathogenesis of autoimmune and inflammatory rheumatic diseases.
- The traditional M1–M2 macrophage classification has been substantially expanded over time to include transcriptional, metabolic and functional features beyond surface markers, better reflecting the complexity and continuum of macrophage phenotypes and their functional interplay.
- In rheumatoid arthritis, synovial tissue macrophages (STMs) show disease stage-specific phenotypes, with MerTK^{+}CD206^{+} subsets associated with remission and MerTK^{-}CD206^{-} clusters dominating active inflammation; furthermore, a distinct subset of osteoclastogenic macrophages contributes to bone erosion and local T cell activation.
- Profibrotic macrophage subsets, such as CD206^{+} and CD163^{+} cells, are among the key drivers of tissue fibrosis in systemic sclerosis (especially of skin and lung fibrosis) and in lupus nephritis, where they contribute to pathological remodelling.
- In polymyalgia rheumatica, macrophages display mixed phenotypes and sustain local inflammation in bursae and synovial tissues; in giant-cell arteritis, distinct pro-inflammatory macrophage subsets contribute to vascular inflammation and remodelling.
- Pharmacological treatments, such as glucocorticoids and antifibrotic drugs (tyrosine kinase inhibitors such as nintedanib) seem to achieve clinical effects partly via the modification of macrophage phenotypes.

Introduction

Macrophages are highly plastic innate immune system cells that are present in almost all tissues and have a crucial role in maintaining immune homeostasis[1]. Dynamic interactions with environmental factors, immune cells, and systemic and tissue-derived signals, including cytokines, regulate macrophage function, especially in autoimmune rheumatic diseases (ARDs)[2,3].

Macrophages recognize, engulf and destroy pathogens through the activation of Toll-like receptors (TLRs) and the production of several pro- and anti-inflammatory mediators[4,5]. Moreover, acting as antigen-presenting cells, macrophages contribute to the induction of T helper (T_H) cell-mediated immune responses[5].

Macrophages originate from either circulating monocytes or tissue-resident cells. Monocytes differentiate from bone-marrow precursor cells. These circulating monocytes are broadly classified into three subsets on the basis of the cell-surface expression of CD14 and CD16: classical monocytes (CD14^{++}CD16^{-}) represent the majority of all monocytes and are rapidly recruited to inflamed tissues; non-classical monocytes (CD14^{+}CD16^{++}) are involved in patrolling endothelial surfaces and tissue repair; and intermediate monocytes (CD14^{++}CD16^{+}) share features of both classical and non-classical monocytes, and have been implicated in inflammatory responses[6] (Box 1). By contrast, tissue-resident macrophage progenitors are of embryonic origin[7].

In the early 2000s, a macrophage classification paradigm was proposed based on the correlation between specific surface markers with a pro- or anti-inflammatory and pro-fibrotic macrophage function. This classification defined two macrophage subsets: the pro-inflammatory M1 subset (also known as classically activated macrophages) and the anti-inflammatory or pro-fibrotic M2 subset (also known as alternatively activated macrophages)[8,9] (Table 1). M2 activation state has been further divided into four subsets according to surface markers, inducing stimuli, cytokine signature and functions: M2a (or alternatively activated), M2b (or regulatory), M2c (or deactivated) and M2d (or tumour-associated) macrophages, which, with the exception of M2d macrophages, all seem to be involved in ARDs[5] (Table 1).

Although the M1–M2 dichotomy has been well-defined in in vitro models, it does not fully reflect the complexity of macrophage plasticity in vivo, as macrophages often exhibit intermediate activation states between the M1 and M2 phenotypes[10] (Table 1). In fact, in 2014, an update to macrophage nomenclature was proposed, based on the expression of not only surface markers but also transcription factors, cytokines, chemokines, extracellular matrix (ECM) proteins and proteins involved in amino acid metabolism[11]. For instance, M1-like macrophages, typically associated with pro-inflammatory responses, commonly express CD80, CD86 and inducible nitric oxide synthase (iNOS), whereas M2-like macrophages, linked to anti-inflammatory and tissue repair functions, are mainly characterized by expression of CD206 (also known as mannose receptor), CD163 (also known as haemoglobin scavenger receptor) and arginase-1 (ARG1)[12]. This substantially expanded the M1–M2 macrophage classification, highlighting intermediate phenotypes between the two extremes of pro- and anti-inflammatory macrophage subsets[11] (Table 1). Subsequent studies based on cytokine targeting and detailed immune-cell profiling have shown that in ARDs macrophages do not always fit within strictly defined phenotypes, but range across cell clusters with a continuum of phenotypes[8].

The pathophysiology of several ARDs, including rheumatoid arthritis (RA), psoriatic arthritis (PsA), systemic sclerosis (SSc), systemic lupus erythematosus (SLE), polymyalgia rheumatica (PMR) and giant-cell arteritis (GCA) involves an increase in macrophage plasticity[4,13,14]. This plasticity is particularly evident in affected tissues and organs, as in the synovial tissue in RA, the fibrotic skin and lung in SSc, the inflamed kidney in SLE, and the bursal tissue and large vessels in PMR and GCA[15–17]. In addition to macrophage plasticity during disease progression and the modified localization of macrophages within the affected tissues, ARDs show distinct profiles of macrophage activation and function both during the development of disease and upon treatment, linked to different environmental signals.

In this Review, we summarize the latest understanding of how macrophage differentiation is a dynamic and, often, reversible process defined by environmental, cytokine and cellular signals in organs affected by inflammatory arthritis, SSc, SLE, PMR or GCA. In addition, we discuss how pharmacological treatments that are currently in use in patients with ARDs might shape macrophage phenotypes, and examine how macrophage plasticity can potentially be further targeted to profoundly influence the pathophysiology of chronic ARDs and their clinical outcomes.

Macrophage plasticity in inflammatory arthritis

RA and PsA both involve progressive joint damage and subsequent bone destruction, and are defined by chronic synovitis and the presence of immune cell infiltration[18–20].

Synovial tissue consists of a sublining layer and a lining layer. The sublining layer is a vascularized connective tissue that can be infiltrated by blood-derived lymphocytes, monocytes and macrophages[4,21].

Box 1 | Monocyte plasticity in patients affected by rheumatic diseases

- Human monocytes are classically categorized into three subsets (classical, intermediate, non-classical), but high-dimensional profiling (e.g. mass cytometry, single-cell RNA-seq) has revealed a spectrum of phenotypes shaped by local microenvironments, characterized by overlapping surface markers and gene expression profiles[184–187]. Classical monocytes express both inflammatory (i.e. CD64) and anti-inflammatory markers (i.e. CD163), enabling dual roles in immune activation and tissue repair. Intermediate monocytes, despite being previously considered pro-inflammatory, primarily express antigen presentation (i.e. HLA-DR, CD80, CD86) and repair-related genes (e.g. TGFβ), suggesting a regulatory function. Non-classical monocytes show mixed inflammatory and stress-response gene expression (e.g. HMOX1), reflecting both inflammatory and anti-inflammatory potential[184,188]. Monocyte recruitment to inflamed tissues is guided by chemokines (e.g. CCL2, CX3CL1) and receptors (CCR2, CX3CR1), via PI3K-Akt and MAPK signalling[189–192]. Aberrant chemotaxis sustains chronic inflammation in immune-mediated rheumatic diseases[193].
- In rheumatoid arthritis (RA), circulating monocyte subsets have modified gene expression profiles in RA compared with monocytes from healthy individuals; CCL2, IL-5, PPARγ, VEGF and IL-8 expression is increased in both $CD16^+$ and $CD16^-$ monocytes in RA.
- In patients with systemic sclerosis, peripheral classical monocytes ($CD14^+$) show significant expression of CD163 and CD204 (M2 markers) compared with monocytes from healthy individuals. In addition, the downregulation of the transcription factor IRF8 (Interferon regulatory factor 8) in peripheral monocytes has been identified as crucial in early M2 polarization, as confirmed in a cohort of systemic sclerosis patients with diffuse cutaneous involvement (Fig. 2).
- In patients with systemic lupus erythematosus, transcriptomic analyses have demonstrated that classical $CD14^+$ peripheral monocytes overexpress type I interferon-stimulated genes and display a functional M1 phenotype signature that leads to the release of pro-inflammatory cytokines (such as IL-1β).
- In polymyalgia rheumatica, circulating monocytes show a skewed subset distribution and altered activation profiles, with expanded intermediate monocytes, activated non-classical monocytes (increased expression of TLR2, CD86) and classical monocytes with reduced expression of activation markers (CD11c, HLA-DR)[143].
- In giant-cell arteritis, all circulating monocyte subsets are expanded, reflecting systemic inflammation. Non-classical monocytes are recruited into the inflamed vessel wall via CX3CL1–CX3CR1 signalling and differentiate into $CD68^+CD16^+$ macrophages that actively contribute to tissue damage[144].

Monocytes are recruited from the circulation into the synovial tissue through interactions with fibroblast-like synoviocytes (FLSs) and other immune cells of the sublining layer[18] (Box 1). The lining layer is a protective barrier populated primarily by tissue-resident macrophages. Macrophages of the lining layer are involved in the maintenance of tissue homeostasis and express tight junctions to form a protective cell layer. In homeostatic conditions, these tissue-resident macrophages prevent joint infiltration by pro-inflammatory cells[19,21].

Hyperplasia of the intimal lining layer and an influx of inflammatory cells and neo-angiogenesis at the level of the sublining layer are shared features of chronic synovitis in RA and PsA[21]. Although the pathological mechanisms of RA and PsA are not fully elucidated, the abundance of synovial tissue macrophages (STMs) has been correlated with increased synovial inflammation and joint damage in RA, on the basis of histological and clinical evidence[18–20].

Synovial tissue macrophage subsets in active rheumatoid arthritis

In the synovial tissue of patients with active RA, the number of $CD68^+$ macrophages correlates with clinical outcomes as evaluated by 28-joint disease activity score (DAS28)[22]. Moreover, synovial inflammation seems to be mediated and sustained by macrophages showing a pro-inflammatory M1-like profile, characterized by the high expression of HLA class II, co-stimulatory molecules CD80 and CD86, TLR2 and TLR4, as well as by the secretion of pro-inflammatory cytokines, such as TNF, IL-6, IL-1β, IL-8 and IL-12, and chemokines, such as CCL2 (refs. 4,23,24). Pro-inflammatory macrophages interact with and activate T cells and fibroblasts in the synovial tissue, thereby contributing to inflammation and bone damage[23,25,26].

Arthritis-associated osteoclastogenic macrophages (AtoMs) have been identified near the bone and shown to interact with the synovial tissue or differentiate into osteoclasts in patients with RA[27]. AtoMs are phenotypically characterized as $CX3CR1^+HLA\text{-}DR^{hi}CD14^+CD64^+$ cells that express CD80 and CD86, and are present in the synovial fluid and tissue, but not in the blood, of patients with RA[27] (Fig. 1a). Two subsets of AtoMs have been described in RA synovial tissue based on the levels of CD86 expression: $CD11c^+CD86^{low}$ cells and $CD11c^+CD86^{hi}$ cells. The continuous range of CD86 expression indicates that AtoMs might be involved in antigen presentation in local foci of the arthritic joints and in the activation of TNF-producing $CD4^+$ T cells. AtoMs highly expressing CD86 and FoxM1 can differentiate into osteoclasts and contribute to bone erosion and amplification of inflammation, and constitute a potential target for the treatment of RA[22,27,28] (Fig. 1a).

Single-cell multi-omics analyses have revealed further heterogeneity in tissue-resident and tissue-infiltrating macrophages in the synovial tissue of patients with RA[29,30]. Two major sub-populations were distinguished in RA synovial tissue based on the cell-membrane expression of tyrosine-protein kinase Mer (MerTK) and CD206 (also known as mannose receptor-1): $MerTK^-CD206^-$ and $MerTK^+CD206^+$ STMs. $MerTK^+CD206^+$ STMs have been linked to the M2-like spectrum, as CD206 is a marker of M2 phenotype and MerTK is involved in the activation of the anti-inflammatory cytokine transforming growth factor β1 (TGFβ1)[29,31] (Fig. 1b). $MerTK^-CD206^-$ and $MerTK^+CD206^+$ STMs include various phenotypic subclusters with unique pathogenic mediator signatures. $MerTK^-CD206^-$ STMs include $CD48^+S100A12^+$, $CD48^+SPP1^+$ and $CD48^+ISG15^+$ macrophages, all of which are involved in the acute inflammatory phase of synovitis in RA[29,30]. $MerTK^+CD206^+$ STMs include $TREM2^+CX3CR1^+FOLR2^+$ macrophages and $LYVE1^+FOLR2^+$ macrophages, with both subclusters being primarily involved in the control and resolution of synovial inflammation[31,32] (Fig. 1).

At initiation of synovial inflammation during the early stages of inflammatory arthritis, the $TREM2^+$ macrophage cluster switches

into a TREM2^{low} cluster that proliferates in the lining layer in response to anti-citrullinated protein antibodies (ACPA)[29]. MerTK$^-$CD206$^-$ STMs become predominant in synovium of patients with active RA, both those who are naïve to treatment and those who are resistant to DMARDs. MerTK$^-$CD206$^-$ STMs produce pro-inflammatory cytokines and chemokines, including TNF, IL-6, IL-1β and CCL2, and drive the activation and pathogenic responses of FLSs, which in turn contribute to maintaining these STMs in an active pro-inflammatory status[25,29].

The reciprocal interaction between MerTK$^-$CD206$^-$ STMs and FLSs seems to contribute to chronic synovitis in RA[29,32]. In the active inflammatory phase of RA, pro-inflammatory CD48$^+$S100A12$^+$ macrophages highly express alarmins and IL-8 (CXCL8), which are all potent inducers of monocyte and fibroblast activation[32]. Moreover, CD48$^+$SPP1$^+$ macrophages, which are characterized by a high level of glycolytic activity, are implicated in bone damage related to their activated migratory phenotype[32]. CD48$^+$ISG15$^+$ macrophages contribute to synovial tissue inflammation through the activation of interferon (IFN) signature[29,32] (Fig. 1a).

Persistence of CD48$^+$S100A12$^+$ macrophages in the synovium of patients who have discontinued RA treatment seems to be associated with an increased risk of flare[29] in these patients. CD48$^+$S100A12$^+$ macrophages maintain a high expression of S100 family members (S100A8, S100A9 and S100A12) in these patients, similar to macrophages detected in patients with active RA[33].

Overall, macrophage plasticity seen in RA is regulated by several intracellular signalling pathways. The nuclear factor-κB (NF-κB) pathway includes canonical and non-canonical signalling and is activated by lipopolysaccharide, hypoxia inducible factor-1α (HIF1α), and TLR4. The canonical NF-κB signalling results in the production of reactive oxygen species and favours macrophage differentiation into pro-inflammatory phenotypes[34] (Fig. 1c). The non-canonical NF-κB signalling pathway,

Table 1 | Frequently reported macrophage phenotypes in autoimmune rheumatic diseases

Macrophage subsets[a]	Surface markers	Inducing stimuli	Cytokine profiles	Functions	Refs.
Pro-inflammatory phenotypes					
M1 (also known as classically activated)	CD80, CD86, MHC-II, TLR-2, TLR-4, iNOS	IFNγ, LPS, IL-12, IL-23	TNF, IL-1β, IL-6, IL-12, IL-23, NO, ROS	Pro-inflammatory, microbicidal, antigen presentation, promotion of T_H1 responses Dominant in affected tissues in the inflammatory phases of active RA, PsA, early inflammatory phases of SLE and SSc, initial stages of vascular inflammation in GCA	9,179
AtoMs (osteoclastogenic macrophages)	CX3CR1$^+$, HLA-DR^{high}, CD14$^+$, CD64$^+$, CD80$^+$, CD86$^+$ (±CD11c, FoxM1$^+$)	TNF, RANKL, GM–CSF, IL-1β	TNF-α, IL-1β, IL-6	Pro-inflammatory; osteoclast precursors; drive bone erosion and T cell activation in RA	
Anti-inflammatory phenotypes					
M2a (also known as alternatively activated)	CD206, IL-1R, ARG1, FIZZ1, Ym1/2	IL-4, IL-13	IL-10, TGFβ, CCL17, CCL18, CCL22	Tissue repair, fibrosis, ECM remodelling, angiogenesis; constitutively expressed in SSc; involved in fibrotic stages of SLE and in RA remission	42,43
M2b (also known as regulatory)	CD86, IL-6R, IL-10R, IL-12R	Immune complexes, TLR ligands, IL-1β	IL-10, IL-6, TNF, IL-1β, CCL1	Modulation of the inflammatory response, T_H2–T_H17 cell balance; present in RA remission	180
M2c (also known as deactivated)[44]	CD163, CD206, TLR1, TLR8, ARG1, MerTK	IL-10, glucocorticoids	IL-10, TGFβ, CCL16, CCL18, CXCL13	Resolution of inflammation, debris clearance; detected in RA and SLE remission; in SSc, they might contribute to fibrosis, especially with glucocorticoid exposure	44
Intermediate phenotypes					
Hybrid M1–M2	Co-expression of CD80, CD163, CD204, CD206, TLR4	Mixed cytokine environment (for example, the presence of both IFNγ and IL-4 or IL-10), chronic inflammation	TNF, IL-10, TGFβ, IL-6, GM–CSF	Context-dependent inflammatory or tissue remodelling functions; detectable in SSc, where they were shown to contribute to tissue fibrosis; in PMR, reported to sustain chronic inflammation; and in PsA synovitis, where CD163$^+$ macrophages display a mixed transcriptional profile	91,142,181
Multinucleated giant cells (also known as multinucleated macrophages)	CD68, HLA-DR, CD163, MMP12	IFNγ, IL-17, GM–CSF, TNF, MMP12	IFNγ, IL-17, GM–CSF, M-CSF, TNF, IL-1β, VEGF, PDGF	Degradation of the arterial wall, promotion of vascular remodelling, vascular occlusion and intimal hyperplasia in GCA	161,182

[a]This classification is adapted from the macrophage activation framework proposed by Murray et al.[11], which recommends defining activation states based on combinations of inducing stimuli, surface markers and effector functions. In autoimmune rheumatic diseases, in vivo macrophage phenotypes frequently span a continuum or display mixed features. ARD, autoimmune rheumatic disease; ARG1, arginase-1; BAI1, brain-specific angiogenesis inhibitor 1; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; CX3CR1, CX3C chemokine receptor 1; ECM, extracellular matrix; FIZZ1, found in inflammatory zone 1; FoxM1, Forkhead box protein M1; FRβ, folate receptor beta; GCA, giant-cell arteritis; GM–CSF, granulocyte–macrophage colony-stimulating factor; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MHC-II, MHC class II; MMP12, metalloproteinase 12; MerTK, tyrosine-protein kinase Mer; NO, nitric oxide; PDGF, platelet-derived growth factor; PMR, polymyalgia rheumatica; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor κB ligand; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TGFβ, transforming growth factor beta; T_H1, T helper type 1; TLR, Toll-like receptor; VEGF, vascular endothelial growth factor; Ym1/2, chitinase-like proteins Ym1 and Ym2.

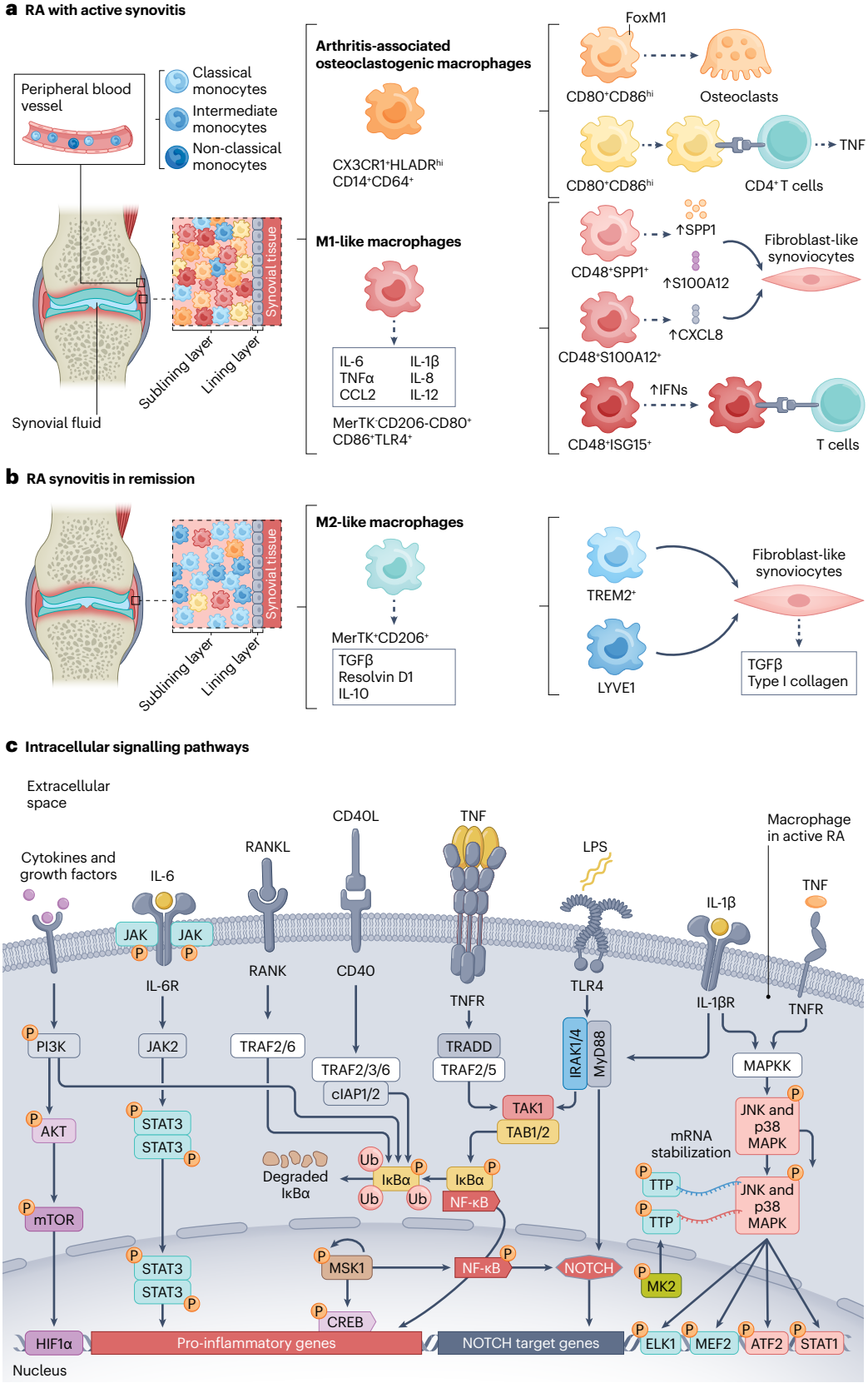
a RA with active synovitis
Peripheral blood vessel
Classical monocytes
Intermediate monocytes
Non-classical monocytes
Synovial tissue
Sublining layer
Lining layer
Synovial fluid
Arthritis-associated osteoclastogenic macrophages
CX3CR1+HLADRhi CD14+CD64+
M1-like macrophages
IL-6 IL-1β TNFα IL-8 CCL2 IL-12
MerTK-CD206-CD80+ CD86+TLR4+
FoxM1
CD80+CD86hi
Osteoclasts
CD80+CD86hi
TNF
CD4+ T cells
↑SPP1
CD48+SPP1+
Fibroblast-like synoviocytes
↑S100A12
↑CXCL8
CD48+S100A12+
↑IFNs
CD48+ISG15+
T cells
b RA synovitis in remission
Synovial tissue
Sublining layer
Lining layer
M2-like macrophages
MerTK+CD206+
TGFβ Resolvin D1 IL-10
TREM2+
LYVE1
Fibroblast-like synoviocytes
TGFβ Type I collagen
c Intracellular signalling pathways
Extracellular space
Cytokines and growth factors
IL-6
RANKL
CD40L
TNF
LPS
Macrophage in active RA
IL-1β
TNF
JAK
JAK
IL-6R
RANK
CD40
TNFR
TLR4
IL-1βR
TNFR
PI3K
JAK2
TRAF2/6
TRAF2/3/6
cIAP1/2
TRADD
TRAF2/5
IRAK1/4
MyD88
MAPKK
AKT
STAT3
STAT3
TAK1
TAB1/2
JNK and p38 MAPK
mRNA stabilization
Degraded IκBα
Ub
IκBα
NF-κB
TTP
TTP
JNK and p38 MAPK
mTOR
STAT3
STAT3
MSK1
NF-κB
NOTCH
MK2
CREB
HIF1α
Pro-inflammatory genes
NOTCH target genes
ELK1
MEF2
ATF2
STAT1
Nucleus

Fig. 1 | Macrophage subsets and functions in synovial tissue during active and remission phases of rheumatoid arthritis. a, Macrophage subsets in the synovial tissue of patients with rheumatoid arthritis (RA) during the active phase of synovitis. Arthritis-associated osteoclastogenic macrophages (AtoMs) maintain expression of the monocyte marker CD14 but are also characterized by the specific expression of CX3CR1, CD64, HLADR and the costimulatory molecules CD80 and CD86, which are involved in mediating T cell activation. AtoMs that highly express CD86 and Forkhead box protein M1 (FoxM1) have the ability to differentiate into osteoclasts. AtoMs are involved in antigen presentation in local foci of the arthritic joints and the activation of TNF-producing $CD4^+$ T cells. $MerTK^-CD206^-$ macrophages have a pro-inflammatory, M1-like phenotype characterized by the expression of CD80, CD86 and Toll-like receptors (TLRs) such as TLR4. These macrophages release IL-6, IL-1β, IL-12 and TNF and chemokines such as CCL2. Three pro-inflammatory $MerTK^-CD206^-$ macrophage subsets are identified during the active phase of RA: $CD48^+ISG15^+$ macrophages show upregulated interferon (IFN) signalling and interact with T cells; $CD48^+S100A12^+$ macrophages release pro-inflammatory mediators protein S100-A12 (S100A12) and IL-8 (CXCL8); and $CD48^+SPP1^+$ macrophages produce high levels of osteopontin (SPP1). Both $CD48^+SPP1^+$ and $CD48^+S100A12^+$ macrophage subsets interact with and activate fibroblast-like synoviocytes. **b**, Macrophage subsets in the synovial tissue of patients with RA synovitis in remission. $MerTK^+CD206^+$ macrophages have an anti-inflammatory, M2-like phenotype, characterized by the production and release of anti-inflammatory mediators including IL-10, transforming growth factor β (TGFβ) and resolvin D1. Two subsets of $MerTK^+CD206^+$ macrophages have been identified, one expressing triggering receptor expressed on myeloid cells 2 (TREM2) and one expressing lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1); both subsets produce type I collagen and interact with fibroblast-like synoviocytes promoting the activation of these cells. **c**, Intracellular signalling pathways activated in synovial macrophages during the active phase of RA synovitis. Growth factors (including the colony-stimulating factors M-CSF and GM–CSF), glycine and cytokines such as IL-4 and TNF activate the PI3K–Akt signalling pathway, which induces the activation of mTOR with subsequent gene expression of hypoxia inducible factor-1 (HIF1α). The interaction of IL-6 with the IL-6 receptor (IL-6R) induces the activation of the JAK–STAT signalling pathway, which directly promotes the transcription of genes involved in inflammation. Receptor activator of nuclear factor κB ligand (RANKL) and CD40 ligand (CD40L), as well as TNF, interact with their receptors on the cell membrane of macrophages and induce the activation of TNF receptor associated factor (TRAF) protein family members, promoting the activation of the nuclear factor κB (NF-κB) signalling pathway. Phosphorylation of the NF-κB inhibitor IκBα leads to IκBa ubiquitylation and degradation, releasing activated NF-κB, which migrates into the nucleus, where it promotes the expression of pro-inflammatory genes and NOTCH-activated target genes either directly or through the activation of NOTCH. The interaction of lipopolysaccharide (LPS) with TLR4 activates MyD88, which in turn promotes the activation of both the NOTCH and NF-κB signalling pathways. The MyD88-dependent activation of the NF-κB signalling pathway is also induced by the interaction of IL-1β with the IL-1β receptor (IL-1βR). IL-1β and TNF also activate the mitogen activated protein kinases (MAPKs) JNK and p38, which phosphorylate inflammation-associated transcription factors ELK1, MEF2, ATF2 and STAT1.

together with the transcription factor FoxM1, is involved in the activation and differentiation of AtoMs into osteoclasts, and reinforces pro-inflammatory cytokine production in AtoMs upon stimulation with receptor activator of NF-κB ligand (RANKL) and TNF[27] (Fig. 1c). In addition, NF-κB interacts with the PI3K–AKT and JAK–STAT signalling pathways; each of these intracellular pathways, as well as their interaction, seems to mediate macrophage differentiation towards a pro-inflammatory M1 phenotype and release of pro-inflammatory cytokines, such as IL-6 and TNF[34–37] (Fig. 1c). Notch activation downstream of TLR2 and TLR4 activation also promotes the differentiation of macrophages towards a pro-inflammatory phenotype that produces high levels of IL-6, IL-1β and TNF[15,38] (Fig. 1c). Emerging evidence suggests that epigenetic mechanisms, including methylation, regulate the activation of NF-κB and JAK–STAT signalling pathways in macrophages and, subsequently, macrophage differentiation into pro-inflammatory phenotypes in models of inflammatory disease[39–41] (Fig. 1c).

Macrophage subsets in arthritis therapy and remission

Accurate knowledge of how the transcriptomic and histological profiles of macrophages and other immune cells differ between the early or active inflammatory phase and remission in RA might inform therapeutic strategies.

Stable RA remission is characterized by the enrichment of M2-like macrophage subsets, including of M2a and M2c macrophages, in synovial tissues[29,30,32]. M2a cells are induced by the T_H2 cell-derived cytokines IL-4 and IL-13 and are phenotypically characterized by the expression of CD206 and ARG1. M2a cells also express anti-inflammatory molecules, including IL-10 and TGFβ[42,43] and are involved in tissue repair and fibrosis, for example, in fibrotic diseases such as SSc[42,43].

M2c macrophages express CD206 and ARG1 together with other phenotypic markers, including CD163 and MerTK[44], and are activated by glucocorticoids. M2c cells are involved in the resolution of inflammation via the production of IL-10, TGFβ and CCL18 (ref. 44).

At remission, the synovial tissue is enriched with both $TREM2^+$ and $LYVE1^+$ $MerTK^+CD206^+$ STMs that have lost expression of VISG4, a B7-related co-inhibitory molecule, and produce mediators implicated in the resolution of inflammation, such as resolvin D1[29] (Fig. 1b). In addition, $MerTK^+CD206^+$ STMs induce FLSs to secrete TGFβ1, which further activates the tissue-repair programme. As $MERTK^+$ macrophages seem to mediate the resolution of inflammation[29,32], RA treatments that promote the shift from a pro-inflammatory macrophage phenotype (primarily $MerTK^-CD206^-$ STMs) towards an anti-inflammatory phenotype ($MerTK^+CD206^+$ STMs) might be fundamental for restoring a homeostatic balance during the management of the disease (Table 2 and Fig. 1b). For example, treatments that target the MerTK signalling pathway are likely to enable disease remission in people with RA[45]. Indeed, glucocorticoid therapy has correlated positively with increased MerTK expression on the cell surface of monocyte-derived macrophages (MDMs), revealing an additional mechanism via which glucocorticoids might attenuate RA flares[45].

Several other RA therapies have been shown to affect macrophage phenotypes. TNF inhibitors (such as infliximab, etanercept, adalimumab, golimumab and certolizumab) decrease the activation of intracellular pathways involved in macrophage signalling and cytokine production, such as the NF-κB pathway[46] and the STAT3–GAS6–SOCS3 pathway in cultured M1-like MDMs obtained from individuals with RA[47]. Also, the CTLA4-Ig fusion protein abatacept, which targets the CD80–CD86 and CD80–CD28 co-stimulatory signalling pathways, seems to inhibit activation of the NF-κB signalling pathway and expression of IL-6, IL-12 and IL-1β, thereby promoting in vitro macrophage differentiation towards a MerTK-expressing macrophage subset[48–51]. The Janus kinase (JAK) inhibitors upadacitinib and baricitinib were able to reprogram human macrophages in vitro, favouring the upregulation of genes that define the $TREM2^+$ and $LYVE1^+$ macrophage subsets[52]. The vitamin D metabolite $1,25(OH)_2D3$, also known as calcitriol, has a dose-dependent anti-inflammatory effect on human

Table 2 | Effects of the various treatments on macrophage plasticity in rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus

Type of treatment	Study design	Effects on macrophage plasticity	Ref.
Calcitriol	THP-1 cells treated with calcitriol and with or without 17β-oestradiol	Calcitriol downregulates the release of pro-inflammatory cytokines (IL1-β, IL-6 and TNF) by MDMs both unstimulated and stimulated by 17β-oestradiol	54
Glucocorticoids	In vitro treatment of MDMs from healthy donors differentiated with GM–CSF or M-CSF	Glucocorticoids increase surface expression of MerTK (M2 marker) on MDMs	45
Endothelin receptors A and B antagonist (bosentan)	In vitro treatment of macrophages derived from both THP-1 cells and peripheral blood monocytes from healthy donors, differentiated with PMA and incubated with endothelin-1 with or without bosentan	Endothelin-1 induces upregulation of gene expression and protein synthesis of M2 markers (CD163, CD204 and CD206), which is counteracted by in vitro incubation of macrophages with bosentan	65
Phosphodiesterase-4 inhibitor (rolipram)	In vitro treatment of murine skin (bleomycin-induced dermal fibrosis, topoisomerase I mouse models and murine sclerodermatous chronic graft-versus-host disease), human fibroblasts and human skin macrophages from individuals with diffuse cutaneous SSc and healthy donors	Rolipram reduces M2 human skin macrophages polarization and release of profibrotic cytokines (TGFβ, IL-6)	103
Anti-TNFα inhibitors (etanercept, adalimumab, certolizumab pegol), IL-6-receptor antagonist (tocilizumab), CD20 antagonist (rituximab)	In vitro treatment of MDMs from individuals with RA and healthy donors differentiated with M-CSF	TNF inhibitors significantly decrease markers of M1 activation (CD40, CD80) and increase markers of M2 activation (CD163, MerTK) in MDMs from patients with RA	47
Jak inhibitors (ruxolitinib, tofacitinib, itacitinib)	In vitro treatment of MDMs from healthy donors differentiated with M-CSF, pretreated with ruxolitinib, tofacitinib and itacitinib and incubated with IFNγ (M1i type), IFNγ and LPS (M1Li type), IL4- and IL-13 (M2a type), IL-10 (M2c type), IL-10 with dexamethasone (M2c) Analysis of lung macrophages from mouse HOCl-induced ILD treated with JAK inhibitors in vivo	Ruxolitinib, tofacitinib and itacitinib significantly reduce expression of M1 markers (CD86, TLR4) and M2 marker CD206 in MDMs of healthy donors Ruxolitinib reduces expression of M2 markers (Arg1, Retn1a, Chi3L3, Flt1 and IL-4Ra) in lung macrophages of a mouse model of HOCl-induced SSc	104
CTLA4-IgG (abatacept)	In vitro treatment of MDMs from both individuals with RA and healthy donors differentiated with LPS	Abatacept downregulates gene expression of M1 markers (CD80, CD86, TLR4) and upregulates gene expression and protein synthesis of M2 markers (CD163, CD204, CD206 and MerTK) in RA MDMs	48
PPARγ inhibitor	In vitro treatment of monocytes from both individuals with SLE and healthy donors activated by Pam3CSK4 (M2 polarization) with or without pretreatment with a PPARγ inhibitor	Circulating $CD14^+$ monocytes from SLE patients, treated with glucocorticoids with/without hydroxychloroquine, show increased expression of PPARγ transcription factor (M2 polarization) PPARγ inhibitor prevents M2 polarization of SLE monocytes	116
Anti-CD74 antibody against immunomodulatory macrophage migration inhibitor factor receptor (milatuzumab)	Clinical trial involving 30 individuals with SLE planned to be randomized 1:1:1 for treatment with milatuzumab 250 mg per day, milatuzumab 150 mg per day or placebo	All 7 patients treated with milatuzumab (anti-CD74 prevalently expressed on M1 monocytes/macrophages) showed improvement of SLEDAI and BILAG (trial interrupted owing to poor enrolment)	129
Dextran mycophenolate-based nanoparticles	In vitro treatment of bone-marrow MDMs from MRL/lpr mice (SLE murine model) differentiated with M-CSF Analysis of macrophages from MRL/lpr mice (SLE murine model) spleen and kidneys of mice treated with dextran mycophenolate-based nanoparticles in vivo	Dextran mycophenolate-based nanoparticles reduce M1 markers (CD40, CD80) and increase M2 markers (CD206) expression in vitro (bone marrow MDMs) and in vivo (spleen and kidney) in MRL/lpr lupus-prone mice	131
Recombinant IL-33	Characterization of $CD11b^+$ cells (macrophages) from spleen and kidneys of MRL/lpr mice (SLE murine model) treated with recombinant IL-33 in vivo	Recombinant IL-33 stimulates M2 polarization with elevated mRNA expression of Arg1 and FIZZI and reduced iNOS in splenic and renal tissues	132
Anti-S1P-receptor antibody (fingolimod)	Characterization of macrophages from spleen and kidneys of MRL/lpr mice (SLE murine model) treated with fingolimod in vivo	Fingolimod blocks S1P-receptor expressed on the surface of M1 macrophages, suppresses activation of NLRP3 inflammasome and decreases M1-type macrophage infiltration	120
Calcitriol	In vitro treatment of MDMs from individuals with RA and healthy donors and THP-1 cells differentiated with PMA and treated with LPS and IFNγ with and without calcitriol	Calcitriol downregulates mRNA expression of M1 activation markers (CD80, IL-6, CXCL-10, IFIT1) in vitro in treated MDMs from individuals with RA, healthy donors and THP-1 cell line	183

Table 2 (continued) | Effects of the various treatments on macrophage plasticity in rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus

Type of treatment	Study design	Effects on macrophage plasticity	Ref.
JAK inhibitors (tofacitinib, baricitinib, upadacitinib)	In vitro treatment of MDMs from individuals with RA differentiated with GM–CSF	Tofacitinib, baricitinib and upadacitinib upregulate anti-inflammatory gene expression (MAFB) in MDMs (M2-like genotype)	52
Tyrosine-kinase inhibitor (nintedanib)	In vitro treatment of MDMs from both individuals with SSc-ILD and healthy donors differentiated with PMA	Nintedanib significantly downregulates gene expression and protein synthesis of M2 markers (CD163, CD204, CD206 and MerTK) in SSc-ILD MDMs as well as the release of active TGFβ1	96
Lysophosphatidic acid	Characterization of CD68$^+$ cells from spleen and kidneys of MRL/lpr mice (SLE murine model) treated with lysophosphatidic acid in vivo	Lysophosphatidic acid reduces CD68$^+$ cells in spleen and kidneys (glomeruli), downregulating their release of pro-inflammatory cytokines (TNF, IL-6, IL-18)	133
Anti-IL-6 receptor antagonist (tocilizumab)	In vivo administration of tocilizumab in 8-week-old BALB/c mice (SSc skin fibrosis murine model)	Tocilizumab significantly reduces the percentage of total monocytes/macrophages CD38$^+$ (phenotype M1-like) in peripheral blood and skin biopsies of mice[99]	

BILAG, British Isles Lupus Assessment Group; GM–CSF, granulocyte–macrophage colony-stimulating factor; IFIT1, interferon-induced protein with tetratricopeptide repeats 1; ILD, interstitial lung disease; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; MDM, monocytes-derived macrophage; MerTK, tyrosine-protein kinase Mer; PMA, phorbol myristate acetate; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; SSc, systemic sclerosis.

pro-inflammatory macrophages in vitro[53,54] and downregulates the synthesis of IL-6, IL-1β and TNF, as well as the activity of aromatase, blocking the pro-inflammatory action of oestrogens on macrophages, at least in RA[54]. Consistently, calcitriol reduces mRNA expression of CD80, IL-6, CXCL10 and IFIT-1 ex vivo in macrophages from patients with RA, limiting differentiation into an M1-like subset and supporting an anti-inflammatory macrophage function. In addition, calcitriol was found to ameliorate arthritis in a TNF-transgenic mouse model by inducing expression of fructose-1,6 bisphosphatase 1 (FBP1) in macrophages[55].

Taken together, these observations suggest that mechanisms reducing the production of pro-inflammatory cytokines by macrophages and promoting the transition from a pro-inflammatory to an anti-inflammatory macrophage phenotype might contribute to RA remission in response to available therapies. Spatial biology and single-cell transcriptomics might lead to the identification of further monocyte–macrophage subsets in the synovial tissue and peripheral blood of people with RA that are associated with the various stages of the disease, especially early RA. These findings would further improve our understanding of the roles of monocytes and macrophages in RA, would shed light on potential molecular targets in RA macrophages and would help to optimize the use of the currently available pharmacological options.

Synovial tissue macrophage subsets in psoriatic arthritis

Although CD8$^+$ T cells, CD4$^+$ T_H1 cells, T_H17 cells, T_H9 cells and T_H22 cells are the main protagonists in the pathogenesis of PsA, the plasticity of STMs is crucially involved in undifferentiated arthritis[23,56–58].

CD163$^+$ macrophages are enriched in PsA synovitis[59]. In both the early and established phases of PsA, CD163$^+$ macrophages show an upregulation of granulocyte–macrophage colony-stimulating factor (GM–CSF)[58], and the GM–CSF-related genes encoding activin A (INHBA), metalloproteinase MMP12 and chemokine CCL17 all have pro-inflammatory functions. Similar macrophage gene-expression profiles have been noted in patients with active established RA[58,60,61]. In addition, the percentage of tissue-resident CD163$^+$CD209$^+$ macrophages expressing the pro-inflammatory markers activin A, TNF and MMP12 is significantly increased in synovial tissue from people with PsA[62]. In addition, myeloid cells expressing high levels of SPP1, resembling the tissue-infiltrating CD48$^+$SPP1$^+$ cells described in RA synovial tissue[29,63], have been identified in the synovial fluid of patients with PsA. These findings indicate dynamic plasticity of STMs in patients with PsA based on both cell-surface markers and functional phenotypes. Further analyses, including single-cell transcriptomic and proteomic studies, are needed to understand the potentially pathogenic role of CD48$^+$SPP1$^+$ cells in PsA.

Macrophages in systemic sclerosis

Macrophage subsets in the pathogenesis of systemic sclerosis

SSc is primarily characterized by cutaneous fibrosis with or without SSc-associated interstitial lung disease (SSc-ILD). Endothelial cell damage drives early SSc pathogenesis, as it leads to autoantigen exposure and dysregulated activation of innate immune cells, including monocytes, through the release of IL-4, IL-13, IL-10 and vasoconstrictive peptides such as endothelin-1 (refs. 64,65). Profibrotic monocyte and macrophage subsets seem to be dominant in organs of people with SSc, and promote progressive, almost irreversible, fibrosis with severe impairment of organ function in both human studies and animal models[13,66,67].

The expression of the transcription factor IRF8 in peripheral monocytes has been inversely correlated with cutaneous fibrosis, as evaluated by a modified Rodnan skin score in a cohort of patients with diffuse cutaneous involvement. In addition, downregulation of IRF8 has been associated with monocyte differentiation into CD68$^+$CD163$^+$ macrophages, and IRF8-silenced monocytes display increased production of pro-fibrotic mediators such as TGFβ and pro-inflammatory cytokines such as IL-6 (ref. 68).

In vitro priming of profibrotic MDMs from patients with SSc results in the massive production and release of soluble pro-fibrotic mediators, primarily TGFβ, and subsequent synthesis of collagen and ECM proteins by fibroblasts[69] (Fig. 2). Of note, in a mouse model of bleomycin-induced lung fibrosis, CD206$^+$ macrophages released a large amount of Wnt7a, a protein that activates Frizzled receptor on fibroblasts to support collagen release[13,70,71] (Fig. 2). Among the ECM proteins produced by fibroblasts, periostin has been shown in vitro to increase the expression of pro-fibrotic cytokines and chemokines in

MDMs from patients with SSc, indicating a potential mechanism that perpetuates fibrotic damage[69].

Classical CD14$^+$ monocytes in the peripheral blood of patients with SSc have been found to significantly upregulate expression of CD163$^+$ and CD204$^+$ compared with monocytes from healthy individuals[72] (Fig. 2). In addition, the soluble form of CD163 is present at higher concentrations in the serum and urine of patients with SSc when compared with samples from healthy individuals[73]. Soluble CD163 concentrations have been associated with pulmonary hypertension in a small cohort of patients with early-stage SSc, but any links to other clinical involvements remain elusive[73,74].

CD68$^+$ macrophages that co-express CD163 and CD204 represent an abundant population in skin biopsies from patients with SSc, and particularly in perivascular and collagen-rich areas[72] (Fig. 2). Bioinformatics analysis of gene expression in biopsy-obtained skin from patients with SSc showed a significant upregulation of genes associated with pro-fibrotic macrophage activation, such as CX3CR1 and IL-10 receptor[75]. Moreover, CX3CR1$^+$ MDMs were found to be recruited into damaged lungs in animal models of lung fibrosis, and to promote fibrosis through the IL-10 pathway[76,77].

Additionally, next-generation RNA sequencing of biopsy-obtained skin from individuals with early diffuse cutaneous SSc (dcSSc) linked CD68$^+$ and CD163$^+$ macrophage activation to cutaneous fibrosis[78] (Fig. 2). Transcriptomic analysis of myeloid cells in biopsy-obtained skin from individuals with dcSSc also identified a cluster of macrophages with high expression of Fcγ receptor IIIA, suggesting a role for this macrophage subset in the pathogenesis and severity of skin fibrosis[79].

In addition to skin fibrosis, monocytes and macrophages are actively involved in SSc-ILD, although the distinct contributions of monocyte-derived alveolar macrophages and resident alveolar macrophages have not yet been clearly elucidated[80].

The frequency of CD14$^+$CD163$^+$ monocytes in the blood correlated significantly with the presence of SSc-ILD. CD14$^+$CD163$^+$ monocytes respond to in vitro stimulation with lipopolysaccharide by releasing profibrotic mediators, such as CCL18 and IL-10 (ref. 81). Moreover, resident alveolar macrophages of fibrotic lungs usually show increased expression of CD206 on their cell surface and upregulated production of profibrotic chemokines CCL17, CCL18 and CCL22, as found in the bronchoalveolar lavage fluid of patients with fibrotic lung disorders compared with healthy controls[82]. SSc pulmonary macrophages were also reported to express CD14 and high levels of fibronectin and TGFβ[83].

Another pro-fibrotic macrophage subset identified in the lungs of patients with SSc-ILD expresses SPP1 in response to stimulation with IL-6 and TGFβ. As single-cell RNA sequencing identified clear differences in chromatin structure and transcription factor regulation between SPP1$^+$ macrophages from patients with SSc-ILD and SPP1$^-$ macrophages from the lungs of healthy individuals, a pro-fibrotic lung macrophage phenotype might involve epigenetic regulation in response to local environmental factors[84].

Although macrophage populations have been scarcely analysed in lung samples from patients with SSc-ILD, most observations derived from mouse models confirm a prevalence and role of pro-fibrotic macrophage phenotypes in the development of ILD[85,86] (Fig. 2). Similarly, profibrotic monocytes and macrophages seem to be involved in other organ manifestations of SSc, including gastrointestinal and cardiac fibrosis, although data are, again, based on animal models[87].

Of note, the dichotomous pro-inflammatory–anti-inflammatory macrophage classification, based on surface markers, does not fully

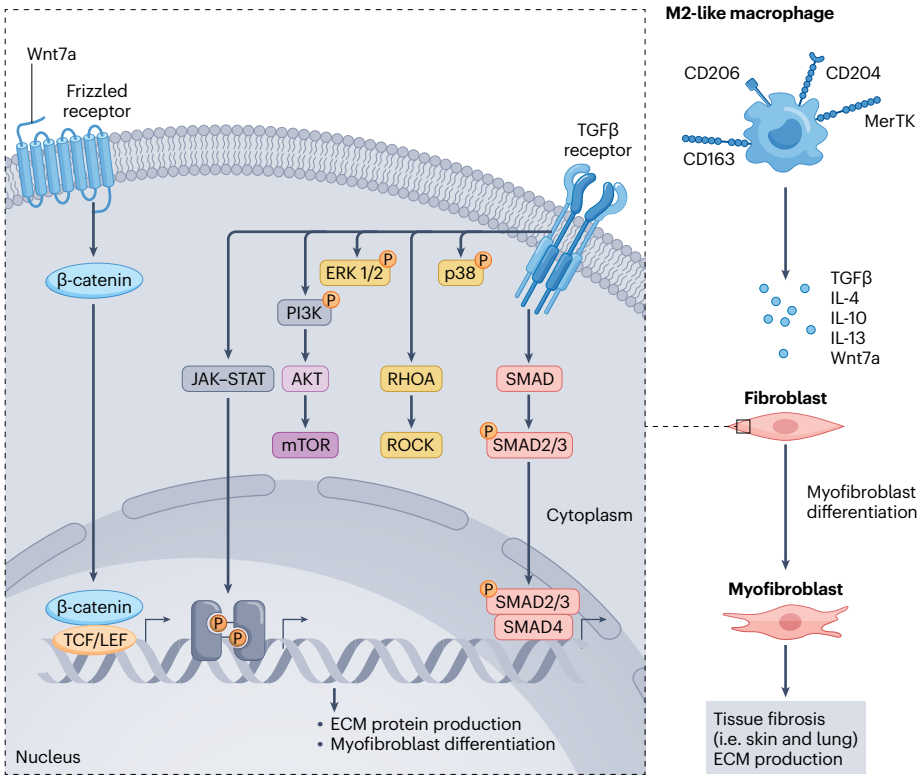

Fig. 2 | Pro-fibrotic macrophage–fibroblast interactions in systemic sclerosis. During systemic sclerosis (SSc), pro-fibrotic macrophages usually express CD163, CD204, CD206 and tyrosine-protein kinase Mer (MerTK) and release large amounts of the pro-fibrotic mediators Wnt7a, transforming growth factor β (TGFβ), IL-4, IL-10 and IL-13. These mediators engage with their receptors on fibroblasts to induce differentiation into myofibroblasts. Wnt7a binds to the Frizzled receptor and promotes the translocation of cytoplasmic β-catenin into the nucleus of the fibroblast. β-catenin binds to the T cell factor–lymphoid enhancer factor (TCF–LEF) complex, stimulating the transcription of several genes involved in fibroblast-to-myofibroblast differentiation. TGFβ activates SMAD-dependent and SMAD-independent pathways (including p38, RHO–ROCK, ERK1 and ERK2, PI3K–AKT and JAK–STAT), which promote fibroblast differentiation into myofibroblasts and the production of extracellular matrix (ECM) proteins, ultimately leading to tissue fibrosis.

describe the complexity of monocyte and macrophage plasticity in SSc. In fact, subpopulations of peripheral monocytes co-expressing pro-inflammatory (CD80, CD86, TLR4) and anti-inflammatory (CD163, CD204, CD206) surface markers have been described in patients with SSc[88]. These 'hybrid' subpopulations of peripheral monocytes correlate with SSc organ involvement, and specifically with the presence of SSc-ILD and extent of skin fibrosis[89,90]. Moreover, hybrid $CD68^{+}TLR4^{+}CD163^{+}CD204^{+}CD206^{+}$ macrophages have been identified in lung biopsies and peripheral blood from patients with SSc-ILD[91]. The chemokines that drive macrophage differentiation towards this phenotype, as well as the cytokines produced by hybrid macrophages, have not yet been precisely characterized and are still under investigation.

Treatments that affect macrophages in systemic sclerosis

Recommended therapies for SSc have been shown to limit progression of tissue damage potentially by interfering with the differentiation of profibrotic monocyte and macrophage subsets[92] (Table 2). Bosentan is an oral antagonist of endothelin-1 receptor isoforms A and B and has been approved for the prevention of ischaemic digital ulcers in people with SSc. Bosentan is also used as part of combination therapy for pulmonary arterial hypertension. Bosentan has been shown to lower the expression of CD204, CD206 and CD163 on MDMs in vitro, as well as to counteract the role of endothelin-1, thereby limiting the transition of cultured MDMs into profibrotic phenotypes[65].

The tyrosine kinase inhibitor nintedanib is an oral antifibrotic drug recommended for SSc-ILD, particularly for rapidly progressive forms, as it slows the decline in respiratory function[93,94]. Nintedanib reduces signalling downstream of the receptors for platelet-derived growth factor (PDGF), fibroblast growth factors 1–3 (FGF1–FGF3) and vascular endothelial growth factors 1–3 (VEGF1–VEGF3)[93,94]. Moreover, circulating fibrocytes collected from patients with SSc-ILD showed a downregulation of gene and protein expression of the markers of fibroblast and myofibroblast activation fibroblast-specific protein-1 (S100A4) and α-smooth muscle actin (αSMA), as well as of type I collagen (COL1) and fibronectin, when treated with nintedanib in vitro[95]. Thus, nintedanib seems to downregulate in vitro the transition of fibrocytes into myofibroblasts and their pro-fibrotic activity as well as macrophage differentiation, particularly in cells isolated from patients with anti-topoisomerase I (antiScl70) autoantibody seropositivity.

In addition, macrophages differentiated in vitro from peripheral monocytes of patients with SSc-ILD display a substantially lower gene and protein expression of profibrotic surface markers (CD163, CD204, CD206) and MerTK when treated with nintedanib[96]. These findings suggest a potential direct role for nintedanib in downregulating the profibrotic phenotype of macrophages in SSc-ILD[96] (Fig. 2).

The anti-IL-6 receptor antagonist tocilizumab has been suggested as a second-line treatment for early inflammatory dcSSc with or without ILD, although the quality of evidence for its use is currently low[97,98]. Evidence from a mouse model of bleomycin-induced fibrosis indicates that tocilizumab treatment significantly reduces the number of $CD38^{+}$ monocytes and macrophages in peripheral blood and skin[99–102]. The phosphodiesterase 4 (PDE-4) inhibitor rolipram has also been demonstrated to ameliorate skin fibrosis in the bleomycin-induced fibrosis mouse model, reducing the release of IL-6 and TGFβ from profibrotic macrophages[103]. Limited evidence from in vitro studies of MDMs from healthy donors or from mouse models of SSc-associated ILD indicate that JAK inhibitors including ruxolitinib, tofacitinib and itacitinib might also partly block macrophage differentiation, warranting further research[104]. Several other experimental drugs have demonstrated effects on macrophage plasticity in murine models of SSc, but their discussion is beyond the scope of this Review.

Glucocorticoids are not routinely used in the treatment of SSc, as they have been associated with an increased risk of developing a scleroderma renal crisis when administered at a dose of >15 mg prednisone-equivalent per day. Renal interstitial fibrosis is often found in the kidneys of patients with SSc who have experienced a scleroderma renal crisis and is treated with high doses of glucocorticoids[105]. In addition, low doses of glucocorticoids (5 mg prednisone-equivalent per day), although safe from a renal perspective, are not associated with a favourable clinical effect on skin fibrosis in patients with dcSSc and are not routinely prescribed[106]. The ability of glucocorticoids to promote differentiation of macrophages into a pro-fibrotic M2c subset might partly explain these unfavourable effects of glucocorticoids in SSc[107].

In conclusion, pharmacological treatments currently used in SSc, including vasodilatory treatments (such as bosentan) and immunosuppressive agents (glucocorticoids, tocilizumab, JAK inhibitors), have substantial effects on tissue macrophages. Antifibrotic drugs such as nintedanib might also slow tissue damage in SSc by preventing the differentiation of pro-fibrotic macrophages.

Macrophages in systemic lupus erythematosus

Macrophage plasticity in systemic lupus erythematosus

SLE is a complex autoimmune rheumatic disease with severe involvement of multiple organs, including the kidneys as in the case of lupus nephritis. Infiltration of the affected tissues and organs by pro-inflammatory $CD14^{+}CD163^{-}$ monocytes and macrophages is an early feature of SLE[14]. The resolution of the initial inflammatory reaction has also been associated with macrophages, and particularly with the presence of M2a, M2c and $CD206^{+}$ macrophages in the tissues, all of which seem to contribute to irreversible damage, as for example in lupus nephritis[108].

Innate immunity has a pivotal role in the pathogenesis of SLE, as the failure to clear autoantigens leads to altered activation of antigen-presenting cells including monocytes and macrophages[109]. SLE macrophages exhibit impairment in the phagocytosis of apoptotic cells, leading to the intracellular accumulation of debris, such as DNA and other nuclear components. These autoantigens are then displayed on the macrophage surface, stimulating positive selection of autoreactive lymphocyte clones, production of autoantibodies, formation of immune complexes, activation of the complement system and tissue damage[100]. In addition, macrophage-exposed autoantigens contribute to the activation of plasmacytoid dendritic cells (DCs), and subsequent secretion of large amounts of type I interferons. The overexpression of type I interferon genes by peripheral immune cells in SLE, known as the 'interferon signature', amplifies the inflammatory response and promotes autoantibody production[101].

TLR signalling contributes to the 'interferon signature' of SLE, and expression of fatty acid synthases (FASN) in circulating monocytes was shown to increase activation of TLR4, TLR7 and TLR9 in these cells via the de novo synthesis of long-chain fatty acids[102]. Type I interferon-stimulated genes (ISGs) have also been found to be substantially upregulated in classic $CD14^{+}$ monocytes from the blood of patients with active SLE. These cells displayed a pro-inflammatory phenotype, including cell-surface expression of CD80 and production of pro-inflammatory cytokines such as IL-1β[110,111].

Non-classical $CD16^{+}$ monocytes from the blood of patients with active SLE also express the surface marker CD80 and display

an interferon signature[112]. In addition, non-classical monocytes of patients with SLE show high expression of the surface receptor for macrophage colony-stimulating factor (M-CSF), and other markers such as cell-surface protein CD115 and 6-Sulpho LacNac (SLAN), along with low expression of the GM–CSF receptor alpha chain (CD116). $CD115^+$ non-classical monocytes have been associated with lupus nephritis[113] (Fig. 3). Furthermore, longitudinal single-cell transcriptomic analysis of classical and non-classical monocytes collected from the peripheral blood of patients with SLE at baseline and upon treatment initiation indicated that classical monocytes are primed towards a pro-inflammatory phenotype that was characterized by the expression of TNF and IL-1, whereas non-classical monocytes are reprogrammed towards a T_H17 cell-like phenotype, characterized by expression of IL-17 and IL-12 (ref. 114).

In addition to circulating monocytes, macrophages isolated from skin lesions of patients with SLE or localized cutaneous lupus (discoid lupus erythematosus) had transcriptomic profiles indicative of cellular interactions with fibroblasts. Moreover, epidermal $CD68^+CD163^+CD206^+$ macrophages in patients with SLE showed a higher interferon signature than patients with discoid lupus erythematosus[115]. A separate study reported downregulation of peroxisome proliferator-activated receptor gamma (PPARγ) expression in pro-inflammatory $CD80^+$ peripheral monocytes and skin macrophages in patients with SLE with active skin lesions[116].

In summary, monocytes and macrophages drive systemic inflammatory response in SLE, as well as cutaneous involvement of disease. Their role in lupus nephritis, which is one of the major causes of mortality and morbidity in SLE, is discussed in the following subsection[117].

Macrophage plasticity in lupus nephritis

In patients with lupus nephritis, multiple metabolic pathways regulate the plasticity of MDMs and activated kidney-resident macrophages (KRMs) during both the inflammatory and reparative phases of the disease[112]. Transcriptomic analyses of kidney biopsies from patients with lupus nephritis or mouse models of lupus nephritis and mouse lupus nephritis tissues suggest that MDMs and KRMs have distinct functions. MDMs seem to be primarily involved in the presentation of immune complexes, whereas KRMs seem to be involved in leukocyte recruitment[118].

Regardless of the diagnosed class of lupus nephritis, acute damage is characterized by a pro-inflammatory macrophage infiltrate in all kidney structures – tubes, interstices and glomeruli. Signalling via TLRs and intracellular proteins, such as the NF-κB cofactor NFAT5, promote this pro-inflammatory macrophage phenotype in the kidney[119] (Fig. 3).

Moreover, in lupus nephritis, pro-inflammatory macrophages highly express sphingosine-1 (SP-1) receptors (SP1R), that react to elevated serum concentrations of SP-1 and activate NLRP3 inflammasome with transcription and release of several pro-inflammatory cytokines[120].

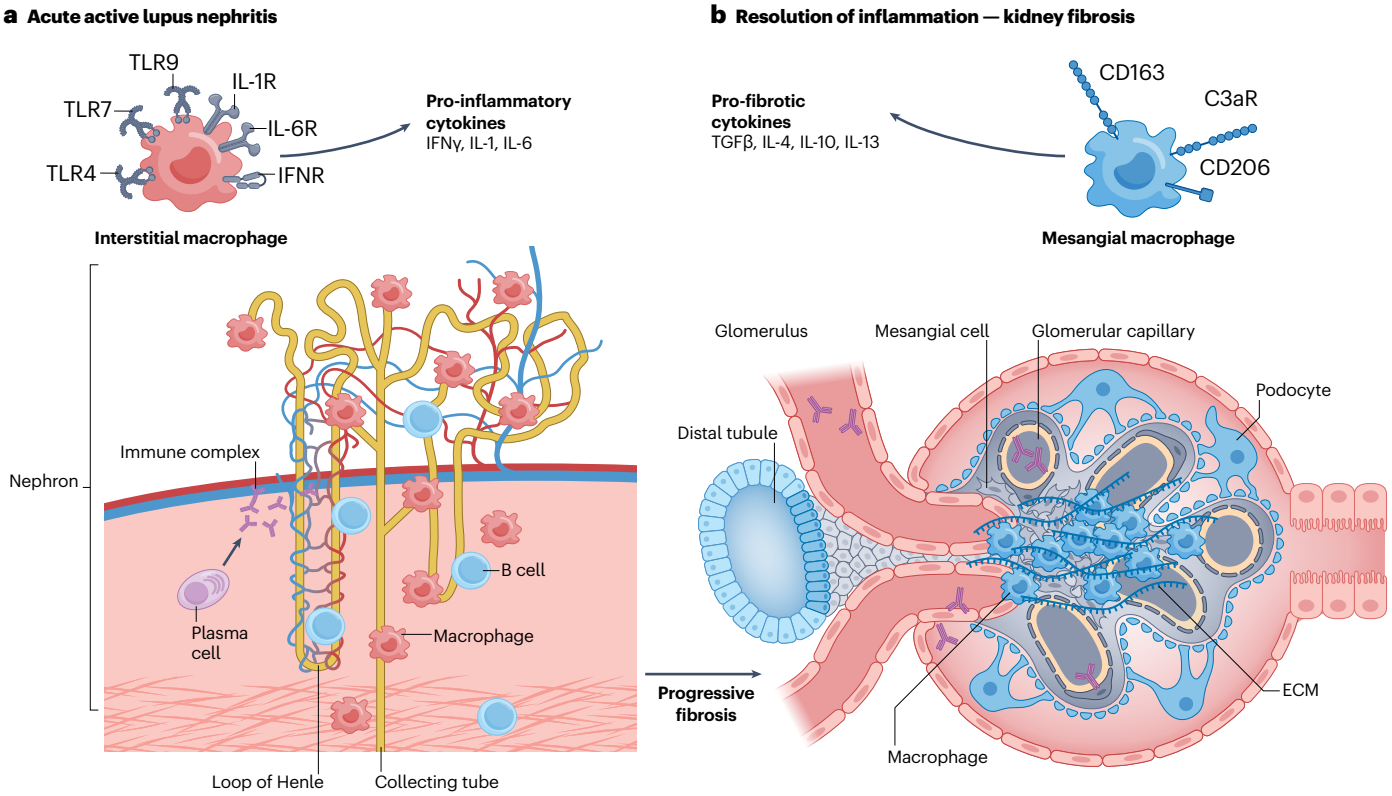

Fig. 3 | Macrophage subsets in lupus nephritis. a, Active lupus nephritis is characterized by an abundant pro-inflammatory monocyte–macrophage infiltrate at the tubulointerstitial and glomerular levels. **b**, During the resolution phase, a pro-fibrotic monocyte–macrophage infiltrate predominates, promoting extracellular matrix (ECM) deposition and fibrosis. In the glomerulus, pro-fibrotic macrophages contribute to mesangial cell activation and ECM production, which may lead to glomerulosclerosis. Pro-fibrotic macrophages have been associated with crescent formation, which primarily involves the proliferation of parietal epithelial cells, ultimately resulting in fibrosis and loss of organ function. IFNR, interferon receptor; IL-1R, IL-1 receptor; IL-6R, IL-6 receptor; TGFβ, transforming growth factor-β; TLR, Toll-like receptor.

A bioinformatics analysis of kidney biopsies from patients with lupus nephritis or mouse models of lupus nephritis identified IFI16, EGR1 and MX1 as key upregulated genes in disease, and associated this upregulation with the presence of pro-inflammatory macrophages at the tubulointerstitial level[121].

The density of the CD68$^+$ macrophage infiltrate at the tubulointerstitial level was also substantially associated with a low likelihood of response to immunosuppressive therapy, as demonstrated with immunohistochemistry in kidney biopsies from 430 patients affected by lupus nephritis[122] (Fig. 3).

The pro-inflammatory gene *LGALS9* has also been reported to be upregulated in lupus nephritis macrophages compared with macrophages residing in healthy kidneys[123].

Kidney-resident NKp46$^+$ innate lymphoid cells amplify inflammation in lupus nephritis by promoting the expansion of MDMs in a GM–CSF-dependent manner, thereby causing damage to renal epithelial cells[124]. Moreover, macrophages activate mesangial cells, through the CXCL12–DPP4 axis, and perpetuate tissue damage, as suggested in mouse models of lupus nephritis[125] (Fig. 3).

Pro-fibrotic macrophage phenotypes are typically associated with the resolution of renal inflammation. However, CD68$^+$ and CD163$^+$ macrophages showed expression of the C3a receptor on their surface, and C3a receptor activation has been correlated with the presence of kidney crescents and loss of renal function, as demonstrated in a cohort of 301 Chinese patients with proliferative lupus nephritis (class III or IV, variably associated with class V)[126] (Fig. 3). Notably, the levels of soluble CD163 in both serum and urine of patients with SLE have emerged as promising biomarkers of lupus nephritis. In fact, patients with SLE with high serum and urine concentrations of soluble CD163 are significantly more affected by lupus nephritis than those with low concentrations of CD163 (refs. 127,128).

Thus, macrophage plasticity in lupus nephritis represents an important pathogenic factor in the development of both acute and chronic damage and may serve as a target for specific pharmacological therapies.

Treatments that affect macrophages in systemic lupus erythematosus

The impact of SLE treatments on monocyte and macrophage plasticity has been explored in both humans and animal models of disease (Table 2).

The clinical advantages of glucocorticoids in active SLE are well recognized and seem to be partly based on mechanisms affecting macrophage plasticity. Analyses in a small cohort of patients with SLE linked use of glucocorticoids (prednisone) in combination with and without hydroxychloroquine with the upregulation of PPARγ expression and downregulation of NF-κB in monocytes. Monocytes from patients with SLE receiving prednisone had profibrotic phenotypes (high expression of ARG1 and low expression of CD80, NF-κB, IL-1β, IL-6, IL-12 and CCR7)[116]. In addition, a small, randomized placebo-controlled clinical trial in patients with active SLE indicated that milatuzumab, an antibody targeting the macrophage migration inhibitory factor (MIF) receptor CD74 on macrophages, might ameliorate disease activity scores (measured based on British Isles Lupus Assessment Group and the Systemic Lupus Erythematosus Disease Activity Index), although the low enrolment limited the statistical significance of the drug's positive effects. This study further emphasized the importance of macrophages in the pathogenesis of clinical SLE manifestations[129].

The potential impact of other treatments has been tested in mouse models of SLE. Mycophenolate mofetil and its metabolite mycophenolic acid are widely used for the treatment of renal and non-renal SLE and seem to interfere with monocyte and macrophage plasticity[130]. Renal macrophages in MRL/lpr lupus-prone mice that had been treated with dextran mycophenolate-based nanoparticles showed a phenotypic shift towards an anti-inflammatory phenotype, with upregulation of CD206 and downregulation of CD80 (ref. 131). Treatment with recombinant IL-33 also reduced the percentages of pro-inflammatory macrophages and increased the percentages of pro-fibrotic macrophages in the kidneys of these mice[132]. In this study, pro-inflammatory phenotype was reported based on the expression of monocyte chemoattractant protein 1 (MCP-1; also known as CCL2) and inducible nitric oxide synthase (iNOS), whereas the pro-fibrotic phenotype was defined through mRNA expression of arginase 1 (ARG1) and FIZZ1, although CD206 expression remained low[132]. Moreover, the experimental use of lysophosphatidic acid, a biolipid involved in several intracellular signalling pathways, for 3 weeks, in MRL/lpr lupus-prone mice, significantly reduced the numbers of CD68$^+$ cells in the glomeruli and improved glomerular macrophage-driven inflammation[133]. The SP-1 receptor inhibitor FTY720 (fingolimod) that acts on SP1R-positive macrophages has also shown a therapeutic effect in this mouse model of lupus nephritis by improving nephritis symptoms[120].

In conclusion, modulating macrophage plasticity seems to have a beneficial effect in the course of SLE, and it will be interesting in the future to investigate whether other currently used drugs, such as calcineurin inhibitors, anti-CD20, anti-BAFF and anti-IFNγ receptor agents, influence macrophage phenotypes and functions.

Macrophages in polymyalgia rheumatica

Macrophage plasticity in polymyalgia rheumatica

PMR is one of the most common inflammatory rheumatic disease among people over 50 years of age and manifests as musculoskeletal pain and stiffness in the neck, shoulders and hips, along with systemic manifestations of the inflammatory status such as asthenia, low-grade fever and weight loss[134,135]. Hallmark symptoms of PMR include intense peri-articular inflammation, involving the synovial tissue of bursae and tendon sheaths[136].

Early studies demonstrated that shoulder synovitis in PMR is characterized by mild-to-moderate infiltration of immune cells, predominantly macrophages and CD4$^+$ T cells[137]. Macrophages infiltrating the shoulder synovial tissue of patients with PMR expressed high levels of HLA-DR and localized primarily in the transitional zone between the synovial lining and blood vessels. Importantly, vascular proliferation and increased expression of HLA class II antigens suggest that PMR might involve an antigen-driven immune process[137,138].

Subsequent studies analysing synovial biopsies from the glenohumeral joints of patients with active PMR have identified macrophages as the predominant immune-cell type, substantially contributing to the local production of pro-inflammatory cytokines, including IL-6, GM–CSF, TNF, IL-12 and IL-23 (ref. 139) (Fig. 4).

In the affected bursae, macrophages were found to outnumber other immune system cells, such as B cells[140] (Fig. 4). T cells, particularly T_H1 cells, were found to be the second most abundant immune-cell type in PMR joints, and T_H1 cell-derived IFNγ seems to amplify macrophage activation in this context[139,140]. By contrast, T_H17 cells, although present in circulation at elevated levels, were not found to be enriched in the bursae, suggesting that the local inflammatory environment might be more conducive to T_H1 cell responses[140] (Fig. 4).

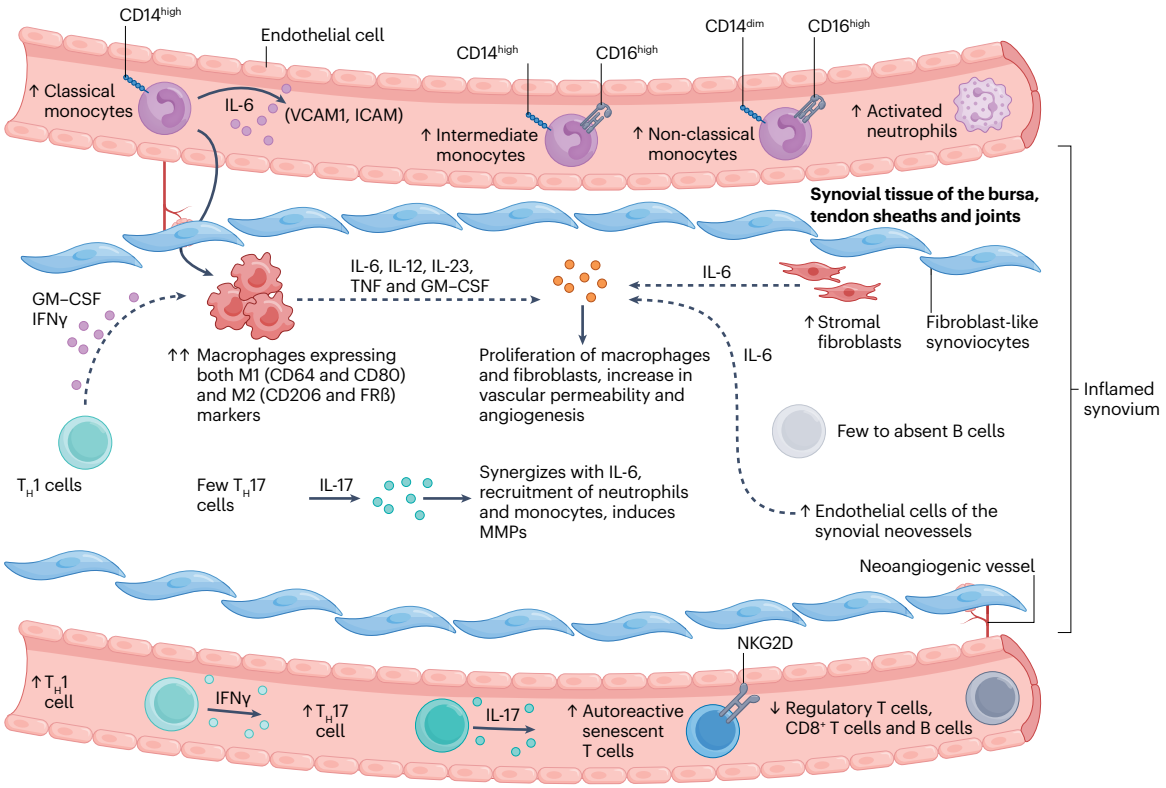

Fig. 4 | Key macrophage-driven mechanisms and pathways involved in the pathophysiology of polymyalgia rheumatica. This figure illustrates the complex inflammatory network in polymyalgia rheumatica (PMR), focusing on the central role of macrophages and their crosstalk with other immune and stromal cells in the synovial tissue of bursae, tendon sheaths and joints. Intermediate monocytes ($CD14^{++}CD16^{+}$) are increased in circulation and are thought to migrate into the inflamed synovial environment via interactions with adhesion molecules such as vascular cell adhesion protein 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1) expressed on endothelial cells, and in response to pro-inflammatory cytokines such as IL-6. This subset also expresses the chemokine receptor CCR2, and its ligand CCL2 is produced by stromal and endothelial cells within inflamed periarticular tissues, supporting a chemokine-driven recruitment mechanism. Classical ($CD14^{++}CD16^{-}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes are not increased in number but exhibit altered activation states: classical monocytes show reduced HLA-DR and CD11c expression, whereas non-classical monocytes display increased CD86 and TLR2 expression, indicating systemic immune priming. Within the inflamed tissue, macrophages exhibit a hybrid phenotype co-expressing both pro-inflammatory (CD64, CD80) and tissue remodelling (CD206, FRβ) markers, suggesting functional plasticity. Despite this mixed phenotype, these macrophages produce high levels of IL-6, TNF, IL-1β, IL-12 and IL-23, reflecting a predominantly pro-inflammatory function. GM–CSF and IFNγ, primarily derived from T helper 1 (T_H1) lymphocytes, contribute to skewing macrophages towards an inflammatory phenotype, marked by activation of the JAK2–STAT5 pathway and downregulation of folate receptor beta (FRβ). Stromal fibroblasts and $CD34^{+}$ endothelial cells support this inflammatory environment by producing IL-6 and expressing adhesion molecules that promote leukocyte recruitment and retention. IL-6, in turn, contributes to macrophage and fibroblast proliferation, increased vascular permeability and induction of matrix metalloproteinases (MMPs). T_H1 cells are abundant in the inflamed synovium and amplify macrophage activation via IFNγ, whereas T_H1 cells are relatively scarce in the tissue but present in the circulation. B cells are notably few or absent in affected tissues. Additionally, $NKG2D^{+}$ senescent T cells, associated with immune ageing, are expanded in the blood of people with PMR and may contribute to systemic inflammation, although their precise role in the tissue microenvironment remains unclear. dim, diminished; GM–CSF, granulocyte–macrophage colony-stimulating factor; high, highly expressed; MMP, metalloproteinase; NKG2D, natural killer group 2 member D.

In PMR, macrophage phenotypes may be influenced by the local pro-inflammatory signals originating from both stromal and immune cells in inflamed bursae (microenvironmental factors). For instance, fibroblasts and endothelial cells have been shown to produce high levels of IL-6 and express adhesion molecules, contributing to leukocyte recruitment and the maintenance of local inflammation[139]. Immunofluorescence studies demonstrated IL-6 expression by $CD90^{+}$ fibroblasts and $CD34^{+}$ endothelial cells in bursal tissue, highlighting the potential role of these cells in shaping the inflammatory milieu[139] (Fig. 4). Moreover, circulating T cells expressing NKG2D, a receptor linked to immune ageing and autoreactivity[141], are increased in PMR, although their tissue distribution and potential interactions with resident macrophages remain to be defined.

Macrophages in PMR exhibit a complex and heterogeneous phenotype, co-expressing major pro-inflammatory (CD80 and CD64) and tissue remodelling-associated (CD206 and folate receptor beta (FRβ)) surface markers[142] (Fig. 4). This complex status potentially reflects both macrophage plasticity and the dynamic interplay of local environmental factors such as cytokines and growth factors within the inflammatory milieu, which is shaped by GM–CSF, M-CSF and IFNγ (Fig. 1). Notably, despite this mixed expression profile, macrophages in PMR-affected tissues exhibit a predominantly pro-inflammatory

functional programme, characterized by robust production of IL-6, IL-1β, TNF and IL-23.

GM–CSF and IFNγ produced in the bursa microenvironment, partly by T_H1 cells, have a pivotal role in skewing macrophages towards a pro-inflammatory phenotype, characterized by heightened IL-6 production[142]. Macrophages in PMR-affected bursae exhibit a GM–CSF-skewed transcriptional and signalling profile, including activation of the JAK2–STAT5 pathway and downregulation of FRβ, suggesting that GM–CSF is a key driver of the inflammatory phenotype[142]. These macrophages also express elevated levels of CD68, CD86 and CD64, and this finding further indicates a pro-inflammatory potential.

Monocytes from the synovial fluid of affected joints and bursae in patients with PMR also display a GM–CSF-driven profile and an upregulated expression of CD206, the expression of which is known to be induced by GM–CSF signalling[142]. In addition, circulating monocytes, which differentiate into macrophages only upon tissue recruitment, also exhibit an aberrant phenotype in PMR[143,144] (Fig. 4). In people with PMR, the frequency of intermediate monocytes is increased, non-classical monocytes are reduced and classical monocytes remain unchanged compared with healthy individuals. Notably, both non-classical and classical subsets display altered activation states, including elevated CD86 and TLR2 on non-classical monocytes and reduced HLA-DR and CD11c on classical monocytes[143,144] (Box 1). This suggests a state of systemic monocyte priming that might enhance monocyte ability to promote tissue inflammation upon recruitment (Fig. 4).

Effects of glucocorticoids on macrophages in polymyalgia rheumatica

Treatment of patients with PMR with glucocorticoids has been shown to reduce markers of inflammation, such as vascular proliferation and expression of HLA class II, in synovial tissue[137]. At the systemic level, treatment with glucocorticoids has also been associated with a decrease in the absolute numbers of all circulating monocyte subsets, classical, intermediate and non-classical, in patients with PMR in remission[144]. This systemic effect aligns with the broader immunosuppressive action of glucocorticoids and further underscores their fundamental role in modulating key immune pathways in PMR[145]. However, the lack of detailed data on the effect of glucocorticoids in situ or on bursal macrophage phenotypes and function leaves an important gap in understanding the glucocorticoid-dependent mechanisms of full disease resolution.

Overall, the dominant role of macrophages in the inflammatory landscape of PMR highlights their importance as both markers of disease activity and potential therapeutic targets. A deeper understanding of macrophage dynamics and cytokine networks might pave the way for new targeted treatments, possibly sparing glucocorticoid treatments to mitigate the associated systemic adverse effects[146–148].

Macrophages in giant-cell arteritis

Macrophage plasticity in giant-cell arteritis

GCA is the most common systemic vasculitis in adults over 50 years of age[149], and the disease name already highlights the crucial involvement of multinucleated giant cells that arise from the fusion of activated macrophages and are detected in the affected temporal arteries on biopsy[150]. Characterized by granulomatous inflammation of medium- and large-sized arteries, GCA often leads to ischaemic complications such as vision loss, stroke, or aortic aneurysms[151,152]. From a clinical perspective, many observational studies have shown that 21–25% of patients with PMR also display symptoms and imaging features of GCA, whereas PMR features are observed in 40–60% of patients diagnosed with GCA[153–155]. This overlap has supported the concept of the GCA–PMR spectrum disease, suggesting shared epidemiological, clinical and immunopathogenic mechanisms, in which macrophages seem to have a pivotal role[156].

Indeed, comparative analyses of subacromial bursa from patients with PMR and temporal artery sections from patients with GCA revealed overlapping immune profiles, including comparable absolute numbers of $CD68^+$ macrophages and similar expression of co-stimulatory molecules such as CD86 and CD206. Both diseases also exhibit comparable tissue levels of pro-inflammatory cytokines, as assessed by semi-quantitative scoring, such as GM–CSF, IL-1β, IL-6, IL-12, IL-23 and TNF, highlighting shared inflammatory pathways[157].

The close immunopathological links between PMR and GCA also suggest shared macrophage-driven mechanisms, as similar pro-inflammatory macrophage phenotypes have been observed in both conditions[157]. Indeed, the interplay between macrophages and vascular components leads to tissue destruction and vascular remodelling[158]. Pro-inflammatory macrophages release reactive oxygen species, proteolytic enzymes and cytokines that degrade the ECM, enabling immune-cell infiltration. Concurrently, other macrophage subsets contribute to angiogenesis and fibrosis through VEGF, PDGF and chitinase-3-like protein (YKL-40) signalling[159]. The functional diversity of macrophages in GCA reflects their plasticity, with pro-inflammatory macrophages dominating early inflammation and mixed phenotypes (hybrids) emerging in chronic lesions[160]. These subsets, influenced by GM–CSF, M-CSF and IL-6, highlight the role of macrophage plasticity in disease progression.

The activation of vascular DCs that reside in the adventitia of large arteries has been hypothesized to underlie the initiation of GCA pathology[161] (Fig. 5). Under homeostatic conditions, vascular DCs maintain vascular immune privilege by remaining quiescent. However, in GCA, unknown environmental triggers, such as infections or damage-associated molecular patterns (DAMPs), disrupt this equilibrium, leading to DC activation[161].

Activated DCs upregulate HLA molecules and costimulatory signals, and present antigens to naïve $CD4^+$ T cells[162], promoting their differentiation into T_H1 and T_H17 cells[156]. T_H1 and T_H17 cell-derived IFNγ and IL-17 activate both resident macrophages and monocyte-derived macrophages recruited from the circulation through chemokine gradients[158]. Together, these macrophage populations amplify inflammation through the production of cytokines such as IL-6, IL-1β and TNF, proteolytic enzymes and chemokines, perpetuating the breakdown of vascular integrity and sustaining the inflammatory process[163] (Fig. 5).

Monocyte recruitment to the inflamed vessel wall in GCA is driven by chemokine gradients, with CCL2 and CX3CL1 guiding classical and non-classical monocytes via CCR2 and CX3CR1, respectively[164]. Recruited classical $CD14^+CD16^-$ monocytes seem to sustain the inflammatory response, whereas non-classical $CD14^+CD16^{++}$ monocytes migrating into the vessel wall differentiate into $CD68^+CD16^+CX3CR1^+$ macrophages, which actively contribute to tissue damage[144] (Fig. 5).

This differentiation of non-classical monocytes into inflammatory macrophages seems to be influenced by local signals such as GM–CSF and IL-6 (ref. 144) (Fig. 5). Once recruited within the arterial wall, macrophages adopt distinct roles, depending on their localization and the microenvironment[158]. In the adventitia, macrophages express CD206 and produce MMPs, VEGF and YKL-40 (a glycoprotein involved in inflammation, tissue remodelling and angiogenesis)[159,165]. These macrophages interact with IFNγ-producing T_H1 cells, enhancing inflammation.

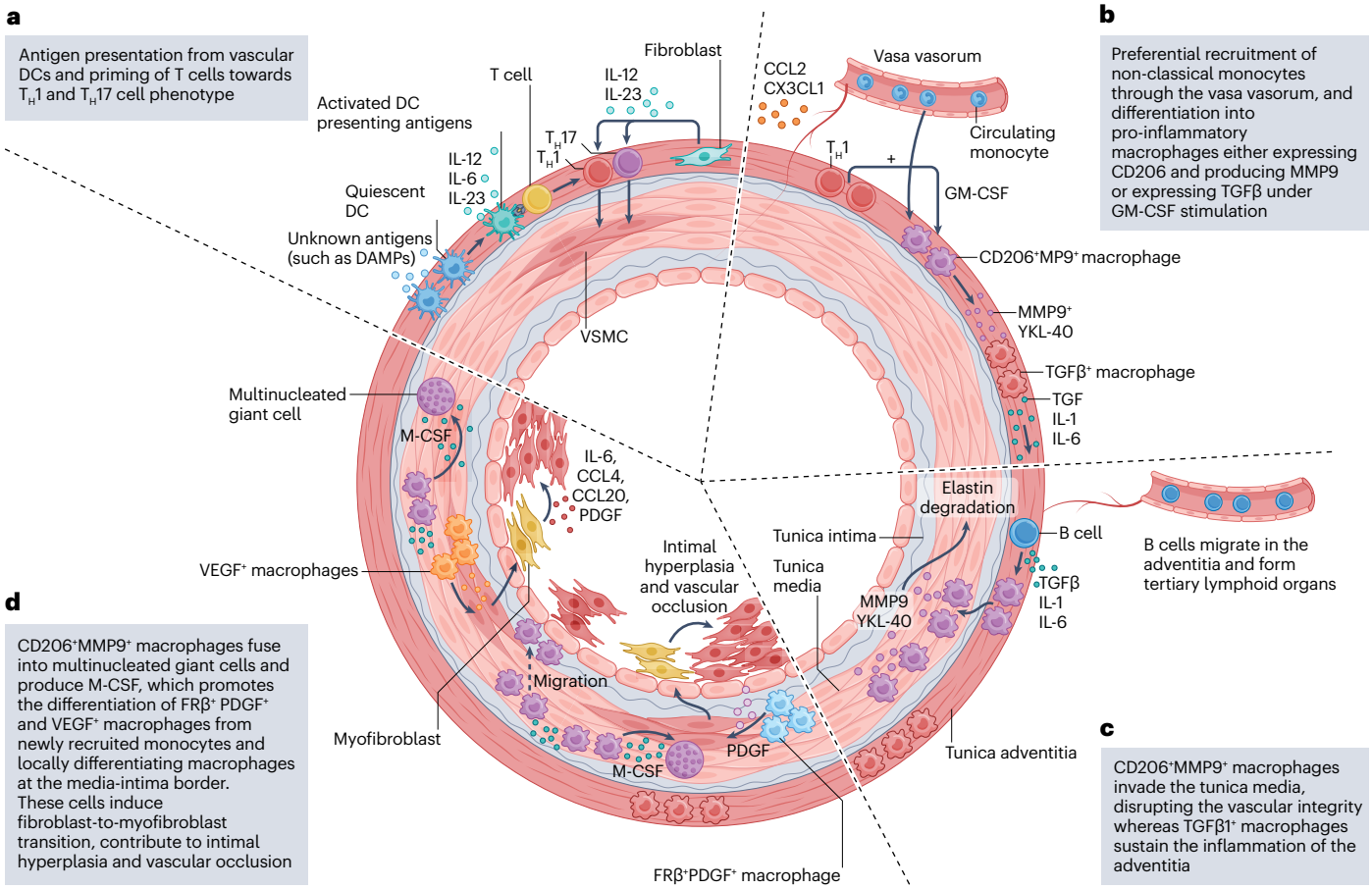

Fig. 5 | Macrophage-dependent mechanisms driving the pathophysiology of giant-cell arteritis. The pathogenesis of giant-cell arteritis (GCA) progresses over four consecutive stages. **a**, Yet undefined antigens and damage-associated molecular patterns (DAMPs) activate quiescent vascular dendritic cells (DCs). Activated vascular DCs present these antigens to naïve $CD4^+$ T cells in the vessel wall, particularly in the adventitia, inducing their differentiation of T helper 1 (T_H1) and T_H17 cells. T_H1 and T_H17 cells in the vessel wall produce cytokines that amplify macrophage activity and promote vascular smooth-muscle-cell proliferation. **b**, Circulating non-classical monocytes are recruited to adventitia from the vasa vasorum. Within adventitia, monocytes differentiate into pro-inflammatory macrophages: a subset that expresses CD206 and produces metalloproteinase 9 (MMP9); and a subset that expresses transforming growth factor β (TGFβ) upon stimulation with granulocyte–macrophage colony-stimulating factor (GM–CSF). **c**, B cells migrate from the vasa vasorum to the adventitia, where they form tertiary lymphoid organs (not shown). These ectopic lymphoid structures within the inflamed vessel wall are characterized by the compartmentalization of T cells and B cells and the expression of CXCL13 and BAFF, further contributing to the chronic inflammatory cascade. $CD206^+MMP9^+$ macrophages invade the tunica media, disrupting vascular integrity, whereas $TGFβ^+$ macrophages sustain inflammation in the tunica adventitia. **d**, In the last stage of GCA pathogenesis, $CD206^+MMP9^+$ macrophages fuse into multinucleated giant cells that produce macrophage colony-stimulating factor (M-CSF). M-CSF promotes the differentiation of folate receptor β (FRβ) + macrophages that produce platelet-derived growth factor (PDGF) and macrophages that produce vascular endothelial growth factor (VEGF). These macrophage subsets that stem from newly recruited monocytes and locally differentiating macrophages at the media–intima border promote fibroblast-to-myofibroblast differentiation. Resident stromal cells, such as vascular smooth-muscle cells (VSMCs) and fibroblasts, also contribute to the inflammatory cascade in GCA. VSMCs express pattern-recognition receptors, secrete pro-inflammatory chemokines (such as CXCL9 and CXCL10) and recruit T_H1 cells. Fibroblasts differentiate into myofibroblasts, promoting extracellular matrix remodelling and intimal hyperplasia through cytokines such as IL-12 and IL-23.

In the early phases of GCA pathogenesis, monocytes recruited to adventitia differentiate into $CD206^+MMP9^+$ macrophages under the influence of T cell-derived GM–CSF[166]. During disease progression, $CD206^+MMP9^+$ macrophages migrate towards the media and the media–intima border; TGFβ1-producing macrophages remain in the adventitia, where they further enhance the inflammatory milieu, secreting IL-1β and IL-6 (ref. 160). Macrophages in the media and intima show increased expression of iNOS and MMP9, driving elastic lamina degradation and intimal hyperplasia[167]. In the inner intima, a gradient of M-CSF favours the differentiation of $FRβ^+$ macrophages, which promote fibroblast proliferation and intimal thickening via PDGF-AA secretion and fibroblast activation (Fig. 5). In parallel, GM–CSF also supports the emergence of $CD206^+MMP9^+YKL\text{-}40^+$ macrophages, which are strongly implicated in tissue destruction and elastic lamina breakdown.

In the late stages of GCA, IFNγ and TLR2 signalling, among others, facilitate the formation of multinucleated giant cells[168] (Fig. 5); macrophages fuse to form $CD68^+CD163^+MMP12^+$ multinucleated giant cells that produce M-CSF and PDGF and further promote intimal hyperplasia and vascular remodelling[163]. In addition, VEGF-producing macrophages, preferentially located in the media and intima, stimulate

neoangiogenesis[158]. Biopsies of internal elastic lamina from patients at late stages of GCA featured senescent macrophages marked by the expression of senescence markers p16 and p21, and these senescent macrophages were found to secrete IL-6. These findings further emphasized the link between ageing and chronic vascular inflammation in GCA[169,170].

Resident stromal cells, such as vascular smooth muscle cells (VSMCs) and fibroblasts, also contribute to the inflammatory cascade in GCA[171]. Fibroblast activation protein (FAP), a serine protease with a key function in ECM remodelling, is abundantly expressed by both fibroblasts and macrophages in inflamed arteries. Increased FAP expression, which has been detected in the adventitia, media and intima of GCA-affected vessels, substantially contributes to fibroblast activation, tissue remodelling and local production of IL-6 and MMP9 (ref. 172). FAP^+ macrophages, particularly those expressing $FR\beta^+$, contribute to vascular remodelling by promoting fibroblast proliferation and intimal hyperplasia, whereas FAP^+ fibroblasts at the adventitia–media interface also display a pro-inflammatory phenotype[172].

Although B cells do not seem to contribute to GCA pathology via the production of autoantibodies, B cells and B cell-rich areas of arteries produce high levels of IL-6, GM–CSF, TNF, IFNγ, LTβ and IL-10 in GCA[17]. B cell-derived cytokines have been shown to skew macrophages towards a pro-inflammatory phenotype in vitro[17].

The interplay between macrophages and vascular components leads to tissue destruction and vascular remodelling[158]. Pro-inflammatory macrophages (M1-like) release ROS, proteolytic enzymes and cytokines that degrade the ECM, enabling immune-cell infiltration. Concurrently, other macrophage subsets contribute to angiogenesis and fibrosis through VEGF, PDGF and YKL-40 signalling[159].

The functional diversity of macrophages in GCA reflects their plasticity, with pro-inflammatory macrophages dominating early inflammation and mixed phenotypes (hybrids) emerging in chronic lesions[160]. These subsets, influenced by GM–CSF, M-CSF and IL-6, highlight the role of macrophage plasticity in disease progression.

Macrophage responses to giant-cell arteritis treatments

In GCA, glucocorticoid treatment leads to a selective reduction of circulating non-classical monocytes, whereas classical and intermediate monocytes persist in circulation. Additionally, most macrophages found in the temporal artery biopsies of GCA patients express CD16 and CX3CR1, markers typical of non-classical monocytes, suggesting that this subset contributes prominently to vascular inflammation and is less effectively targeted by current therapies.

A particular feature of GCA is the persistence of arterial wall inflammation, detected at temporal artery biopsy, in about half of patients after 1 year of standard therapy with systemic glucocorticoids[173]. Although glucocorticoids reduce systemic inflammation and the number of circulating non-classical monocytes, tissue-resident macrophages seem to remain active, driving local inflammation and vascular remodelling at least in a subset of patients[144]. This persistence is partly attributed to trained immunity, that is, the epigenetic reprogramming and metabolic shifts that take place in monocytes and macrophages, including enhanced glycolysis and oxidative phosphorylation, that enable sustained pro-inflammatory cytokine production despite treatment with glucocorticoids[174]. These adaptations underline the resilience of the inflammatory response in GCA, particularly within the arterial microenvironment.

Imaging studies confirm the persistence of smouldering vascular inflammation in a substantial subset of patients with GCA who are treated with glucocorticoids and tocilizumab. Interestingly, PET or CT scans have revealed active vasculitis in 33% of these patients, and vascular ultrasonography showed intima-media thickening in approximately 30% of patients with GCA treated with glucocorticoids and tocilizumab for a median period of 2.4 years[175]. Biopsy analyses also detected persistent inflammation in one-third of patients with GCA after 6 months of the same combined therapy, indicating that although tocilizumab reduces systemic inflammation, it might not resolve localized vascular inflammation[175]. Thus, given the central and complex role of macrophages in vascular inflammation and remodelling in GCA, therapeutic strategies beyond IL-6 blockade should be explored[176].

GM–CSF blockade has emerged as such a potential therapeutic strategy in GCA. Mavrilimumab, a monoclonal antibody targeting the GM–CSF receptor α chain, has demonstrated efficacy in reducing vascular inflammation in patients with GCA. Mavrilimumab decreased the recruitment of inflammatory cells in ex vivo cultured arteries from patients with GCA, reducing the levels of key pro-inflammatory cytokines such as IL-6, TNFα, IL-1β and vascular remodelling markers, such as MMP9 and VEGF[177]. In a phase II clinical trial, mavrilimumab, combined with a 26-week prednisone taper, significantly reduced the risk of flare (hazard ratio 0.38; $P = 0.026$) and significantly increased sustained remission rates at week 26 (83% vs 50%; $P = 0.0038$) compared with placebo in patients with GCA. In the same study, mavrilimumab maintained a favourable safety profile[178]. These findings support the continued development of GM–CSF inhibitors as a promising therapeutic approach to GCA.

Addressing gaps in the understanding of macrophage plasticity, particularly the transitions between pro-inflammatory and reparative states, might reveal key targets for intervention in GCA[158].

Conclusions

Monocyte and macrophage plasticity, as reflected by cell-surface and functional marker combinations, is crucially implicated in the early steps, progression and final outcomes of ARDs. This plasticity represents the adaptive response of monocytes and macrophages to environmental signals and specific pharmacological treatments. Indeed, transcriptomic and proteomic studies have demonstrated that phenotypic and functional transitions of macrophages occur in a continuum rather than as distinct states, especially in chronic inflammatory conditions such as RA, SSc, SLE, PMR and GCA[29,87,120,142,158]. Hybrid functional states marked by the co-expression of M1 and M2 markers might also reflect transitional phases in ARD pathophysiology. Although the M1–M2 nomenclature has offered a simplified reference model, a more accurate characterization of macrophage states requires integration of specific molecular markers, transcriptional signatures, and functional profiles. These elements should be contextualized within disease-specific cytokine networks and pathophysiological mechanisms to better capture macrophage heterogeneity and function in vivo. Understanding the intermediate states of macrophage plasticity is essential for designing targeted therapies that selectively modulate macrophage functions to promote resolution of inflammation or limit progression of fibrosis. Some standardized drugs used to treat ARDs already seem to achieve clinical effects in part by targeting macrophage plasticity. Thus, both established and newly devised therapeutic approaches targeting macrophages have the potential to profoundly influence the pathophysiology of several chronic ARDs and to improve clinical outcomes, especially when implemented during the early stages of disease.

Published online: 28 July 2025

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Acknowledgements

The authors thank the EULAR Study Group on NeuroEndocrine Immunology of the Rheumatic Diseases (NEIRD), the EULAR Study Group on Microcirculation in Rheumatic Diseases and the European Network on Rare and Complex Connective Tissue Diseases (ERN ReCONNET) for their continuous cultural support. V.S. is a Senior Clinical Investigator of the Research Foundation Flanders (grant number 1802920N and 1802925N). The authors also acknowledge Rosanna Campitiello for her contribution to the presentation of Figs. 2 and 3.

Author contributions

M.C., S.S., E.G. and E.H. wrote the first draft and contributed equally to the final version of the article. V.S. contributed substantially to discussion of the content. All authors reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Rheumatology* thanks Maria Sandovici, Stefan Uderhardt and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01277-y

Perspective

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Addressing the challenge of global delays in diagnosis and treatment of systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus is a complex and increasingly prevalent disease that presents substantial challenges in both diagnosis and management. Diagnostic delays frequently lead to irreversible organ damage, and remain a crucial concern, mainly among vulnerable populations, such as minority ethnic groups and those living in the global south. These delays are exacerbated by the clinical heterogeneity of systemic lupus erythematosus, the lack of specific diagnostic biomarkers, gaps in disease awareness or medical training, and persistent health care disparities, particularly in low-resource settings. This Perspective highlights the urgent need for a standardized definition of diagnostic delay that accounts for both clinical and socio-economic factors. By prioritizing early intervention and expanding access to specialized care, we can improve patient outcomes and reduce the long-term burden of the disease.

Sections

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Introduction

Insidious and heterogeneous diseases such as systemic lupus erythematosus (SLE) present a substantial challenge in both their recognition and management, and a race against time is required to limit disease progression. As health professionals, our goal is to minimize organ damage and, ultimately, to enhance prognosis and quality of life for patients. The global prevalence of SLE continues to rise worldwide regardless of ethnicity, socio-economic status, or geographical location[1–3]. As of 2021, the overall global incidence of SLE ranged between 1.5 and 11 cases per 100,000 person-years, whereas global prevalence estimates varied from 13 to 7,713.5 cases per 100,000 individuals[4]. An increasing trend in both incidence and prevalence has been reported across various populations. For example, American population studies over several decades showed that the incidence of SLE rose from 1.51 (95% confidence interval (CI) 0.85–2.17) per 100,000 person-years in the period 1950–1979 to 5.56 (95% CI 3.93–7.19) in 1980–1992 (ref. 5). An expanded analysis in the same population found that incidence rates increased from 3.32 (95% CI 2.03–4.60) per 100,000 person years in 1976–1988 to 6.44 (95% CI 4.97–7.91) during 2009–2018, representing an annual increase of 2% (95% CI 1–3%, $P < 0.001$). Prevalence similarly rose, from 28 cases in 1985 to 153 per 100,000 cases in 2015, corresponding to an increase from 30.65 (95% CI 18.86–42.44) to 97.4 (95% CI 81.61–113.19) per 100,000 individuals[6]. Similar upward trends in incidence have been described in the UK and Greece[7–10]. These increases might reflect heightened disease awareness among patients and physicians, improvements in immunological testing, and the increased sensitivity and specificity of the current classification criteria, which are capable of identifying milder cases that might have been overlooked in the past[3].

The time to diagnosis for any disease is generally defined as the interval between the initial recognition of clinical symptoms or signs of disease and the diagnosis. According to some authors, delays occur when the physician is unable to make a correct diagnosis, despite having sufficient clinical information from the patient; this results in preventable damage, rendering the condition potentially untreatable or even terminal[11,12].

The factors influencing diagnosis delay of SLE are multifaceted[3]. Although delays in diagnosis and treatment are not new to rheumatic and musculoskeletal diseases, their impact is particularly pronounced in SLE when compared with other conditions such as rheumatoid arthritis or axial spondyloarthritis[13–15], potentially owing to the complex and heterogeneous clinical presentation of SLE. Given the substantial burden of disease and the well-established correlation between diagnostic delay and an increase in organ damage, it is concerning that relatively few efforts have been made to explore and mitigate this phenomenon[16–18]. Clearly, a call to action is long overdue.

Several studies have reported a median time between 9 and 78 months from the onset of symptoms or disease signs to the formal diagnosis of SLE, an alarmingly long period, during which substantial organ damage might accumulate. This diagnostic delay seems to disproportionately affect vulnerable populations, including women, members of minority racial and ethnic groups, and those with lower socio-economic status[19–35].

In this Perspective, we aim to provide an overview of the current state of knowledge on factors that contribute to a delay in SLE diagnosis, and the implications of this delay for the treatment, quality of life and mortality of individuals with SLE. In addition, we highlight the urgent need to define diagnostic delays, depending on the regional context, as this step is crucial for improving care for patients with this complex and challenging disease.

Factors contributing to diagnostic delays in systemic lupus erythematosus

Clinical heterogeneity and lack of diagnostic markers

SLE is characterized by substantial clinical heterogeneity, with non-specific manifestations that might mimic other diseases, subclinical organ damage and variable disease severity across the sexes and the various age, racial, ethnic and socio-demographic groups[3,4].

Sex-dependent heterogeneity in the clinical manifestations of SLE is largely thought to depend on sex hormones, although definitive evidence is still lacking. Oestrogen and oestrogen receptors promote autoantibody production and modulate the differentiation of T helper 17 (T_H17) cells, dendritic cells, as well as the type 1 interferon signature of the disease[36]. By contrast, androgens inhibit B cell activation, which might explain the sex ratio seen in SLE, as well as the distinct clinical features that are seen in women versus men with SLE[37] and that might sometimes complicate diagnosis.

The presence of autoantibodies often precedes the onset of inflammation and clinical manifestations by as long as 3.4 years[21], but it is only after the onset of symptoms that individuals seek medical consultation. Moreover, non-classical SLE symptoms, such as fatigue and arthralgias, have been associated with long delays in diagnosis, as they are often misattributed to alternative conditions or comorbidities[31,35,38]. Once the typical SLE manifestations are recognized, irreversible and often severe organ damage might have already occurred[21] (Fig. 1). The time from onset of symptoms to organ damage accrual varies greatly. Approximately 30–50% of individuals with SLE develop organ damage within the first 5 years of disease onset, with an additional subset of at least 25% of the patients developing damage within 10 years[2] (Fig. 1). The development of lupus nephritis is a key prognostic factor in SLE, but might not be detected until approximately 4 years after the initial onset of symptoms[39]. Many patients exhibit histological changes in renal biopsies, even in the absence of clinical evidence of renal disease, a phenomenon known as 'silent lupus nephritis'[40].

Clinical heterogeneity is further increased when considering specific patient groups, such as pregnant women or paediatric patients. In pregnancy, a high hormonal burden has been associated with SLE onset or severe flares, as well as with obstetric complications and risks for the newborn[41,42]. Additionally, in children with SLE disease severity correlates inversely with age at SLE onset[41,42]. Notably, onset of lupus during pregnancy or childhood is associated with increased disease activity and potentially more severe outcomes. Individuals with SLE onset in childhood and adolescence represent 15–20% of all individuals with SLE, and these patients have higher disease activity levels, more severe organ damage manifestations and a higher incidence of renal, cardiovascular and neuropsychiatric complications than individuals with adult-onset SLE[43]. Despite these facts, the study of delay in diagnosis in these populations remains limited.

Structural gaps

A variety of structural factors, including some health authorities neglecting to recognize SLE as a public health issue, have resulted in a lack of policy focused on allocating financial and material resources to specialized training for health professionals[13]. The participation of various health care providers in the early recognition of symptoms is a crucial contribution to a timely diagnosis[1]. General practitioners are frequently the primary point of contact for patients, but their inadequate training in how to identify signs and symptoms of SLE exacerbates the risk of misdiagnosis and delays referral to rheumatologists[33]. For example, an African survey revealed that many primary care physicians

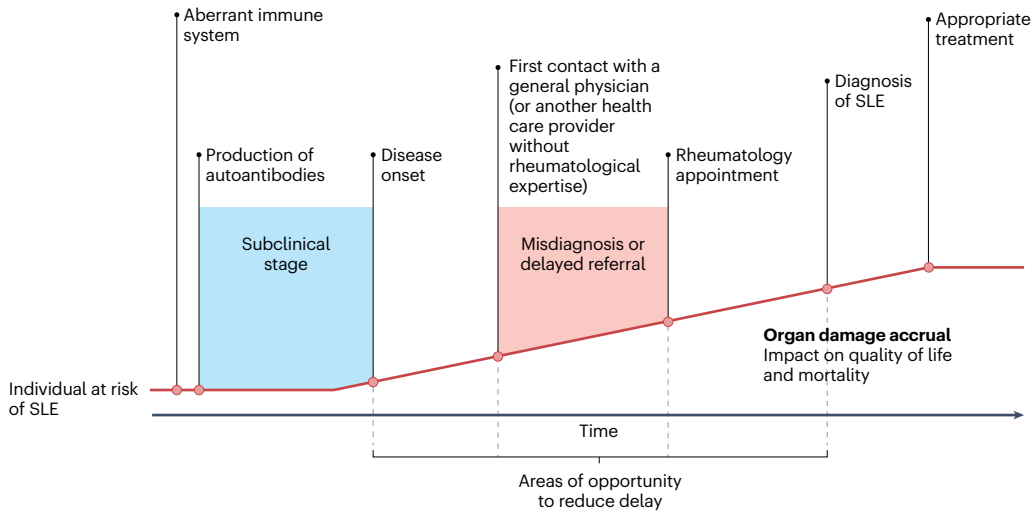

Fig. 1 | Delay in diagnosis and care of people with systemic lupus erythematosus. Individuals at risk of systemic lupus erythematosus (SLE) have an aberrant immune system that might be variably demonstrated via the production of autoantibodies during the subclinical stage of the disease. Disease onset does not always correlate with markers of the subclinical stage, and patients navigate across varied trajectories after the onset of symptoms. As these symptoms are often non-specific, first-contact health consultants, who are general physicians or medical professionals without a specialization in rheumatology, might misdiagnose the disease or delay referral to a rheumatologist. Disparity in access to both initial and specialized consultations also contributes to a delay in diagnosis, with serious consequences for the patient's quality of life.

had a limited understanding of SLE manifestations[44]. The misdiagnosis of SLE often delays appropriate treatments, as frequently documented in late-stage presentations of paediatric SLE in South Africa[45]. These barriers have similarly been documented in Latin America and Asia[46,47].

Even when diagnosis occurs relatively 'early', that is within 6 months after onset of symptoms or signs of disease, many countries still lack a sufficient number of certified adult rheumatologists to confirm the diagnosis and manage the disease. Several studies highlight the global shortage of rheumatologists and the need to improve access to rheumatology services, especially in low- and middle-income countries[48–51]. For example, in 2022, a total of 2,970 rheumatologists served 1.19 billion inhabitants in 44 countries in Africa, and these numbers represent a ratio of only 0.25 rheumatologists per 100,000 inhabitants[52]. Similarly low ratios of rheumatologists per number of inhabitants are seen in Asia, where we estimate a ratio of 0.17 rheumatologists per 100,000 inhabitants in China, 0.02 rheumatologists per 100,000 inhabitants in India and 0.84 rheumatologists per 100,000 inhabitants in South Korea[53]. In Latin America, ratios of rheumatologists range from 0.47 (Mexico) to 0.78 (Brazil) per 100,000 inhabitants[49]. Paediatric rheumatologists are even scarcer, as documented in South Africa. In 2021, fewer than 30 specialists were registered across the African continent[54]; this trend could possibly be extrapolated to the rest of Africa and the world[39]. Additionally, the frequently observed concentration of specialists in tertiary care or large urban areas contributes to health disparities[51,55]. This inadequacy of health care infrastructure is a substantial barrier in many countries. Limited access to diagnostic tools, including immunological assays such as antinuclear antibody (ANA) and anti-dsDNA testing, also impedes early detection of SLE[56].

Socio-economic factors

Socio-demographic characteristics, including culture, education, health literacy (specifically, awareness of SLE) and access to health care (either by social coverage or insurance) can determine the timing of SLE diagnosis[3]. Moreover, patients with limited economic resources often experience prolonged delays in accessing specialized care, and geographical barriers — such as long distances from health care centres — further complicate timely diagnosis and treatment[11,12,57,58]. Poverty and associated economic barriers disproportionately affect individuals with SLE compared with other rheumatic diseases. Diagnostic procedures, similar to long-term medication regimens, are financially prohibitive for many, particularly in rural areas. In South Africa, research demonstrated that over 40% of women with SLE discontinued care because of economic constraints[59].

The stigma associated with chronic illnesses in certain communities might discourage individuals from seeking timely medical care, thereby further delaying diagnosis and treatment[13]. Cultural beliefs and stigma might also discourage patients from seeking medical care. In some communities, chronic illnesses are perceived as spiritual afflictions attributed to witchcraft, which leads patients to prioritize traditional healers over biomedical interventions[60]. Women, especially in patriarchal societies, often deprioritize their health care needs owing to societal roles and financial dependency[61,62].

Paucity of research

The paucity of research and data on SLE in under-resourced settings contributes to delayed diagnosis, primarily owing to the limited availability of region-specific epidemiological information and the absence of diagnostic guidelines and treatment protocols that are tailored to health care systems. Most available clinical data originate from studies conducted in high-income countries and might not adequately capture the distinct genetic, environmental and socio-economic factors that influence SLE diagnosis and management in other populations. SLE is a complex disease, often with polygenic inheritance, involving over 100 common risk alleles that contribute to individual variation in disease susceptibility, making it challenging to create global risk models of SLE[63]. Moreover, environmental factors such as alcohol consumption,

Table 1 | Studies focused on the delay of diagnosis in SLE

Author	Geographic area (year)	Definition of diagnostic delay	Diagnosis delay (in months)	Factors associated with delay	Ref.
Petri et al.	USA (1991)	NA	Estimated: 26.4 months	NA	19
Nossent et al.	Antilles (1993)	NA	Reported: 18 (range 0 to 56 months)	NA	20
Ka et al.	Senegal (1998)	NA	The mean symptom duration of diagnosis was 24 months	Lack of diagnostic tools; SLE expertise among physicians	91
Arbuckle et al.	USA (2003)	NA	Reported: 19.2 months ± 33.6 months	African American men were diagnosed earlier than other patient groups owing to disease severity	21
Font et al.	Spain (2004)	NA	Estimated: 19.2 months	NA	22
Houman et al.	Tunisia (2004)	Diagnostic delay: from symptom onset until diagnosis	The delay of the diagnosis was on average 13 months (extremes of 1 and 72 months)	Misdiagnosis; lack of education; limited access to health care	92
Feng et al.	China (2010)	NA	Reported: 8.0 ± 14.75 months	Men had shorter diagnostic delays than women	23
Oglesby et al.	USA (2014)	Diagnosis lag time: interval between SLE onset date and the first SLE diagnosis date	Reported: early group (<6 months): 1.96 months ± 1.96; late group (>6 months): 9.4 ± 1.66 months	Early diagnosis of SLE is associated with improved clinical outcomes and reduced use of healthcare resources	24
Zomalheto et al.	Benin and West Africa (2014)	Diagnostic delay: from symptom onset until diagnosis	The mean delay before diagnosis was 15 months (1 to 84 months)	Misdiagnosis; challenges in serological profile assessment	93
Gonçalves et al.	Portugal (2015)	Diagnosis delay: time between first symptom/manifestation attributable to SLE and the date of clinical diagnosis established by the treating physician	Reported: patients without damage: 4.33 ± 9.7 months; patients with severe damage: 6.2 ± 11.0 months	Delay in diagnosis associated with severity of organ damage	25
Peñaranda-Parada et al.	Colombia (2015)	NA	Reported: late-onset SLE=16.08 months vs conventional SLE=13.56 months	NA	26
Jeleniewicz et al.	Poland (2015)	NA	Reported: late-onset SLE=31.7 months (range 0–144 months)	Late-onset SLE is associated with increased delays	27
Elfving et al.	Finland (2016)	NA	Reported: 6.0 months IQR (3.0, 16.0 months)	Lack of patient education; atypical disease symptoms	28
Stummvoll and Stamm	Austria (2017)	Early diagnosis: diagnosis of SLE within the first year of clinical manifestations; delayed diagnosis: diagnosis of SLE after 6 years of clinical manifestations	Reported: 41.5% of patients <12 months, 21.2% between 12 and 36 months, 9.3% between 36 and 72 months, 16.1% between 72 and 144 months, 11.9% >144 months	NA	29
Lewandowski et al.	South Africa (2017)	NA	Mean time to diagnosis was 23.5 weeks (5.8 months)	Financial status; access to transport; lack of patient education; social stigma	45
Morgan et al.	UK (2018)	NA	Reported: 76.8 ± 114) months	NA	30
Sebastiani et al.	Italy (2018)	Diagnostic delay: from symptom onset until diagnosis	Reported: 20 months	Two patterns of diagnostic delay: one associated with concomitant musculoskeletal and cardiorespiratory involvement; and one with concomitant mucocutaneous and haematological involvement	31
Wang et al.	China (2018)	Diagnosis delay from onset of symptoms to diagnosis	Reported: urban areas diagnosed 5 years earlier than rural areas (median 41 vs 46 years)	Earlier diagnosis in urban versus rural areas	32
Dey et al.	Ghana (2019)	Diagnosis delay from onset of symptoms to diagnosis	Reported: 21 to 31 months	Financial status; little to no disease awareness amongst health care workers; lack of diagnostic tools	94
Kernder et al.	Germany (2021)	Diagnosis delay of SLE: more than 6 months from symptom onset	Reported: 47.2 ± 72.6 months	Shorter diagnostic delays were associated with better outcomes (quality of life, disease-related damage, and disease activity)	33
Adelowo et al.	Africa (2021)	Delay before presentation to a rheumatologist	The delay before presentation to a rheumatologist was 15.6 months in Tunisia and 30 months in Nigeria	Misdiagnosis; lack of patient education; limited access to health care services and facilities	95

Table 1 (continued) | Studies focused on the delay of diagnosis in SLE

Author	Geographic area (year)	Definition of diagnostic delay	Diagnosis delay (in months)	Factors associated with delay	Ref.
Amissah-Arthur et al.	Ghana (2022)	Time to diagnosis, from onset of symptoms until diagnosis	The mean duration of symptoms until diagnosis was 17.5 months	Lack of patient education and disease stigma; gap in medical expertise; lack of access to health care	60
Kapsala et al.	Greece (2023)	Delayed diagnosis: diagnosis of SLE 6 months after symptom onset	Median (IQR): 12 (34) months in patients consulting 1 physician, to 60 (156) months	Diagnosis delay by >6 months in three-quarters of patients, leading to more damage accrual	34
Nieto et al.	Latin America (2024)	Time to diagnosis was defined as the time elapsing from when a patient met the first ACR criterion to SLE diagnosis	Time to diagnosis: 6.0 months	No apparent negative impact on disease outcome (damage accrual and mortality) for up to 24 months of diagnostic delay	35

IQR, interquartile range; NA, not available. Reference to 'estimated' diagnostic delay indicates that diagnostic delay was not described as a primary objective in the original study[46,47].

tobacco smoking, pollution, ultraviolet radiation and exposure to viral or bacterial infections contribute to variable incidence and prevalence of SLE worldwide[64,65].

In summary, a global shortage of trained health care professionals, structural deficiencies, socio-economic barriers to health care access and a paucity of research on the genetic and environmental factors affecting disease onset, progression and epidemiology, particularly in low-income countries, represent a substantial challenge to the early and accurate diagnosis of this complex disease[51,58].

Impact of diagnostic delays in systemic lupus erythematosus

Delay or early interruption of treatment

Delays in initiating appropriate treatment have profound consequences for patient outcomes, even though these consequences have been inadequately studied. Evidence shows that early initiation of specific therapies is crucial for controlling disease activity, preventing flares and minimizing irreversible organ damage. When treatment is delayed, patients are more likely to experience active disease, to rapidly accumulate organ damage and to require high doses of corticosteroids, which, in turn, increase the risk of long-term complications and comorbidities[66–68]. Early interruptions in targeted treatment, which are often caused by the same factors as those causing diagnostic delays, might also lead to disease flares, hospitalizations and increased use of health care resources[66,69,70].

Although current guidelines emphasize the importance of the early initiation of treatment, they often overlook structural barriers that affect both access to care and treatment continuity. Factors contributing to delays in diagnosis and treatment, as well as to treatment interruption, exist at multiple levels: the patient; the health care provider; and the health care system[71,72]. At the patient level, limited awareness of SLE, beliefs about health and disease, perception of symptom severity, socio-demographic characteristics, economic status, access to health insurance and adherence to treatment might substantially prolong the time to diagnosis and treatment initiation, or affect adherence to treatment. For health care providers, especially general practitioners and non-rheumatology specialists, insufficient training in rheumatological diseases contributes to delayed referrals, misdiagnosis and inappropriate management. Poor communication between the patient and the health care provider further impairs the patients' understanding of the chronic nature of SLE and the necessity of long-term, sometimes aggressive, treatments. Finally, structural deficiencies in health care systems, particularly in countries without universal health coverage or specific policies for early detection and treatment, create substantial barriers to timely and appropriate care[66,69,70].

Two key concepts should be incorporated into the training of health care professionals to enhance the timeliness and effectiveness of care, while also minimizing delays in diagnosis and treatment. First, the well-established principle of 'treat to target' (T2T)[73–75] involves four critical steps: establishing a relevant, individualized treatment goal; implementing strategies to achieve that goal; monitoring progress towards the goal; and adjusting therapy if the goal is not met. This approach emphasizes the importance of achieving remission or low disease activity as key milestones in the management of patients with SLE[73–75]. Achieving these goals has been associated with better outcomes in individuals with SLE and other chronic diseases[76–79]. An international consensus has emphasized the importance of early diagnosis and close follow-up for a T2T strategy in SLE that achieves low disease activity or remission[73–75]. However, in vulnerable populations, the feasibility and viability of implementing T2T is severely limited by the various barriers to accessing the health care system[80]. The second key concept related to delay in treatment and care is disease modification, which is defined as "minimizing disease activity with the least treatment-associated toxicity, while slowing or preventing

Box 1 | Proposed definition for delay in the diagnosis of SLE

The interval between the onset of systemic lupus erythematosus (SLE)-related manifestations and the establishment of a clinical diagnosis is defined as a delay in diagnosis when it adversely affects the patient's health trajectory. Diagnostic delays are considered clinically relevant when they are associated with negative outcomes in patient well-being, function and survival. Increased disease activity and frequency of flares, prolonged use of corticosteroids, often at increased doses, delayed initiation of immunomodulatory or disease-modifying therapies, reduced access to appropriate care, increased rates of hospitalization, poor health-related quality of life, accelerated accrual of irreversible organ damage and increased mortality are all outcomes of a delay in SLE diagnosis. By incorporating both a temporal dimension and the clinical consequences of diagnostic delay, this proposed definition has potential as a modifiable factor to improve long-term outcomes in SLE.

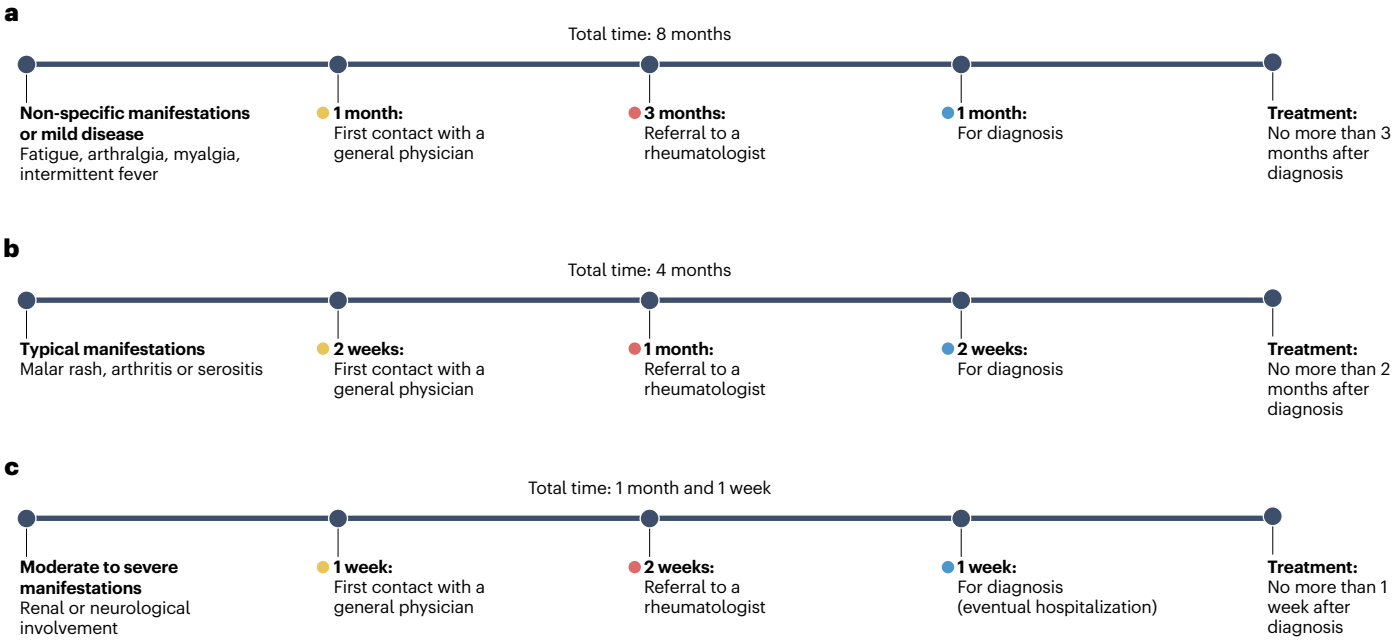

Fig. 2 | Hypothetical trajectories to a diagnosis of SLE and treatment initiation. Depending on the type and severity of the initial manifestations of systemic lupus erythematosus (SLE), there might be three ideal scenarios for the patient trajectory towards diagnosis and treatment initiation. **a**, If affected by non-specific manifestations or mild disease, such as fatigue, arthralgia, myalgia and intermittent fever, the patient would ideally contact a general physician within a month after symptom onset. The optimal time period until referral to a rheumatologist would be 3 months, and the rheumatologist would ideally be able to make a diagnosis within 1 month and initiate treatment no later than 3 months after diagnosis. **b**, The ideal trajectory of a patient with typical SLE manifestations (such as malar rash, arthritis or serositis) would be much shorter than in the first scenario, taking a total of 4 months until treatment initiation (2 weeks for the first visit to a general physician; 1 month for referral to a rheumatologist, 2 weeks for a diagnosis and 3 months for treatment initiation). **c**, In patients with moderate to severe manifestations with organ involvement, such as renal or neurological features, the total time from onset to treatment initiation would ideally be limited to just over 1 month (1 week until first contact with a general physician, 2 weeks for a referral to a rheumatologist, 1 week for diagnosis and eventual hospitalization and no longer than 1 week for treatment initiation). This model was constructed based on a qualitative study conducted in Mexico using focus groups with board-certified rheumatologists and patients[90]. The study emphasized that defining diagnostic delay in SLE requires accounting for disease severity, clinical heterogeneity, access to rheumatologists, risk of organ damage, treatment availability and institutional barriers.

the progression of organ damage in SLE"[81]. The incorporation of this concept into clinical practice focuses on altering the disease course, emphasizing individualized and comprehensive management for each patient.

Quality of life and mortality

A delayed diagnosis and treatment of SLE and the associated increases in disease activity, organ damage or risk of severe complications and comorbidities have a profound negative effect on patients' quality of life. Individuals having received a delayed diagnosis experience disabling symptoms such as chronic fatigue, persistent joint pain, cutaneous involvement, neuropsychiatric manifestations and mood disorders[66–68]. These manifestations substantially interfere with daily functioning, limit social interactions and impair the ability to participate in work, education and family life[67,68].

Delayed diagnosis has also been associated with an increased risk of death, particularly in health care settings characterized by limited access to rheumatologists or by the underutilization of appropriate immunosuppressive agents and emerging therapeutic options. Vulnerable populations, including socio-economically disadvantaged and minority ethnic groups, are disproportionately affected, often facing additional barriers such as geographic isolation and fragmented health care systems[33,39,82]. Delays in initiating effective treatment also frequently lead to prolonged reliance on high-dose corticosteroids, which are associated with substantial long-term adverse effects, including cardiovascular disease and infections. Consequently, the cumulative burden of untreated or undertreated disease and treatment-related morbidity further worsens overall prognosis[83–85]. Addressing barriers to timely diagnosis and early intervention in lupus is not only an imperative for advancing health equity but also a crucial strategy for improving patient- and patient-family-centred outcomes, reducing morbidity and mortality, decreasing the economic burden on health care systems and ultimately enhancing the overall quality of life for individuals living with lupus[80,86,87].

A global perspective on diagnostic delays

The tackling of diagnostic delays is an emerging topic in the context of SLE. Whereas efforts and policies have been implemented globally to reduce diagnostic delays in other rheumatic diseases that might be more prevalent than SLE, such as rheumatoid arthritis, the concept of delay in SLE diagnosis remains to be precisely defined[88]. Furthermore, there is a substantial lack of understanding of the barriers, facilitators and needs at local, population-specific and global levels. The available data on diagnostic delays in SLE vary substantially depending on

the cohorts studied, and, importantly, most of these data are derived from the so-called global north. Systematic reviews about diagnostic and treatment delay in SLE found that 76% of quantitative studies and 66.7% of qualitative or mixed-method studies about this topic come from the global north, although the most relevant factors associated with delay varied across regions[46,47]. As such, these findings might not fully capture the nuances of diagnostic delays in regions with different disease presentations and health care infrastructures. Table 1 presents a summary of the diverse concepts used to define diagnostic delay, with a particular focus on socio-demographic and geographic factors. A definition that considers a global health perspective of SLE would facilitate the development of screening strategies for early detection that are applicable across a variety of settings[3,4,64,89].

A call to action

This Perspective constitutes a call to action for stakeholders to recognize diagnostic and treatment delays as crucial and unmet needs in the care of patients with SLE. It is increasingly urgent for international organizations and rheumatology specialists to establish a consensus definition of diagnostic delay in SLE, a definition that integrates the perspectives of both the patient viewpoint, including genetic, socio-demographic and cultural factors, as well as support networks, and the health care professionals and systems[10,13,36,37] (Box 1). Moreover, a definition of diagnostic delay in SLE must account for disease heterogeneity, as well as for variations in genetic, socio-cultural, and health care contexts. Disparities in health care access, especially in low- and middle-income countries, including limited universal health care coverage, inadequate SLE education in medical curricula, workforce shortages and insufficient infrastructure and resources, render the integration of the health care context into a definition of diagnostic delay in SLE particularly relevant. Marginalized populations, who not only experience more severe manifestations of SLE than the general population but also face substantial health care inequities, require special attention.

Given the complexity of SLE, a comprehensive definition of diagnostic and treatment delay must be adaptable to the specific realities of each country or region, health care system and patient population. The definition of diagnostic and therapeutic delay in SLE should take into account the clinical severity at presentation and the urgency of the required intervention. Based on consensus definitions derived from qualitative interviews with rheumatologists and patients in Mexico[90], three prototypical clinical scenarios may be outlined. Each scenario is characterized by distinct target timeframes for both diagnosis and initiation of therapy, helping to guide clinical decision making and system-level interventions aimed at reducing delay.

In the first scenario, patients present with non-specific manifestations or mild disease, such as fatigue, photosensitivity, arthralgia, or transient rash, that do not immediately trigger suspicion of an autoimmune disease. In such cases, the diagnostic process is prolonged owing to the subtle and heterogeneous nature of the initial clinical manifestations. Ideally, the total duration from symptom onset to diagnosis should be limited to 5 months, and effective pharmacological treatment should commence no later than 3 months following diagnosis.

The second scenario involves patients who present with more characteristic SLE manifestations, such as persistent arthritis, serositis, mucocutaneous lesions, serological abnormalities or cytopenias. These features should enable faster clinical recognition and referral compared with the first scenario. In this context, the time to diagnosis should ideally be no longer than 2 months from symptom onset, and treatment should commence within 2 months of establishing the diagnosis. These parameters are aimed at balancing the time required for a thorough diagnostic work-up with the need for timely therapeutic intervention to prevent organ involvement or systemic complications.

In the third scenario, patients present with severe or organ-threatening manifestations at onset, such as lupus nephritis, neuropsychiatric manifestations, or severe haematological abnormalities. In these high-risk settings, rapid action is crucial. The diagnosis should be established within a maximum of 4 weeks from the initial presentation, and treatment should commence no later than 1 week after the diagnosis is confirmed. Any delay in this context represents a serious threat to patient safety and prognosis, underscoring the need for early recognition at the primary care or emergency level and immediate referral to specialized care.

These scenario-based definitions of diagnostic and treatment delay offer a pragmatic framework that aligns clinical urgency with expected timelines of care (Fig. 2).

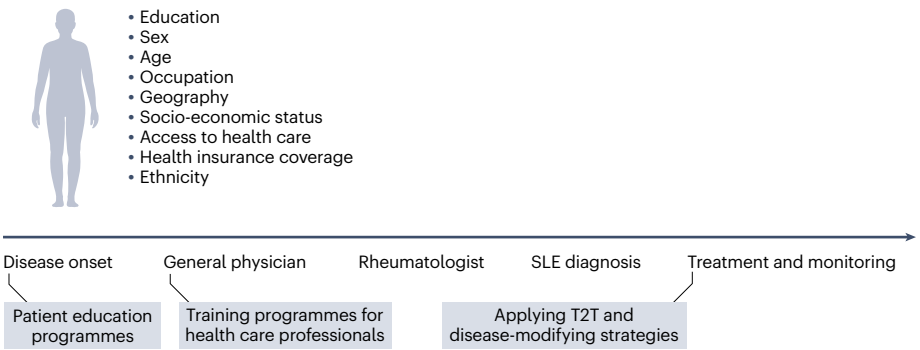

Fig. 3 | Intervention points to improve the patient journey towards the diagnosis of SLE and initiation of treatment. The journey of a patient with systemic lupus erythematosus (SLE) from initial symptom onset to SLE diagnosis and long-term control and monitoring includes several transition points in care, ranging from the first consultation with a general physician and a rheumatology referral to a diagnosis by a rheumatologist and follow-up within a specialized health care setting. Systemic barriers across this trajectory include low health literacy, disparities related to socio-economic status and ethnicity, limited access to care and gaps in health insurance coverage. To improve this journey, three strategic interventions are proposed: first, patient education programmes to foster early symptom recognition and help-seeking behaviour; second, training for primary care professionals to enhance timely referral; and third, the application of treat-to-target (T2T) strategies and disease-modifying therapies during monitoring. This patient-centred framework is aimed at shortening the diagnostic journey, reducing inequities and improving long-term care for individuals with SLE.

Although these definitions might have limited generalizability, as they have been based on the Mexican population, this study demonstrates the feasibility of developing context-specific definitions of diagnostic delay that are both locally grounded and globally informative[82].

Additionally, recognizing and reducing diagnostic delay is a key clinical strategy to lower disease activity and prevent or delay organ damage[81]. Informed by previous research that identified actionable gaps at the patient, provider and health-system levels, we propose a strategy to improve access to specialized care (Fig. 3) This strategy addresses factors contributing to misdiagnosis or delayed recognition[46,47]. Patient education programmes, training programmes for medical students and health care professionals, as well as the inclusion of SLE as a public health priority for resource allocation and campaigns, are key aspects of such a strategy[46,47,90].

Conclusions

Diagnostic delays in SLE contribute substantially to disease progression, irreversible organ damage and long-term complications. Addressing this issue requires the establishment of a unified definition of diagnostic delay that incorporates both clinical and socio-economic dimensions. Although health care systems and access to care undoubtedly differ across regions, a global effort to define and standardize what constitutes a clinically meaningful delay is essential. Such a definition would enable consistent measurement across settings, facilitate international comparisons and guide targeted interventions. Importantly, insights from under-represented regions, often characterized by pronounced health disparities, might offer valuable lessons applicable to other areas facing similar challenges. A coordinated global approach will be key to improving early detection, reducing inequities and ultimately enhancing outcomes and quality of life for all individuals with SLE.

The Global Health approach provides a valuable tool for tracking health inequities over time, ensuring that interventions align with the specific needs of each country. Future efforts will focus on using standardized definitions, identifying barriers, facilitators and needs, and co-designing strategies with authorities to address both medical and structural determinants, thereby effectively reducing the delay in diagnosis and treatment burden in SLE.

Published online: 21 July 2025

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Acknowledgements
We would like to thank A. Manrique de Lara for her critical reading, comments and editing. We also thank L. Grasso for the design and illustration of the graphics.

Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
The authors declare that they have no competing interests related to the research, authorship and/or publication of this article.

Additional information
Peer review information *Nature Reviews Rheumatology* thanks Amita Aggarwal, Anisur Rahman and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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