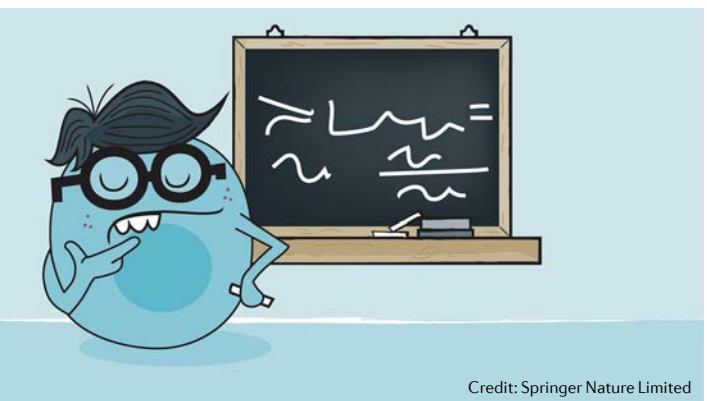
THERAPY

Implanted ‘smart’ cells release biologic drugs on demand

> “Mice implanted with the IL-1Ra-producing constructs had … lower clinical arthritis scores and less pain sensitivity”

CRISPR–Cas9 genome engineering of induced pluripotent stem cells (iPSCs) has enabled researchers to produce ‘smart’ cells that can sense inflammation and respond proportionally by producing anti-inflammatory biologic drugs. In a new study, these smart cells were engineered into small implants that, when placed subcutaneously in mice with experimental arthritis, ameliorated disease via autoregulated delivery of IL-1 receptor antagonist (IL-1Ra). The approach could provide an effective way to treat a range of diseases, such as rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA), while mitigating the risks associated with continuous administration of immunosuppressive drugs.

“One of the biggest issues facing patients with inflammatory arthritis such as RA or JIA is that biologic drugs are given continuously at high concentrations, greatly increasing the risk for adverse events such as infection,” explains co-author Christine Pham. “We hypothesized that a method for delivering biologic drugs automatically could provide a more effective treatment while minimizing the risk of adverse events.”



Credit: Springer Nature Limited

The engineered iPSCs incorporate a synthetic gene circuit that expresses IL-1Ra, an inhibitor of IL-1, in response to activation by inflammatory mediators. A stable cell-based implant was created by seeding the cells onto a porous 3D woven scaffold, after which they differentiated into chondrocyte-like cells and produced a cartilaginous matrix within the scaffold. In culture, these constructs responded to stimulation with IL-1α by producing IL-1Ra, and they decreased IL-1Ra production when IL-1α stimulation was withdrawn. Similarly, constructs containing iPSCs engineered to produce soluble TNF receptor 1 responded dynamically to TNF stimulation.

The IL-1Ra-producing constructs were then evaluated in the K/B × N serum-transfer model of arthritis. “We were excited to find that the implants could survive for months in vivo, and responded very rapidly to flares of inflammation by producing IL-1Ra,” corresponding author Farshid Guilak reports. Notably, the implants could be repeatedly reactivated by serum transfer.

Mice implanted with the IL-1Ra-producing constructs had less swelling, lower clinical arthritis scores and less pain sensitivity than mice that received non-functional scaffolds or no treatment. The reduction in arthritis severity was accompanied by changes in cytokine profiles, with decreased serum concentrations of IL-1α and IL-6, increased serum concentration of IL-10 and reduced concentrations of IL-1α, IL-6, TNF and other pro-inflammatory cytokines in the paw lysate of mice treated with the IL-1Ra-producing constructs.

“One exciting finding of our study was that the implant completely prevented bone erosions, which no other drug treatments have been able to do,” highlights co-first author Kelsey Collins.

In the K/B × N serum-transfer model, the constructs outperformed methotrexate and tofacitinib, neither of which significantly mitigated arthritis. Interestingly, treatment with the bioengineered constructs and with twice daily injections of anakinra (recombinant human IL-1Ra) resulted in similar circulating concentrations of IL-1Ra, but only the former reduced arthritis scores after serum transfer.

The researchers are now developing implants that deliver different anti-inflammatory drugs, which could be used to treat a variety of inflammatory diseases. They are also exploring the potential to build in genetic ‘switches’ that would enable drug production by the implants to be turned on and off, for example if a patient needed to discontinue treatment before surgery or in case of infection.

“We are very excited about the possibility of changing the way that patients with RA or JIA are being treated. By creating ‘smart’ cells that can sense different inflammatory molecules, we hope to create a completely different approach where the cells automatically create the right biologic drugs, at the right amount, and the right time to enable the most effective therapy for patients with arthritis,” concludes Guilak.

Sarah Onuora

ORIGINAL ARTICLE Choi, Y. R. et al. A genome-engineered bioartificial implant for autoregulated anticytokine drug delivery. *Sci. Adv.* **7**, eabj1414 (2021)
RELATED ARTICLE Apparailly, F. Breaking Prometheus’s curse for cartilage regeneration. *Nat. Rev. Rheumatol.* **13**, 516–518 (2017)

IN BRIEF

PSORIATIC ARTHRITIS

Secukinumab reduces synovitis in PsA

IL-17A inhibition with secukinumab rapidly reduced synovitis as well as signs and symptoms of psoriatic arthritis (PsA) in the phase III, placebo-controlled ULTIMATE study. Patients in the study had active PsA despite treatment with conventional synthetic DMARDs and were naive to biologic DMARDs. Mean change from baseline to week 12 in Global EULAR-OMERACT Synovitis Score was greater in patients treated with secukinumab than in those who received placebo (-9 versus -6; $P = 0.004$), with the difference between the groups apparent as soon as 1 week after the start of treatment.

ORIGINAL ARTICLE D'Agostino, M. A. et al. Response to secukinumab on synovitis using power Doppler ultrasound in psoriatic arthritis: 12-week results from a phase III study, ULTIMATE. *Rheumatology* https://doi.org/10.1093/rheumatology/keab628 (2021)

THERAPY

Rituximab shows potential for treatment of PMR

Results of the proof-of-concept BRIDGE-PMR trial suggest the B cell depleting agent rituximab could have steroid-sparing effects in polymyalgia rheumatica (PMR). Among patients who received a single intravenous infusion of rituximab (1,000 mg), 11 (48%) of 23 were able to achieve glucocorticoid-free remission at 21 weeks, compared with five (21%) of 24 patients who received placebo ($P = 0.049$). The effects of rituximab seemed to be more pronounced in patients with newly diagnosed disease than in those whose PMR had relapsed on glucocorticoid therapy.

ORIGINAL ARTICLE Marsman, D. E. et al. Efficacy of rituximab in patients with polymyalgia rheumatica: a double-blind, randomised, placebo-controlled, proof-of-concept trial. *Lancet Rheumatol.* https://doi.org/10.1016/S2665-9913(21)00245-9 (2021)

MYOSITIS

Profiling comorbidities of inclusion body myositis

In a population-based, case–control study using medical records from the expanded Rochester Epidemiology Project, patients with inclusion body myositis (IBM; $n = 50$) were 2.7 times more likely to have peripheral neuropathy, 3.9 times more likely to have a haematologic malignancy and 6.2 times more likely to have Sjögren syndrome than individuals in the general population ($n = 294$). IBM was also associated with increased mortality, with 10-year survival rates of 36% compared with 59% in the general population and 67% in patients with other inflammatory myopathies.

ORIGINAL ARTICLE Naddaf, E. et al. Survival and associated comorbidities in inclusion body myositis. *Rheumatology* https://doi.org/10.1093/rheumatology/keab716 (2021)

GENETICS

New OA risk factors and drug targets revealed

A multi-cohort genome-wide association study meta-analysis, involving 177,517 cases of osteoarthritis (OA) and 649,173 controls among individuals of European and East Asian descent, identified 100 OA-associated variants, 52 of which had not been previously reported. Some of the reported variants were associated with specific OA phenotypes (for example, thumb or spine OA) and others overlapped across phenotypes, including OA in both weight-bearing and non-weight bearing joints; notably, some were linked with pain-related phenotypes. Integrated functional genomics analyses from OA-relevant tissues identified putative effector genes, many of which could be targets for therapeutic intervention.

ORIGINAL ARTICLE Boer, C. G. et al. Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. *Cell* https://doi.org/10.1016/j.cell.2021.07.038 (2021)

OSTEOARTHRITIS

Nasal chondrocytes enable cartilage repair in OA joints

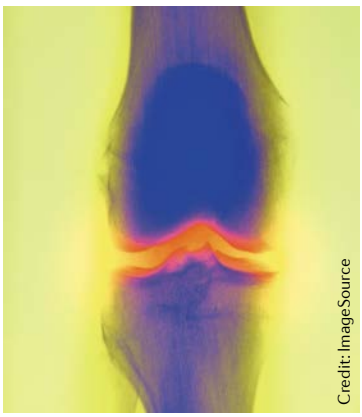
A new study shows that nasal chondrocyte-based tissue-engineered cartilage (N-TEC) is stable in inflammatory environments. Preliminary clinical results demonstrate the potential for N-TEC grafting to be developed as a safe and effective alternative to knee arthroplasty in defined osteoarthritis (OA) conditions.

Joint degeneration in OA causes pain and disability, and end-stage disease necessitates total joint replacement. "For patients with OA no disease-modifying or regenerative treatments are yet available," says corresponding author Ivan Martin, explaining the motivation behind the study.

Previous results showed that nasal septum chondrocytes have greater capacity to generate functional cartilaginous tissues than articular chondrocytes, and that N-TEC contributes to repair of non-OA knee defects in animal models. In the current study, N-TEC maintained cartilaginous properties on exposure in vitro to pro-inflammatory cytokines associated with OA, including TNF, IL-1β and IL-6.

Compared with articular chondrocytes, gene expression in nasal chondrocytes favoured anti-inflammatory and biosynthetic activity, with downregulation of WNT signalling. Notably, conditioned media from N-TEC caused reduction of expression of pro-inflammatory cytokines and matrix metalloproteinase 13 in articular chondrocytes from OA joints.

The performance of N-TEC was evaluated in animal models. Human osteochondral constructs were generated in vitro by combining N-TEC with bone-like tissues derived from culture of OA osteoblasts. The constructs were ectopically implanted into nude mice, and after 8 weeks they had developed into avascular cartilaginous tissue that was integrated with the underlying bone. N-TEC was also placed in cartilage lesions that were generated in vitro in osteochondral explants from human OA knee joints. The constructs were implanted into nude mice, and after 8 weeks the N-TEC had integrated with the OA cartilage and bone.



Credit: ImageSource

The ability of N-TEC to restore cartilage defects was further tested in sheep. Knee OA was induced by generating full-thickness cartilage defects, resulting in elevation of synovial fluid volumes and concentrations of pro-inflammatory cytokines. Following implantation of autologous N-TEC, these effects were reversed.

Extending the model to human disease, autologous N-TEC grafts were evaluated in two patients with advanced OA, resulting in improvements in pain, knee-joint function and quality of life, with no adverse reactions, 14 months after surgery. According to Martin, the next steps will be to initiate controlled, suitably powered trials to extend the treatment to other joints, and to further investigate the mechanism underlying the action of N-TEC.

Robert Phillips

ORIGINAL ARTICLE Rua, L. A. et al. Engineered nasal cartilage for the repair of osteoarthritic knee cartilage defects. *Sci. Transl Med.* https://doi.org/10.1126/scitranslmed.aaz4499 (2021)

OSTEOARTHRITIS

Targeting articular *Mmp13* in OA

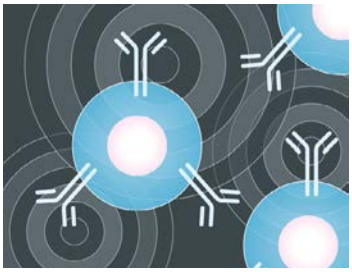
Pharmaceutical management of osteoarthritis (OA) is currently limited to pain relief with agents such as glucocorticoids, which might have negative effects on disease progression if used in the long term. No approved disease-modifying OA drugs (DMOADs) are yet available. Results of a study published in *Nature Biomedical Engineering* have now shown that targeted nanoparticles that deliver therapeutic RNA silencing specifically to articular cartilage can protect cartilage integrity and joint structure in a mouse model of post-traumatic OA (PTOA).

Joint injury predisposes to PTOA, inducing a degenerative cycle of inflammation and degradation of cartilage by proteinases such as matrix metalloproteinases (MMPs). Unfortunately, nonselective, systemic targeting of MMPs can cause pain and nephrotoxicity. By contrast, intra-articular injection has the potential for localized treatment, but the high rate of drug clearance from synovial fluid currently limits its usefulness.

In the new study, a monoclonal antibody to type II collagen (anti-CII) was attached to the surface of nanoparticles that contained small interfering RNA targeting *Mmp13*. Intra-articular injection of the nanoparticles into mice with PTOA induced by repetitive knee-joint loading resulted in greater nanoparticle retention than in uninjured joints, because injury exposed the articular type II collagen. The anti-CII antibody-conjugated nanoparticles reduced articular expression of *Mmp13* compared with antibody-free nanoparticles.

In mice with PTOA, single or weekly intra-articular treatments over 6 weeks with the glucocorticoid methylprednisolone, a current standard therapy for OA, did not affect joint structural degradation. By contrast, nanoparticle-based *Mmp13* silencing prevented PTOA-associated cartilage proteoglycan loss, synovial hyperplasia, osteophyte formation and meniscal mineralization, and suppressed expression of genes involved in OA progression.

> “… nanoparticle-based *Mmp13* silencing prevented PTOA-associated cartilage proteoglycan loss …”



Credit: S.Harris/ Springer Nature Limited

These results demonstrate that selective targeting of *Mmp13* following joint injury can prevent cartilage degradation and its associated downstream pathological changes. According to Craig Duvall, the corresponding author of the study, “this approach has the potential to be developed as the first clinically available DMOAD.”

Robert Phillips

ORIGINAL ARTICLE Bedingfield, S. K. et al. Amelioration of post-traumatic osteoarthritis via nanoparticle depots delivering small interfering RNA to damaged cartilage. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-021-00780-3 (2021)

RHEUMATOID ARTHRITIS

NK cells induce a pro-inflammatory phenotype in RA synovial fibroblasts

In rheumatoid arthritis (RA), distinct pro-inflammatory cell subsets are thought to be mediators of pathogenesis. One such subset consists of synovial fibroblasts that are characterized by expression of CD90 (also known as Thy-1) and HLA-DR. The results of a study of this cell population, which is greatly expanded in active RA, suggest that these pro-inflammatory, IL-6-secreting cells are activated by IFNγ derived from synovial natural killer (NK) cells.

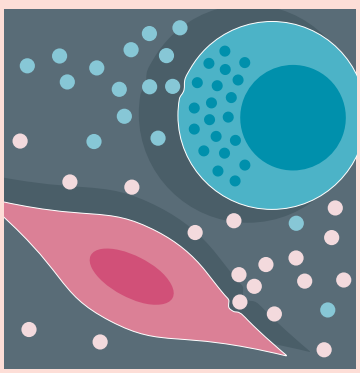
Several alleles of *HLA-DRB1* have been linked to seropositive RA, and could have roles in the formation of autoantibodies via antigen presentation by synovial fibroblasts. In the new study, the researchers found that HLA-DR expression could be induced by treatment with IFNγ in human fibroblasts derived from arthritic synovia or from healthy or sclerotic skin.

One source of IFNγ is activated NK cells, which were found in synovial fluid from patients with RA. The presence of immune complexes, which are recognized by the low affinity Fcγ receptor IIIa, can contribute to the activation of NK cells. Notably, in tissue samples from patients with RA or osteoarthritis, HLA-DR expression on synovial fibroblasts correlated with the percentage of activated synovial NK cells.

> “HLA-DR expression on synovial fibroblasts correlated with the percentage of activated synovial NK cells”

“The role of synovial immune complexes in activating resident Fc-receptor-bearing cells like NK cells is presently underexplored, and these results further our understanding of the initiation and perpetuation of autoimmunity in RA,” says the study’s corresponding author Wolfgang Merkt.

In vitro, culture of synovial fibroblasts with activated NK cells or with recombinant IFNγ induced HLA-DR expression, production of IL-6 and the ability to activate T cells. IFNγ signals via Janus kinases (JAKs), and JAK1 and JAK2 were both expressed by synovial fibroblasts, and particularly by the HLA-DR$^+$CD90$^+$ subset. In synovial fibroblasts stimulated with NK cell supernatants, the JAK1 inhibitor upadacitinib blocked induction of HLA-DR expression.



Credit: S.Harris/Springer Nature Limited

“The inducibility of inflammatory HLA-DR$^+$CD90$^+$ synovial fibroblasts is a targetable feature, potentially enabling fibroblast-centred therapeutic strategies in the future,” suggests Merkt.

Robert Phillips

ORIGINAL ARTICLE Zhao, S. et al. JAK inhibition prevents the induction of pro-inflammatory HLA-DR$^+$CD90$^+$ RA synovial fibroblasts by IFNγ. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41958 (2021)

RHEUMATOID ARTHRITIS

Stromal cells implicated in RA genetic risk

Genome-wide association studies in rheumatoid arthritis (RA) have identified more than 100 genetic risk loci, some of which are associated with genes related to immune functions, but many of which reside in noncoding regions. To date, functional genomics studies have mostly focused on unravelling the role of these noncoding variants in immune cells. However, as understanding of RA pathogenesis has advanced, more attention has been given to stromal cells such as synovial fibroblasts (also known as FLS), and the results of a new study now suggest that up to 24% of the genetic risk of RA could actually be attributed to synovial fibroblasts.

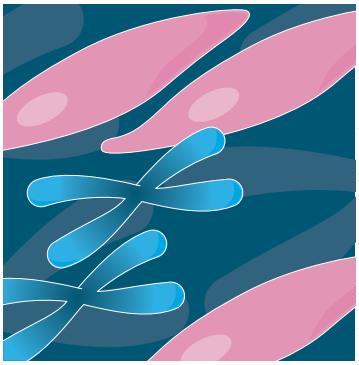
"The question has always been whether synovial fibroblasts are actually causal in the development of RA or whether they are just responding to an external initiating stimulus," explains corresponding author Caroline Ospelt. "Researchers in the fibroblast field, of course, always propagated the first theory and argued that the gap in treatment response that we see with immunosuppressive therapies in RA is based on this intrinsic fibroblast activation. However, that was difficult to prove. With our data, we can now for the first time show a causal relationship between stromal cell activation in the joint and the development of RA."

The researchers used several techniques, including ChIP-seq, ATAC-seq and capture HiC, to analyse the genomic and epigenomic landscape of synovial fibroblasts and to fine-map RA-associated risk loci. Furthermore, by comparing their data on synovial fibroblasts with data on immune cell subsets, the research team were able to assign signals to specific cell types.

Using this approach, the researchers were able to identify chromatin interactions that link RA risk variants with receptors for type I, II and III interferons, suggesting an important role for stromal cell interferon signalling in RA. Notable additional findings include confirmation of a vital role for the transcription factor AP-1 in gene expression changes after stimulation with TNF and a potential repressive role for developmental transcription factors in distal joints.

> "up to 24% of the genetic risk of RA could actually be attributed to synovial fibroblasts"

"The genes and signalling pathways affected by genetic risk factors in synovial fibroblasts in RA are excellent candidates for the development of therapies that modulate stromal cell activation in RA," states Ospelt. She suggests that the development of such therapies could be helpful for patients with RA who do not respond well to current therapies.



Credit: S.Harris/Springer Nature Limited

Joanna Clarke

ORIGINAL ARTICLE Ge, X. et al. Functional genomics atlas of synovial fibroblasts defining rheumatoid arthritis heritability. *Genome Biol.* **22**, 247 (2021)

INFLAMMATION

IRAK4 inhibitor attenuates inflammation

Inhibition of IRAK4, a kinase involved in innate immune signalling, blocks pro-inflammatory cytokine production in humans and mice and thus represents a promising therapeutic approach in rheumatic diseases. A new study shows that the IRAK4 small-molecule inhibitor PF-06650833 reduces inflammation in rodent models of rheumatoid arthritis (RA) and lupus and basal inflammation in healthy people.

The researchers assessed the effects of PF-06650833 on RA pathogenetic processes in vitro. PF-06650833 reduced anti-citrullinated protein antibody (ACPA)-induced TNF release from macrophages, pro-inflammatory cytokine and matrix metalloproteinase release by RA synovial fibroblasts (also known as FLS), and innate immune responses in various human primary cells (assessed by phenotypic profiling). Importantly, PF-06650833 also blocked RA pathogenesis in vivo, reducing inflammation in rats with collagen-induced arthritis (CIA).

> Inhibition of IRAK4 … represents a promising therapeutic approach in rheumatic diseases

Next, the effects of PF-06650833 on systemic lupus erythematosus (SLE) pathophysiological processes were tested in vitro and in vivo. PF-06650833 blocked neutrophil NETosis (a response to immune complexes in SLE) and the maturation of plasmacytoid dendritic cells in response to this process. PF-06650833 also blocked pro-inflammatory cytokine release by peripheral blood mononuclear cells and B cells, as well as B cell maturation.

This ability to inhibit SLE pathogenetic processes in vitro was confirmed in vivo in two mouse models of lupus. PF-06650833 administration markedly decreased anti-nuclear autoantibody titre in mice with pristane-induced lupus and kidney inflammation in MRL/lpr mice, supporting the efficacy of PF-06650833 in ameliorating SLE pathogenesis.

The authors then assessed the effects of PF-06650833 on inflammation markers in a phase I trial. "In healthy human volunteers, the basal tone of inflammation (interferon signature genes and circulating C-reactive protein (CRP) concentration) is dependent on IRAK4 kinase activity, highlighting IRAK4 as a central node of human inflammatory signalling, even in the absence of an autoimmune disease diagnosis," explains lead author Aaron Winkler.

"Our work provides confidence in the rationale and pharmacology for advancing PF-06650833 into phase II studies in RA," concludes Winkler.

Credit: S.Harris/Springer Nature Limited

Grant Otto

ORIGINAL ARTICLE Winkler, A. et al. The IRAK4 kinase inhibitor PF-06650833 blocks inflammation in preclinical models of rheumatologic disease and in humans enrolled in a randomized clinical trial. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41953 (2021)

Credit: Jeffrey Coolidge/Stone

SYSTEMIC LUPUS ERYTHEMATOSUS

Are DNA–HLA class II interactions the missing link in SLE?

David S. Pisetsky

New evidence has emerged that DNA can bind to cell surface HLA class II molecules. If true, this surprising interaction could lead to T cell and B cell activation by DNA, and surface DNA could also provide a target for cell killing by anti-DNA antibodies in systemic lupus erythematosus.

Refers to Tsuji, H. et al. Anti-dsDNA antibodies recognize DNA presented on HLA class II molecules of systemic lupus erythematosus risk alleles. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41897 (2021).

DNA is a large polymeric macromolecule that has powerful immunological activities related to its base sequence, backbone structure and context. Systemic lupus erythematosus (SLE) has been a major focus in the study of these activities given that anti-DNA antibodies are the serological hallmark of this disease as well as being markers for diagnosis, classification and disease activity[1]. Although anti-DNA antibodies have features of antigen selection (such as clonal expression and variable region somatic mutations), the nature of T cell help for this response has been elusive, as has been any putative interaction between DNA and HLA class II molecules on antigen-presenting cells. A new study by Tsuji et al. suggests that DNA might bind directly to HLA class II molecules, providing new ideas for the immune recognition of DNA by B cells and T cells in the pathogenesis of SLE[2].

In contrast to the dearth of knowledge about interactions with DNA, a whole treasure trove of information is available about the interaction of protein antigens with HLA class II molecules. In the immune response to proteins, peptides derived from proteins by enzymatic cleavage in an endosomal compartment bind to the groove of the HLA class II molecules for presentation to T cells. These interactions are allele specific. Indeed, algorithms that predict the extent of peptide binding can help in the development of vaccines to prevent infection or treat malignancy[3,4].

> “these findings … could lead to a re-imagining of the origin of the anti-DNA antibody response in SLE”

Although it is usually peptides that bind to HLA class II molecules, intriguing observations indicate that misfolded proteins, which are ordinarily degraded, can also interact with HLA class II molecules and be presented on the cell surface; these interactions can occur without the cleavage reactions that produce peptides to fit into the antigen-binding groove[5]. Importantly, complexes of the proteins with HLA class II molecules can serve as autoantigens, perhaps explaining the association of particular HLA alleles with autoimmune diseases. Autoantigens showing this pattern of HLA class II association include IgG, β_2-glycoprotein I and myeloperoxidase[6–8].

In their provocative paper, Tsuji et al. extend the paradigm established with misfolded proteins and demonstrate that DNA can bind to HLA class II molecules and be expressed on the cell surface[2]. Briefly, in this study, the investigators incubated cell lines that had been transfected to express HLA class II molecules with DNA and, using a monoclonal anti-DNA antibody, showed binding of DNA on the cell surface that was dependent on the presence of certain HLA class II molecules. Uptake by endocytosis was required for this binding because it occurred at 37 °C but not at 4 °C; the process of endocytosis depends on temperature and, therefore, does not occur at 4 °C, although binding can. Furthermore, the binding groove of the HLA class II molecules had to be open because occupancy by a peptide prevented DNA binding. The relevance of this binding to the induction of autoimmunity was demonstrated by the stimulation of cytokine production by a B cell line bearing a surface immunoglobulin receptor for DNA to allow specific binding.

Most impressively in this study, the binding of DNA to HLA class II molecules was allele specific[2]. The highest level of DNA surface expression occurred with HLA class II molecules encoded by alleles associated with SLE risk (such as HLA-DRB1*15:01, HLA-DRB1*04:01 and HLA-DRB1*09:01). In this regard, although this model system involved a B cell line, it is certainly possible that the same type of stimulation could occur with T cells, assuming that a T cell receptor that recognizes DNA is possible. The implications of these findings are profound and could lead to a re-imagining of the origin of the anti-DNA antibody response in SLE; such re-imagining could also lead to new ideas for therapy.

Two questions arise immediately: do these interactions occur in vivo and what are the functional consequences? The experimental system used by Tsuji et al. involved cells transfected to express HLA class II molecules that were incubated with high concentrations of DNA, either genomic in origin or a synthetic oligonucleotide[2]. In the experiments reported, the concentration of DNA was as high as 50 μg/ml. By contrast, the amount of DNA in the circulation is generally estimated to be in the range of 1–100 ng/ml, depending on the assay that is used and the clinical setting[9]. However, the concentration of DNA at sites of inflammation could be very high as a result of neutrophil extracellular trap production and capable of inducing this type of response. Notably, the presence of surface DNA expression required incubation with an exogenous source of DNA, suggesting that endogenous DNA is insufficient for HLA class II binding, at least under the conditions used by Tsuji et al.

An intriguing aspect of this study concerns the analogy with misfolded proteins. Proteins and DNA differ markedly in structure, and DNA is highly charged, so why the same type of interactions occur with HLA class II molecules is both fascinating and perplexing. Furthermore, it is not clear whether DNA can also be 'misfolded', although this could involve a transition to a single-stranded structure. Given the very extensive studies on how peptides bind to HLA class II molecules, the binding of both intact proteins and intact DNA to HLA class II molecules is surprising; however, the binding groove is open on HLA class II molecules so it could theoretically accommodate molecules larger than peptides.

Studies on the binding of misfolded proteins to HLA class II molecules have provided evidence that these interactions can occur in vivo; for example, in one study, IgG and HLA class II molecules were shown to interact in synovial tissue from a patient with rheumatoid arthritis using a proximity ligation assay[6]. Another study demonstrated that neutrophils from a patient with vasculitis have surface expression of myeloperoxidase that is associated with HLA class II molecules[8]. Future studies will hopefully show whether DNA–HLA class II interactions occur in SLE, although, at present, the best place to look is a matter of speculation. An examination of neutrophils should certainly be considered as these cells are implicated in the pathogenesis of SLE and are easy to obtain.

The second important question relates to the functional consequences of the binding of DNA to HLA class II molecules. Such an interaction could provide a novel autoantigen structure for antibody-dependent or complement-mediated cell cytotoxicity. Such cytotoxicity could represent another mechanism by which anti-DNA antibodies can promote pathogenesis, although the clinical consequences would depend on the cell types that express surface DNA–HLA class II complexes. DNA bound to HLA class II molecules could also lead to T cell recognition and promote autoreactivity. Interestingly, a study from almost 25 years ago also showed an interaction between DNA and HLA class II molecules[10]. That study suggested that DNA bound to HLA class II molecules could prevent peptide from being loaded into the groove and, therefore, limit the induction of immune responses to proteins. Perhaps such binding redirects the immune response to nucleic acids and away from proteins.

The recognition that DNA can interact with HLA class II molecules adds another dimension to the immune properties of nucleic acids. Although these interactions are unexpected, they might be crucial for the induction for autoimmunity and provide a long-missing link to explain the expression of anti-DNA antibodies in SLE and their role in immunopathogenesis.

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Competing interests
The author declares no competing interests.

VASCULITIS

GCA management guidelines — vive la différence?

Bernhard Hellmich and Frank Buttgereit

The ACR have published their first guideline for the management of large vessel vasculitis, which covers giant cell arteritis and Takayasu arteritis. The new guideline differs from the current EULAR recommendations on some important points, but do these different views actually affect patient care?

Refers to Maz, M. et al. 2021 American College of Rheumatology/Vasculitis Foundation guideline for the management of giant cell arteritis and Takayasu arteritis. *Arthritis Rheumatol.* **73**, 1349–1365 (2021).

The ACR, together with the Vasculitis Foundation (a United States patient organization), has released their first ever guideline on the management of giant cell arteritis (GCA) and Takayasu arteritis[1]. Comparison of this new guideline with the 2018 EULAR recommendations for management of and use of imaging in large vessel vasculitis[2,3] reveals many similarities and differences; but which of the divergent recommendations could affect clinical practice, and do they make sense? Here, we compare both guidelines with a focus on GCA.

As an important common feature, it must first be emphasized that both the ACR guideline[1] and EULAR recommendations[2,3] have been developed according to standardized methodologies, which require systematic literature reviews and clearly defined procedures on how recommendations are formulated and graded, as well as how consensus is found. However, the systems used to grade the recommendations give different weight to expert opinion versus evidence in the case of low-quality evidence. These methodological differences affect the wording of the respective recommendations and how they are perceived by the audience. Despite these differences, both societies use a fundamentally similar, modern and generally accepted approach at the highest scientific level. Not surprisingly, many concurring recommendations have been made in the two guidelines. Nevertheless, there are several important differences that could have implications for clinical practice (TABLE 1; Supplementary information).

First, untreated active GCA carries a substantial risk of visual loss[4]. The systematic literature review conducted by EULAR during the development of their recommendations revealed a moderate level of evidence that a diagnostic work-up including ultrasonography within 24 hours in a dedicated referral centre (known as a 'fast-track clinic') substantially reduces the rate of permanent visual impairment compared with conventional practice[4]. Therefore, the EULAR recommendations highlight the need for immediate diagnostic work-up and treatment[2]. Surprisingly, the ACR did not include this, in our opinion very important, aspect in either their systematic literature review or in their guideline[1].

Second, in patients with suspected GCA, the ACR guideline recommends temporal artery biopsy (TAB) over ultrasonography or MRI for establishing the diagnosis[1]. By contrast, EULAR recommends imaging as the first diagnostic test[3]. Although these recommendations seem to be contradictory at first glance, they actually are not. Although the sensitivity of TAB seems to be lower than that of ultrasonography in patients with predominant extracranial GCA, the sensitivity of TAB and ultrasonography compared with clinical diagnosis of GCA was similar in most studies[5]. Ultrasonography can confirm the diagnosis within minutes, presents no burden to patients and is cheaper than TAB. However, like other diagnostic tests, ultrasonography requires training, experience and appropriate equipment to deliver a reliable result. Therefore, the method of choice strongly depends on the setting and expertise provided by the individual site and examiner. As a consequence, the EULAR recommendations emphasize that TAB should be favoured if good quality imaging is not readily available[3]. In the end, this statement is in line with the ACR guideline[1], which states that the major reason for recommending TAB over imaging is the limited experience with temporal artery ultrasonography and MRI in the United States compared with Europe, where the vast majority of studies on imaging in GCA have been conducted.

Third, both the ACR and EULAR agree that patients with newly diagnosed active GCA should be treated with high-dose oral glucocorticoids and that intravenous glucocorticoids should be reserved for patients at risk of visual loss. However, the definition of 'high-dose' glucocorticoids differs somewhat between the two guidelines. The ACR guideline[1] recommends a starting dose of 1 mg per kg prednisone or equivalent, up to 80 mg daily,

Table 1 | **Key differences between the 2021 ACR guideline and the 2018 EULAR recommendations for the management of GCA**

Clinical scenario	2021 ACR–VF guideline[1]	2018 EULAR recommendations[2,3]
Fast-track diagnostic work-up for patients with suspected GCA	Not discussed	Urgent referral to a specialist team
First choice diagnostic test for patients with suspected cranial GCA	Temporal artery biopsy	Ultrasonography of temporal (and axillary) arteries[a]
Glucocorticoid[b] starting dose for induction of remission	1 mg per kg daily up to 80 mg or equivalent	40–60 mg daily
Use of adjunctive immunosuppressive therapy for newly diagnosed GCA	Recommended for all patients[c]	Recommended for patients with presence of or an increased risk of glucocorticoid-related adverse effects
Choice of adjunctive immunosuppressive therapy for newly diagnosed patients	Newly diagnosed GCA: TCZ, although MTX can also be considered	TCZ, although MTX can be used as an alternative
	Extracranial large vessel involvement: TCZ, although MTX can be considered for patients unable to use TCZ	

See Supplementary information for an extended version of this table. GCA, giant cell arteritis; MTX, methotrexate; TCZ, tocilizumab; VF, Vasculitis Foundation. [a]In centres where ultrasonography is readily available and performed well. [b]Prednisone or equivalent. [c]Decision should be based on physician's experience and patient's clinical condition, values and preferences.

over a moderate dose of 0.5 mg per kg daily (corresponding to 10–40 mg daily for most patients), whereas EULAR recommends a dose of 40–60 mg daily[2]. As high-quality data on the optimal starting dose for glucocorticoids in GCA are lacking[4,6], it is difficult to say which approach leads to better outcomes. In fact, it could well be that no difference would be found even in large studies. Notably, body-weight-adjusted dosing of glucocorticoids is common practice for many rheumatic diseases, including small vessel vasculitides[7]. However, the majority of previous studies, as well as clinical trials in GCA that are currently recruiting, use fixed starting doses of glucocorticoids of 60 mg daily, instead of body-weight-adjusted doses[4]. Although there is low-quality evidence that doses of 40 mg daily are superior to lower doses[8], there are no studies comparing doses of 40 mg and 60 mg, and doses of <40 mg have not been systematically studied[4,6]. In clinical practice, a patient with a body weight of >60 kg who was treated according to the ACR guideline[1] would have received a starting dose of glucocorticoids that was up to 20 mg higher daily, compared with if the EULAR recommendations[2] were followed. Evidence showing a better outcome for those patients who receive a higher starting dose of glucocorticoids is lacking, and a higher dose might prolong glucocorticoid tapering, thereby increasing the cumulative dose and potentially the risk of adverse effects.

Fourth, recommendations for the use of adjunctive glucocorticoid-sparing therapies notably differ between the ACR and EULAR guidelines. High-quality evidence exists to show that adjunctive glucocorticoid-sparing therapy reduces cumulative prednisone doses and improves remission rates[4,9]. Both societies therefore recommend adjunctive treatment for patients whose disease relapses. However, the ACR guideline also recommends the use of tocilizumab in combination with glucocorticoids over glucocorticoids alone for all newly diagnosed patients with GCA[1]. By contrast, EULAR recommends limiting the use of adjunctive therapies in treatment-naive patients to those with the presence of or an increased risk of glucocorticoid-related adverse effects[2]. Given that patients without these risk factors would receive glucocorticoid-sparing therapy following a relapse and, unlike in the GiACTA trial[9], would not continue glucocorticoid monotherapy for a whole year, the excess cumulative glucocorticoid exposure for these patients in clinical practice will actually be substantially smaller than that in GiACTA. Thus, the additional benefit of adjunctive therapy in this subgroup is unclear and needs to be balanced against potential adverse effects resulting from the additional treatment.

In addition, the ACR conditionally recommends tocilizumab over methotrexate as an adjunctive therapy in GCA[1]. As data from randomized controlled trials comparing both agents in GCA are lacking, estimates of relative efficacy and safety have to be based on indirect comparisons of individual trials. However, as both the design and quality of trials of tocilizumab substantially differ from those of methotrexate, the evidence available to assess which therapy is more suitable is limited. EULAR delivers a more cautious interpretation of the data than the ACR, stating that methotrexate can be used as an alternative to tocilizumab[2]. Nevertheless, the EULAR task force does note that tocilizumab provides a higher degree of confidence that a clinically meaningful treatment response will be achieved[2].

Overall, the 2021 ACR guideline provides valuable and up-to-date advice on how to manage GCA in clinical practice[1]. Although some statements differ from the 2018 EULAR recommendations[2,3], the majority of differences can be explained by differences in guideline methodology and in the health-care systems between the United States and most European countries, as well as by a somewhat different view as to which areas are lacking high-quality evidence.

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Competing interests

B.H. declares receiving speaker fees and/or consultancy fees from Abbvie, AstraZeneca, Chugai, Novartis and Roche, and was convenor of the 2018 EULAR recommendations and the 2020 Deutsche Gesellschaft für Rheumatologie guideline for the management of large vessel vasculitis. F.B. declares receiving consultancy fees, honoraria and travel expenses from Galapagos, Horizon Therapeutics, Roche and Sanofi, and grant support from Horizon Therapeutics, Mundipharma, Roche and Sanofi. He has served as co-principal investigator and site investigator in a Mundipharma sponsored trial in polymyalgia rheumatica, and as principal investigator and site investigator in giant cell arteritis and polymyalgia rheumatica trials sponsored by Sanofi.

Supplementary information

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Fatigue in inflammatory rheumatic diseases: current knowledge and areas for future research

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Abstract | Fatigue is a complex phenomenon and an important health concern for many people with chronic inflammatory rheumatic diseases, such as rheumatoid arthritis, psoriatic arthritis, primary Sjögren syndrome and systemic lupus erythematosus. Although some clinical trials have shown the benefits of cognitive behavioural therapy in fatigue management, the effect of this approach is relatively modest, and no curative treatment has been identified. The pathogenesis of fatigue remains unclear. Despite many challenges and limitations, a growing body of research points to roles for the immune system, the central and autonomic nervous systems and the neuroendocrine system in the induction and maintenance of fatigue in chronic diseases. New insights indicate that sleep, genetic susceptibility, metabolic disturbances and other biological and physiological mechanisms contribute to fatigue. Furthermore, understanding of the relationships between psychosocial factors and fatigue is increasing. However, the interrelationships between these diverse mechanisms and fatigue remain poorly defined. In this Review, we outline various biological, physiological and psychosocial determinants of fatigue in inflammatory rheumatic diseases, and propose mechanistic and conceptual models of fatigue to summarize current understanding, stimulate debate and develop further research ideas.

Inflammatory rheumatic diseases are a group of multi-system, immune-mediated rheumatic conditions that include primary Sjögren syndrome (pSS), systemic lupus erythematosus (SLE), vasculitis, psoriatic arthritis and rheumatoid arthritis (RA). Although the clinical manifestations of inflammatory rheumatic diseases vary, fatigue is a prevalent and often disabling symptom in many of them[1]. An international study of over 6,000 patients found that approximately half were severely fatigued, defined by a score of ≤35 on the 36-item short-form survey (SF-36) vitality scale[2]. Fatigue represents the largest health economic burden and unmet need to patients and society. Fatigue has been identified as one of the most challenging symptoms to manage for patients with inflammatory rheumatic diseases and is associated with poor quality of life[3–5]. Fatigue is also an important independent predictor of job loss and disability in patients with RA, ankylosing spondylitis, SLE, pSS and vasculitis[6–10]. Considering the widespread personal and economic burden of fatigue, discerning the underlying mechanisms and finding effective treatment options are research priorities.

In this Review, we outline various biological, physiological and psychosocial determinants of fatigue in inflammatory rheumatic diseases. We have taken a biopsychosocial approach[11] to understanding fatigue mechanisms, systematically considering biological, physiological, psychological and social factors and their complex interactions. Owing to the vast number of published articles mentioning "fatigue", and the challenges in drawing comparisons between different research studies on fatigue, we have not performed a comprehensive, systematic literature search. Also, several excellent reviews have focused on the role of inflammation and the brain in fatigue pathogenesis[12–15]. Therefore, in this Review we discuss what we consider to be the relevant evidence in the literature and include potential contributing mechanisms of fatigue that currently do not receive much attention. We also present hypothetical mechanistic and conceptual models of fatigue to summarize current understanding, stimulate debate and support the development of further research ideas.

The challenges of fatigue research

Fatigue is a complex, multifaceted phenomenon, and the many challenges in fatigue research limit our current understanding (BOX 1). Conceptually, no consensus exists on the definition of fatigue. Most people have experienced fatigue during their everyday life, but qualitative research suggests differences between fatigue

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Key points

- Fatigue is a common and disabling symptom of inflammatory rheumatic diseases.
- The mechanisms of fatigue in inflammatory rheumatic diseases are not fully understood but are likely to involve multiple biological, physiological, psychosocial and behavioural mechanisms.
- The mechanisms of fatigue in inflammatory rheumatic diseases might change over time and vary between individuals.
- Fatigue might reflect the body's resource management strategy in response to chronic stressors, favouring rationing and storage over expenditure.
- Studying fatigue has many challenges; consensus on a study framework for fatigue research and a multidisciplinary approach are essential.
- Optimal management of fatigue requires a personalized and holistic approach.

Recall period
The period over which people are asked to recall a prior event (for example, their fatigue experiences, thoughts and/or behaviours).

Sickness behaviour
Adaptive behaviours (such as social withdrawal, reduced activity and increased sleep) developed by animals and humans during an acute infection that are presumed to be beneficial for recovery and survival.

Anhedonia
Loss of interest in activities that were previously enjoyed and a decreased ability to feel pleasure.

associated with chronic diseases and 'usual' or premorbid fatigue. The most distinguishing features of fatigue associated with chronic diseases include the perception of the fatigue as having no obvious 'explanation', a lack of improvement with rest, variability in severity, unpredictability and fatigue being profound or overwhelming[16,17]. Patients often explain their fatigue in relation to the considerable impact it can have on all aspects of their daily lives[18]. Such findings suggest the involvement of different mechanistic pathways in pathological fatigue and the fatigue experienced by healthy individuals, although the mechanisms of fatigue in both settings remain poorly defined. A related consideration is whether occurrences of fatigue associated with different chronic conditions (for example, RA, fibromyalgia, inflammatory bowel disease, Parkinson disease and cancer) are similar phenomena. Studies have shown similarities in the qualities of the fatigue experienced across a range of inflammatory and non-inflammatory chronic diseases[19,20]. Studies in different diseases have also identified similar 'predictors' of fatigue[21–23] (BOX 2). Furthermore, fatigue correlates poorly with the disease activity of the underlying conditions[24–28]. These observations suggest that fatigue might be the same phenomenon across different chronic conditions. In this Review, we focus on fatigue associated with inflammatory rheumatic diseases.

Measuring fatigue accurately and reliably is challenging. Fatigue is inherently a subjective phenomenon, and its assessment relies on the use of self-reported questionnaires. Many questionnaires have been developed to measure fatigue using different approaches and each having strengths and weaknesses[29]. Factors to consider when selecting a method to measure fatigue include whether the instrument should be generic versus disease-specific, whether it should be single-item versus multi-item, and/or whether it should provide a single overall score (usually for the severity of physical fatigue) versus sub-scale scores for different facets of fatigue (for example, physical, cognitive and emotional). The number of questionnaires in use and the lack of an agreed gold standard means that comparisons across studies are difficult, as demonstrated for studies in RA[30]. Additional issues contributing to cross-study comparisons include differing recall periods, variation in wording between questionnaires and the lack of cut-off values to define cases of fatigue in most questionnaires. Moreover, these questionnaires poorly capture the variability of the fatigue. Finally, patient-reported outcomes are prone to recall bias and other psychosocial influences, increasing the subjectivity of patients' responses. Objective assessment of fatigue, if available, would overcome some of these issues, although capturing patients' perceptions is also important to aid the interpretation of the outcomes.

Box 1 | **Challenges in fatigue research**

- No consensus has been reached on a definition of fatigue.
- Difficulties exist in measuring fatigue accurately and reliably.
- Addressing potential confounders of fatigue remains a challenge.
- The premorbid state of fatigue is often poorly described.

Difficulties in fatigue research also arise from potential confounders of fatigue, such as mood disorder and pain, and a lack of information about the premorbid fatigue state. Without knowledge on the premorbid levels of fatigue, one cannot determine whether the level of fatigue changes following the onset of the inflammatory rheumatic disease, thereby making it difficult to evaluate how the inflammatory rheumatic disease contributes (if at all) to the symptom.

Putative mechanisms in fatigue

Many biological, physiological and psychosocial mechanisms have been implicated in fatigue pathogenesis, such as the central nervous system, inflammation, pain and anxiety (FIG. 1). However, the cross-sectional nature of many of the studies investigating mechanisms of fatigue makes establishing causality and directionality a challenge. Furthermore, complex interactions exist between many of these mechanisms. It is likely that multiple mechanisms promote fatigue, with the contribution of each mechanism differing between patients and potentially within patients over time. Therefore, support for fatigue should be tailored to individuals and involve exploration of possible contributing factors. A systematic review of factors associated with fatigue in RA identified a cluster of variables that should be considered as potentially maintaining fatigue, including psychological and physical functioning, pain, sleep disturbance, depression and anxiety[31].

In this section, we describe the evidence supporting each putative mechanism of fatigue in inflammatory rheumatic diseases.

Biological and physiological mechanisms

Immunological mechanisms. Inflammation is arguably the most frequently studied mechanism underlying fatigue. The sickness behaviour model describes conditions such as fatigue, anhedonia, social withdrawal and depression as immune-mediated responses by vertebrates that enhance survival from infection[32]. Energy expenditure is thereby reserved for the immune system, and social withdrawal prevents further disease spread and reduces the risk of predation[33]. The production of type I interferons and other pro-inflammatory cytokines contributes to sickness behaviour[32]. Studies in animals and humans have not only provided strong evidence

for inflammation-induced fatigue but also revealed its potential molecular basis. A large body of data demonstrates the links between inflammation and fatigue in inflammatory rheumatic diseases and is reviewed in the literature[6,12,13,34]. However, how far the acute sickness behaviour model and inflammation explain fatigue in chronic inflammatory rheumatic diseases remains unclear.

If inflammation is the only factor contributing to fatigue, one would expect a strong correlation between fatigue severity and production of pro-inflammatory cytokines and disease activity, and that fatigue should improve with immunomodulatory treatment and subside when the underlying inflammatory rheumatic disease is in remission. However, although circulating concentrations of pro-inflammatory cytokines are typically elevated in patients with inflammatory rheumatic diseases compared with healthy individuals and those with non-inflammatory rheumatic disease[35,36], higher circulating concentrations of these cytokines are not necessarily associated with worse fatigue. In RA, although fatigue often accompanies disease flare[14], no consistent relationship between fatigue and validated disease activity scores has been identified[25–27,37]. In particular, a substantial proportion of patients with inflammatory rheumatic diseases continue to experience disabling fatigue despite seemingly being in clinical and biological remission, as observed in patients with RA[37]. Conversely, as shown in pSS, a small proportion of patients do not experience fatigue despite having active disease[38,39].

In pSS, clinical trial data on the effect of immunemodulating therapies on fatigue are inconsistent. For example, out of seven trials of rituximab, a reduction in fatigue was shown in four[40–42], two studies showed a mild improvement only at certain time points[43,44] and one study showed no effect[45]. In a phase II trial, treatment with RSLV-132 (an RNase-Fc fusion protein) led to improvement of fatigue in patients with pSS, which was intriguingly accompanied by an increase in expression of selected interferon-inducible genes[46]. Several studies even revealed an inverse relationship between fatigue severity and circulating concentrations of the pro-inflammatory cytokines CXCL10 (also known as IP-10), TNF, lymphotoxin-α and IFNγ in patients with pSS, and such inverse relationships between these pro-inflammatory cytokines and fatigue, together with depression and pain, were important predictors of fatigue in a multi-regression model[35,47]. Similarly, an inverse relationship between interferon activation and fatigue was observed in patients with pSS in two separate studies[38,46]. In addition, using gene set enrichment analysis of the genome-wide transcriptomic data of 133 patients with pSS, 19 biological pathways were associated with fatigue, but none was overtly inflammation related[24]. Thus, no consistent relationship between systemic inflammation and fatigue has been demonstrated; one possible explanation is that the role of inflammation varies at different stages of the disease.

Many patients with RA continue to experience disabling fatigue despite having no demonstrable synovitis or systemic responses[37,48]. A meta-analysis of studies on interventions for RA with fatigue as a primary or secondary outcome (most of which ran for 24 weeks or less) demonstrated that both anti-TNF agents and other biologic DMARDs (including rituximab, tocilizumab, canakinumab, abatacept and anti-IFNγ) similarly reduced fatigue in patients with active RA compared with placebo[48]. Whether fatigue improvement is a direct result of reduced disease activity and inflammation, or results indirectly via another mechanism, such as reduced pain, is unclear[49]. Notably, participants in the trials included in the aforementioned meta-analysis had a high level of disease activity, fatigue was measured as a secondary outcome and little adjustment was made for confounding factors. Thus, although immunomodulatory therapies can improve fatigue in some patients with RA, whether this improvement is attributable to the reduction of inflammation remains unclear.

The relationship between fatigue and inflammation in SLE is also unclear. The BLISS-52 and BLISS-76 trials of the B cell-inhibiting biologic DMARD belimumab measured fatigue as a secondary outcome using the SF-36 Health Survey questionnaire over a study period of 52 weeks and 76 weeks, respectively[50,51]. In post hoc analysis, statistically significant improvements from baseline in SF-36 physical component score were demonstrated in the group who received 10 mg/kg belimumab compared with placebo in BLISS-52, but not in BLISS-76 (REF.[52]). In a long-term extension study of BLISS-76 in which all participants who completed the parent trial had the option to continue belimumab or switch from placebo to belimumab, improvement in fatigue scores was maintained[53]. Interestingly, fatigue levels initially rose with belimumab treatment, suggesting that sustained therapy could be needed to see improvement in fatigue.

Overall, these observations indicate a complex relationship between fatigue and disease activity in inflammatory rheumatic diseases, and that the relationships between pro-inflammatory responses and fatigue remain to be fully defined. Inflammation probably has an important function in initiating fatigue responses, particularly in the early stages of the disease and during disease flares. However, the evidence for systemic inflammation in the maintenance of fatigue in chronic disease is less clear. In some individuals, inflammation might not be an important factor, and additional or different mechanisms are the predominant promoters of fatigue. Consistent with this idea, some patients with hepatitis C treated with IFNα therapy reportedly continued to experience fatigue even 6 months after completing treatment, that is, when IFNα would no longer be present[54].

Box 2 | Common predictors of fatigue in chronic diseases[21–23]

- Pain
- Depression
- Sleep disturbances
- Reduced physical activities
- Autonomic dysfunction
- Altered hypothalamic–pituitary–adrenal axis responses

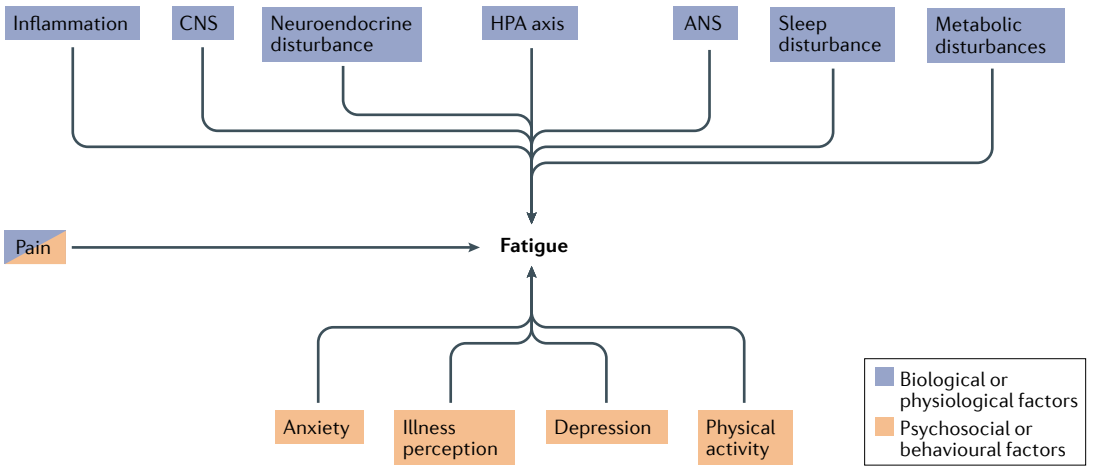


Fig. 1 | **Putative mechanisms implicated in the pathogenesis of fatigue.** Putative pathogenetic mechanisms of fatigue include biological and physiological factors (purple) as well as psychosocial or behavioural factors (orange). Pain involves the conscious interpretation of a physiological signal as 'pain' and therefore can be considered as either type of factor. All these mechanisms could contribute to fatigue pathogenesis, and might also interact with each other. ANS, autonomic nervous system; CNS, central nervous system; HPA axis, hypothalamic–pituitary–adrenal axis.

Arguably, fatigue that correlates closely with activity of the underlying disease and/or systemic inflammation is not a large clinical and scientific unmet need, as such fatigue should improve when the underlying disease is treated or when systemic inflammation is suppressed. By contrast, fatigue dissociated from disease activity or systemic inflammation is a conundrum for clinicians, scientists and the pharmaceutical industry and represents a large health-economic burden and unmet need to society and patients.

Central nervous system changes. Several characteristics of fatigue point to the potential involvement of the central nervous system (CNS). For instance, symptoms such as cognitive impairment and lack of motivation are common among patients with inflammatory rheumatic diseases experiencing fatigue[55]. Furthermore, the CNS can contribute to muscle fatigue during exercise by reducing the neural drive to the muscles[56]. As the neural drive decreases, a smaller number of motor neurons are activated, resulting in a weaker force of muscle contraction.

Potential mechanisms by which inflammation might alter neurochemistry and functional connectivity in the brain, and in turn might contribute to fatigue, have been comprehensively reviewed elsewhere[15]. Bidirectional communication between the immune system and the brain is mediated by multiple signalling pathways[32,57]. Circulating pro-inflammatory cytokines transfer directly across the blood–brain barrier via various direct mechanisms (for example, receptor-mediated transcytosis, leakage across damaged tight junctions or via circumventricular organs), or indirectly via activated vascular endothelial cells or the vagus nerve (FIG. 2a), leading to microglia activation in the brain[58]. Pro-inflammatory activity in the brain in turn results in several changes. First, the release of the neurotransmitter noradrenaline, which is important for increasing arousal, alertness and attention, is inhibited[59]. Second, the uptake and breakdown of monoamines (serotonin, dopamine and noradrenaline) is increased, reducing their availability in the synaptic cleft[15]. These monoamines play a key role in mood, motivation and arousal. Third, tryptophan-2,3-dioxygenase and indoleamine-2,3-dioxygenase 1 (IDO-1), which promotes tryptophan conversion in the kynurenine metabolic pathway, is increased. The metabolites of the kynurenine pathway further induce local inflammation in the brain. In a rat model of induced fatigue, metabolites of the kynurenine pathway were present in the presynaptic neurons of the hypothalamus, hippocampus and cerebral cortex[60]. In patients with SLE or RA, kynurenine pathway activation and elevated kynurenine to tryptophan ratios correlated with fatigue[61,62]. However, in a study of pSS, peripheral levels of *IDO1* mRNA were similar in patients with and without fatigue[63]. Furthermore, in a study of patients with hepatitis C receiving IFNα treatment (a model of inflammation-induced fatigue), although levels of kynurenine metabolites were altered, they were not associated with persistent fatigue[54]. Therefore, whether kynurenine metabolites directly mediate fatigue remains to be elucidated. Tryptophan also acts as a precursor for serotonin synthesis. Therefore, tryptophan depletion could result in decreased serotonin synthesis, which could have important consequences for mood and cognitive functioning. However, in patients with SLE, tryptophan metabolism correlated with fatigue but not with depression[61]. Increased plasma concentrations of kynurenine were associated with exhaustion in athletes and correlated with worse fatigue and depression scores in patients undergoing haemodialysis[64,65], suggesting that kynurenine metabolism could also be implicated in fatigue in other settings. Taken together, these findings support the notion that the CNS is implicated in fatigue pathogenesis in inflammatory rheumatic diseases.

Direct evidence of pro-inflammatory or metabolic changes in the CNS in patients with fatigue remains elusive. Proteomic analysis using liquid

Neural drive
The activation signals from the central nervous system delivered to the innervating motor neurons of the muscle.

chromatography-mass spectrometry and tandem mass spectrometry identified 15 proteins present in cerebrospinal fluid from patients with pSS that could discriminate those with fatigue from those without, but none of the proteins has known pro-inflammatory functions[66]. Instead, many are associated with cellular stress responses, cellular metabolism and depression. However, in another study examining the role of the IL-1 pathway in fatigue in patients with pSS, the concentration of IL-1 receptor antagonist (IL-1RA), a natural inhibitor of IL-1β, was elevated in the cerebrospinal fluid from the patients with fatigue compared with those without, and predicted severity of fatigue (alongside depression and pain)[67,68]. As IL-1RA is implicated in the IL-1β pathway via its role as an IL-1β antagonist, the detection of increased IL-1RA concentrations could indicate activation of the IL-1β pathway in the CNS in fatigue.

Neuroimaging is a promising tool for exploring the role of the CNS in fatigue. MRI can show volumetric changes in different areas of the brain, which might have implications for function, and functional MRI (fMRI) can reveal alterations in neural networks during fatiguing tasks. For instance, fMRI has provided evidence for a positive correlation between fatigue and increased functional connectivity between the dorsal attention network and right medial prefrontal cortex in patients with RA[69]. In another fMRI study, patients with granulomatosis with polyangiitis and fatigue had hyperactivity in several brain regions during a mental challenge task, as compared with those without fatigue[70]. A meta-analysis of fMRI and PET studies that involved either markers of peripheral inflammation or induced inflammation (for example, following vaccination or immunotherapy such as IFNα) showed that these studies have identified changes in brain regions and networks that are associated with peripheral inflammation[71]. Some of these brain regions and networks could provide an explanation of sickness behaviour. However, this meta-analysis did not directly explore the relationship between CNS changes and fatigue. In a systematic review of MRI studies of fatigue in chronic diseases, the brain areas implicated in fatigue were highly heterogeneous, not only between diseases, but also within the same disease[72]. Similarly, another systematic review found conflicting data regarding lesion location and post-stroke fatigue[73]. Thus, more research is needed to characterize the CNS changes relevant in fatigue.

Neuroendocrine disturbances. The neuroendocrine system controls many physiological processes, including stress response, metabolism and energy utilization; therefore, neuroendocrine disturbances may contribute to fatigue. An appropriate cortisol response is important for the body to handle stressors, which can be physical (for example, injuries), physiological (for example, hypotension), pathological (for example, infections) or emotional (for example, significant life events). Cortisol production is primarily regulated by the hypothalamic–pituitary–adrenal (HPA) axis. Interaction between the HPA axis and the immune system is complex. Pro-inflammatory cytokines (particularly gp130 cytokines, such as IL-1 and IL-6) stimulate the production of corticotropin-releasing hormone by the hypothalamus and of adrenocorticotropic hormone (ACTH) by the pituitary glands[74], resulting in increased cortisol production by the adrenal glands. Cortisol in turn suppresses pro-inflammatory responses, completing a feedback loop (FIG. 2b). With persistent inflammation, however, the response of the HPA axis could be blunted[75]. In addition to having immunomodulatory effects, cortisol is important for the regulation of metabolism. Cortisol and other glucocorticoid hormones increase the availability of energy by mobilizing the release of glucose, free fatty acids and amino acids from endogenous stores. Fatigue and lack of motivation are well-recognized features of hypoadrenalism. In clinical studies, HPA dysfunction can be demonstrated by reduced basal cortisol concentrations, relative cortisol insufficiency (for example, reduced cortisol to pro-inflammatory cytokine ratio) or suboptimal cortisol response to stimulation (for example, by exogenous ACTH and/or corticotropin-releasing hormone or by hypoglycaemic states). Blunted HPA axis response has been reported in patients with pSS[76], RA[77,78] and SLE[79] compared with healthy individuals.

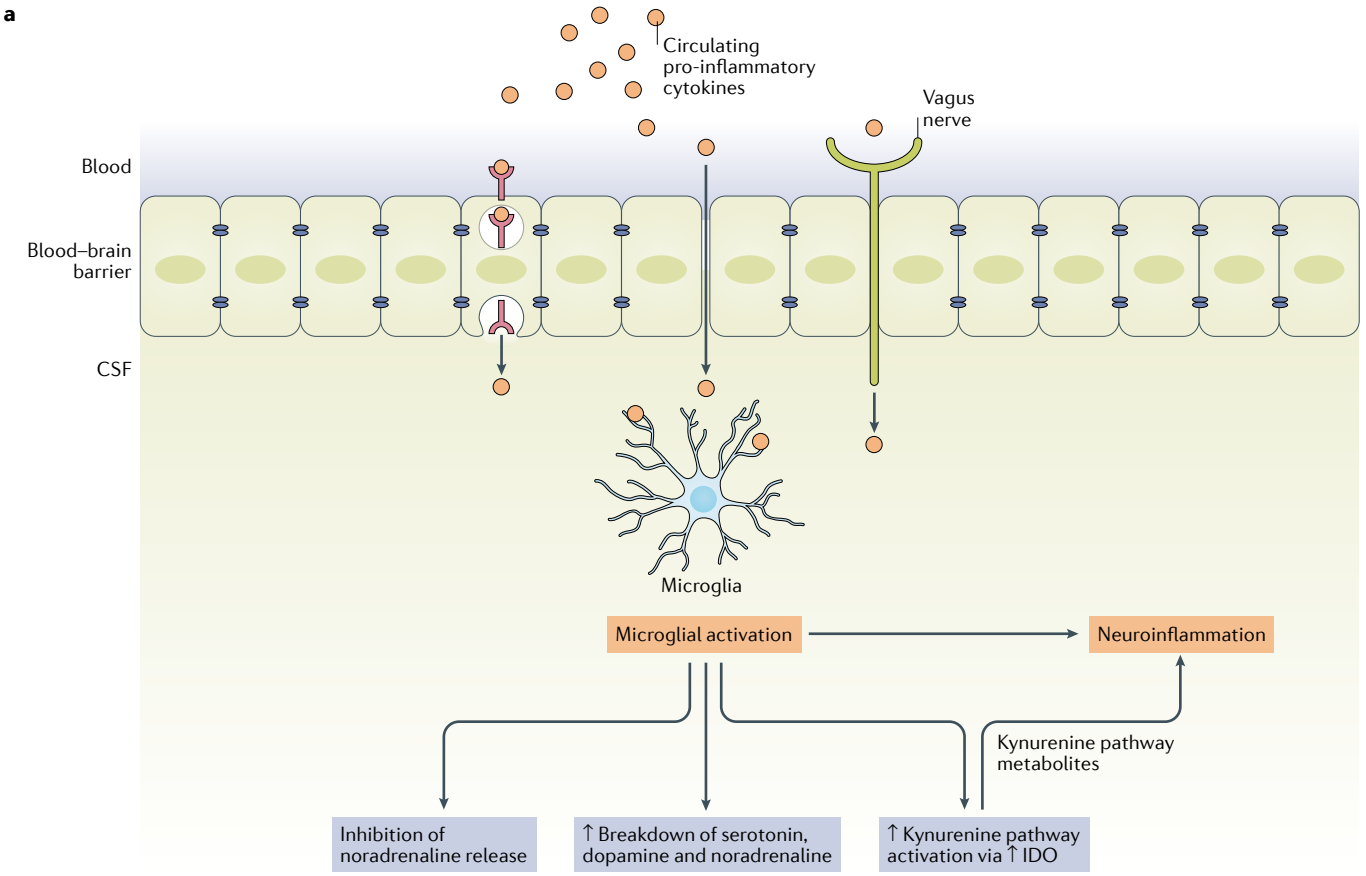
However, few studies have directly explored the link between HPA axis dysfunction and fatigue in patients with inflammatory rheumatic diseases. A longitudinal study exploring the relationships between daily stressors, worrying, the HPA axis (cortisol), pro-inflammatory cytokines, disease activity, pain and fatigue over 6 months in 80 patients with RA showed that daily stressors, IL-1β and IFNγ predicted increased fatigue 1 month later[80]. However, daily stressors, worrying, cortisol and pro-inflammatory cytokines were not included in a single model, so whether they represent independent predictors is not clear. A multivariate analysis of a cohort of patients with SLE without concomitant fibromyalgia showed that stress, depression and pain independently correlated with fatigue[28], with stress being the largest contributor to fatigue whereas disease activity did not contribute. However, another study of patients with SLE concluded that pain, social support and depression predict fatigue, but perceived stress did not[81]. Dysfunction of the hypothalamic–pituitary–gonadal axis and hypothalamic–pituitary–thyroid axis has been reported in patients with pSS, SLE and RA[82,83], although the relationship with fatigue was not examined[84]. Given that fatigue is a symptom of thyroid dysfunction and reduced sex drive is associated with fatigue, investigation into potential links between hypothalamic–pituitary–thyroid or hypothalamic–pituitary–gonadal dysfunction in fatigue is of interest.

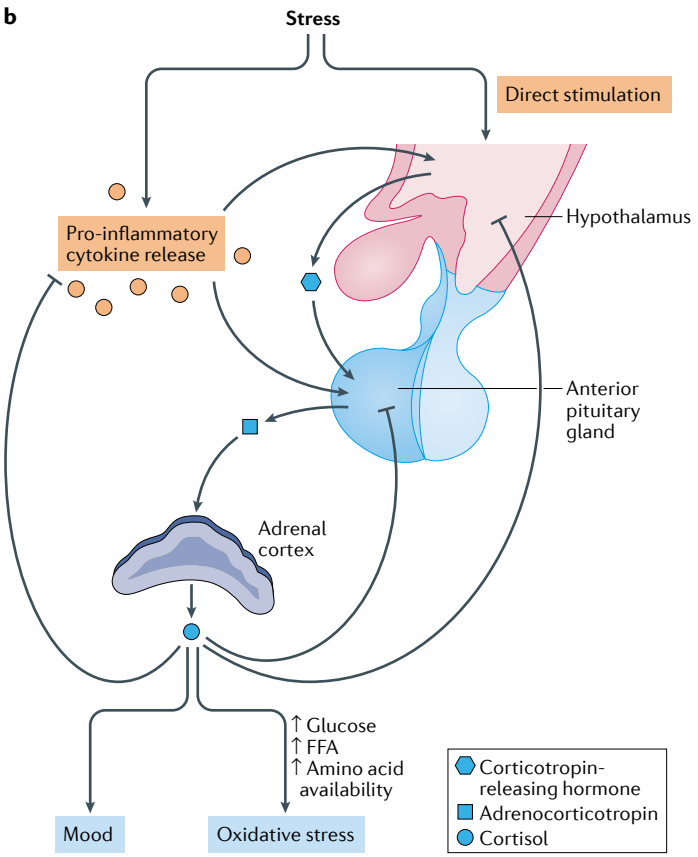
Autonomic nervous system dysfunction. The autonomic nervous system (ANS) has a vital function in responses to stressors through its ability to rapidly implement anticipatory actions. Inflammation activates pattern-recognition receptors on innate immune cells and other cells, which in turn stimulate the vagus nerve, resulting in the release of the neurotransmitter norepinephrine. In animal models, norepinephrine activates T cells that produce the neurotransmitter acetylcholine and inhibit

Hypothalamic–pituitary–adrenal (HPA) axis
Refers to the connections and interactions between the hypothalamus, pituitary gland and adrenal glands.

Hypothalamic–pituitary–gonadal axis
Refers to the connections and interactions between the hypothalamus, pituitary gland and the gonads.

Hypothalamic–pituitary–thyroid axis
Refers to the connections and interactions between the hypothalamus, pituitary gland and the thyroid glands.


a
Circulating pro-inflammatory cytokines
Vagus nerve
Blood
Blood–brain barrier
CSF
Microglia
Microglial activation
Neuroinflammation
Kynurenine pathway metabolites
Inhibition of noradrenaline release
↑ Breakdown of serotonin, dopamine and noradrenaline
↑ Kynurenine pathway activation via ↑ IDO


b
Stress
Direct stimulation
Pro-inflammatory cytokine release
Hypothalamus
Anterior pituitary gland
Adrenal cortex
↑ Glucose
↑ FFA
↑ Amino acid availability
Mood
Oxidative stress
Corticotropin-releasing hormone
Adrenocorticotropin
Cortisol

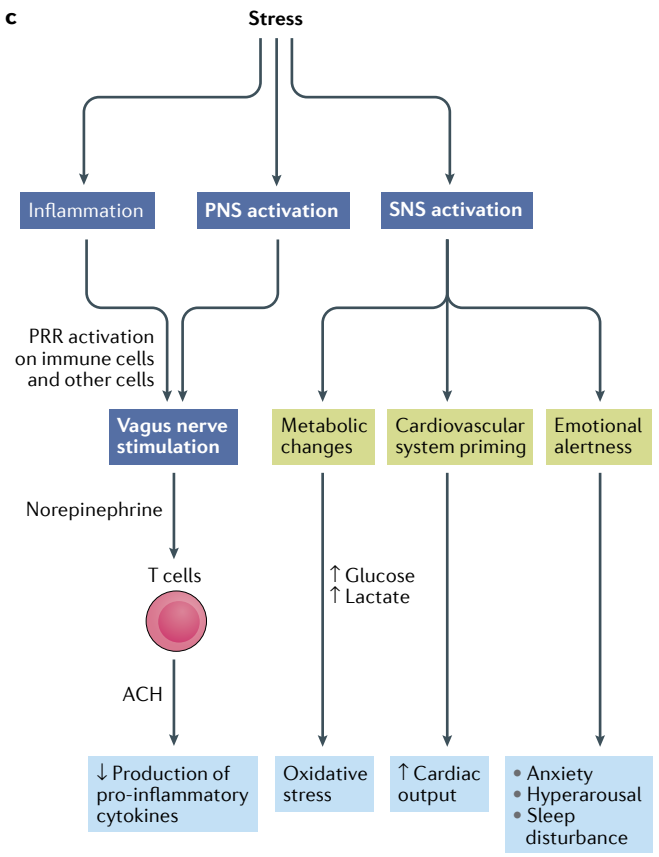

c
Stress
Inflammation
PNS activation
SNS activation
PRR activation on immune cells and other cells
Vagus nerve stimulation
Metabolic changes
Cardiovascular system priming
Emotional alertness
Norepinephrine
T cells
ACH
↑ Glucose
↑ Lactate
↓ Production of pro-inflammatory cytokines
Oxidative stress
↑ Cardiac output
• Anxiety
• Hyperarousal
• Sleep disturbance

Fig. 2 | **The role of the nervous system in fatigue. a** | Central nervous system. Circulating pro-inflammatory cytokines transfer directly across the blood–brain barrier by receptor-mediated transcytosis, leakage across damaged tight junctions or via the circumventricular organ (not shown), or indirectly via the vagus nerve or activated vascular endothelial cells, causing brain microglial activation. Pro-inflammatory activities in the brain inhibit noradrenaline release, which is important for increasing arousal, alertness and attention, and also mediates increased uptake and breakdown of the monoamines serotonin, dopamine and noradrenaline, reducing their availability in the synaptic cleft. Additionally, inflammation increases tryptophan-2,3-dioxygenase and indoleamine-2,3-dioxygenase (IDO), which promotes tryptophan conversion in the kynurenine metabolic pathway, further promoting neuroinflammation. **b** | Hypothalamic–pituitary–adrenal axis. Stress and pro-inflammatory cytokines stimulate release of corticotropin-releasing hormone from the hypothalamus, causing release of adrenocorticotrophin-releasing hormone from the anterior pituitary gland. Adrenocorticotrophin-releasing hormone stimulates the adrenal gland to release cortisol, which affects mood, inhibits inflammation and promotes the release of amino acids, free fatty acids (FFA) and glucose into the circulation, enhancing oxidative stress. **c** | Autonomic nervous system. Stress promotes inflammation, antagonizes the parasympathetic nervous system (PNS) and primes the cardiovascular system to optimize cardiac output. Systemic nervous system (SNS) activation promotes shifts in metabolism, release of glucose and lactate, oxidative stress and emotional alertness leading to hyperarousal, anxiety and sleep disturbance. Inflammation stimulates the vagus nerve, causing acetylcholine (ACH) production by T cells and inhibiting pro-inflammatory cytokine production. The PNS limits stress reactions and restores equilibrium post-threat. Imbalances between the SNS and PNS lead to hyper-arousal, emotional changes and attenuated heart rate variability. CSF, cerebrospinal fluid; PRR, pattern-recognition receptor.

pro-inflammatory cytokine production by macrophages, completing a feedback loop[85] (FIG. 2c). The sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) are part of the ANS; the SNS triggers the 'fight or flight' response to enable rapid reactions to a threat, whereas the PNS tends to limit stress reactions and restore equilibrium once the threat has passed. Catecholamines have an important function in the regulation of energy mobilization and utility as well as cardiovascular function (which control the supply of fuel to tissues). Imbalances between the SNS and PNS can lead to hyper-arousal, emotional changes and attenuated heart rate variability[86]. Symptoms of dysautonomia, such as postural hypotension and exercise intolerance, as well as objective measures of autonomic dysfunction, are both common in patients with pSS and have been associated with fatigue[87–96]. Of note, in one cohort study >40% of patients with pSS had decreased parasympathetic activities[89]. More interestingly, non-invasive vagus nerve stimulation twice daily for 4 weeks was accompanied by improvement in fatigue in patients with pSS[97]. These observations support a role for the ANS, particularly the vagus nerve, in the modulation of fatigue. Autonomic dysfunction has also been reported in other inflammatory rheumatic diseases; however, the relationship with fatigue was not explored[98–100]. In RA, vagus nerve stimulation is associated with clinical improvement and a reduction in pro-inflammatory cytokines, although the effect of this treatment on fatigue was not measured[101,102].

These findings support a role for autonomic dysfunction in the pathogenesis of fatigue in inflammatory rheumatic disease.

Sleep disturbances. Sleep disturbances affect 40–75% of patients with rheumatic diseases and are often associated with fatigue[103]. The relationship between fatigue and sleep is not fully defined but is probably a bi-directional relationship, with poor sleep leading to fatigue and daytime fatigue resulting in sleep disturbances[104,105]. Sleep has a complex relationship with inflammation, the HPA axis, the ANS and mood disorders, and is influenced by factors such as chronicity and type of sleep disruption[104]. As described earlier in this Review, inflammation affects synthesis of neuroendocrine mediators such as monoamines, melatonin, prolactin, and growth hormone, all of which can affect sleep[106]. Sleep disturbance is associated with altered HPA activity and cortisol production, and changes in the circadian pattern of circulating concentrations of cortisol in turn regulate sleep[107]. Sleep disruption is associated with activation of the SNS. Sleep disturbances in patients with RA are characterized by worse sleep efficiency, sleep quality, sleep latency, number of awakenings and time awake after sleep compared with healthy individuals[108,109]. In patients with SLE, sleep disorders seem to be associated with disease activity alongside pain and fatigue[110]. In patients with pSS, poor sleep is prevalent and is associated with a high symptom burden, orthostatic symptoms and fatigue[111].

These findings support a role for sleep disturbance in the pathogenesis of fatigue in inflammatory rheumatic disease.

Metabolic disturbances. Metabolic disturbances, including oxidative stress, are associated with fatigue. Oxidative stress refers to an imbalance of pro-oxidants and antioxidants in favour of the former[112], whereas nitrosative stress is characterized by overproduction of nitric oxide[113]. Inflammation, a key pathological condition in inflammatory rheumatic diseases, increases oxidative and nitrosative stress by inducing the production of free radicals and reactive intermediates of oxygen and nitrogen. Measurement of F_2-isoprostanes has been used to assess oxidative stress. Increased concentrations of urine and plasma F_2-isoprostanes independently predict fatigue levels in patients with SLE[114]. The activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase and the antioxidant molecule glutathione were reduced in patients with SLE compared with healthy individuals[115], and might be responsible for the increased oxidative stress observed. The precise mechanisms by which increased oxidative or nitrosative stress results in fatigue, however, are unclear. Possible mechanisms include attenuation of aerobic metabolic capacity and reduction in muscle force production[116]. Antioxidant supplementation, which theoretically reduces oxidative stress, has been shown to improve exercise performance and reduce muscle fatigue[117,118].

Cardiopulmonary fitness, physical activity and body mass index. Reduced physical activity is a hallmark of fatigue, and cardiopulmonary fitness and BMI are predictors of physical activity. In a cross-sectional study, female patients with pSS had lower VO_{2peak}, muscle strength and function and higher levels of fatigue than healthy women matched for age and habitual physical activity levels[119]. Physical activity levels (measured using an accelerometer) were comparable in the two groups, and no statistically significant correlation between fatigue and VO_{2peak}, muscle strength and

Dysautonomia
An umbrella term used to describe conditions attributable to malfunctioning of the autonomic nervous system.

Sleep disturbances
An umbrella term used to describe the spectrum of sleep disorders, such as difficulty falling asleep, frequent wakening and sleep apnoea.

Somatic focus
Heightened attention to physical symptoms.

function or physical activity was found. However, fatigue was measured using Functional Assessment Chronic Illness Therapy (Fatigue), which may not be the most appropriate tool for analysing the relationship between fatigue and these objective measurements as it contains question items that might not be affected by physical performance or capacity. Furthermore, only bivariate correlation analysis was performed, without taking into consideration potential confounders such as mood or pain[119]. Conversely, a randomized controlled trial of supervised walking in patients with pSS found that improvement in VO_{2max} and cardiovascular fitness was associated with reductions in fatigue and depression[120]. Reduction of cardiopulmonary fitness could be a consequence of reduced physical activity due to fatigue and musculoskeletal pain. Data from 273 patients in the UK Primary Sjögren's Syndrome Registry revealed that self-reported physical activity levels at all intensity levels were reduced among patients with pSS compared with healthy individuals matched for age and sex[121]. Furthermore, reduced levels of moderate or vigorous physical activity were associated with fatigue in patients with pSS[121]. Consistently, reduced physical activity, activity avoidance and somatic focus were associated with fatigue among patients with pSS[122]. Thus, physical activity and physical capacity may contribute to fatigue and vice versa in pSS.

Poor exercise tolerance and reduced maximum aerobic capacity were also observed in patients with SLE or RA[123,124]. In a study of 443 patients with RA, Løppenthin and co-workers showed that 78% of the patients were mainly sedentary or had a low level of physical activity, which was higher than the general Danish population. Physical fatigue is the strongest predictor of reduced physical activity in RA[125]. Increased physical activity is associated with improved cardiorespiratory fitness and reduced fatigue in patients with RA[126]. In a meta-analysis and subsequent review the investigators concluded that physical activity potentially improved fatigue in patients with RA, although the effect size was described as small to moderate[127,128]. Furthermore, although physical activity intervention seemed to improve fatigue in patients with RA, the trials included in the meta-analysis were often performed over a short period of time (typically <12 weeks) without evidence of sustained improvement. In two studies in patients with SLE, home exercise interventions over longer periods (8 and 12 weeks) led to reductions in fatigue[129,130], which were sustained at 3 months if participants continued to exercise[130]. These findings suggest that in RA and SLE, physical activity may contribute to fatigue and vice versa.

Patients with fatigue often describe that their muscles feel weak and unable to sustain prolonged or vigorous activity. Reduced muscle strength and endurance are well-documented in patients with inflammatory arthritis and correlate with disease activity and reduced physical activity[131]. Sarcopenia (reduced muscle mass) and myositis (muscle inflammation) can occur in many inflammatory rheumatic diseases[132–135], which could also contribute to fatigue.

Obesity is a predictor of fatigue in patients with RA[136–138] and SLE[139,140]. The association between obesity and fatigue is interesting from the perspective of energy management because although accumulation of adipose tissues in obesity results from a surplus in energy, there is an imbalance in energy expenditure and conservation in favour of the latter, and individuals with obesity experience fatigue and decreased physical endurance, reflecting an energy-deficient state. The mechanisms linking obesity and fatigue in inflammatory rheumatic disease are unclear. However, a study using electromyography showed a greater reduction of voluntary (that is, CNS-mediated) activation of available motor units in obese participants when fatigued compared with participants who were not obese[141]. Other possible mechanisms linking obesity and fatigue include altered energy distribution or production and mitochondrial dysfunction, particularly in skeletal muscles. Obesity could also contribute to both oxidative stress and fatigue by increasing the inflammatory burden[142]. However, obesity is associated with several determinants of health including the social and economic environment, the physical environment, and the person's individual characteristics and behaviours. Therefore, the relationship between obesity and fatigue is an example of one that involves potentially interacting factors at the intra-individual, inter-individual and societal levels.

Psychosocial determinants

Mood disturbances. Depression is more prevalent in patients with inflammatory rheumatic diseases compared with the general population[143]. Many factors can contribute to the co-occurrence of depression and inflammatory rheumatic diseases, such as common genetic risk factors and shared biological pathways, as well as the influence of social, behavioural and psychological factors[144]. Depression is also strongly linked with fatigue in inflammatory rheumatic diseases[21,28,35,47,49,81,145] as well as in non-inflammatory diseases[146]. Notably, 'marked tiredness' is one of the classification criteria for depression[147]. However, although it is possible that fatigue can contribute to depression via shared mechanistic pathways and consequences of daily life, depression and fatigue are distinct phenomena[148] and many patients with fatigue do not have depression[38]. Distinguishing depression from fatigue in the clinic can be challenging. Depression is primarily psychological, and the main signs include sad mood, social isolation and negative thoughts, sometimes accompanied by physical symptoms such as headaches, cramps and stomach upsets. Fatigue is often a feature, but not the primary symptom, of depression. Patients with depression commonly experience anhedonia and become uninterested in taking part in activities, irrespective of the task or the amount of effort the task requires. By contrast, fatigue is primarily physical, and many patients report wanting to engage in activities but feel too tired to do so.

Inflammation has been implicated in the pathogenesis of depression on the basis of findings such as increased circulating concentrations of pro-inflammatory cytokines and microglial activation in the brain (as demonstrated in post-mortem and in vivo imaging studies)[149]. The extent to which inflammation mediates all depressive illness remains unclear. Notably, adverse childhood

events can result in immune activation[150], raising the possibility of a bi-directional relationship between depressive illness and inflammation. Furthermore, whether the same mechanisms by which inflammation mediates depressive symptoms also mediate fatigue is unclear. In this regard, the IL-1β pathway is a candidate mechanism linking depression and fatigue at a molecular level[151,152].

Pain. Pain occurs as a complex interplay between peripheral and central sensitization, biological influences (such as pro-inflammatory cytokines) and the psychological perception of pain[152,153]. Musculoskeletal pain is often a defining feature of many inflammatory rheumatic diseases[39,154,155] and is an important predictor of fatigue[28,35,47,49,81]. Data from clinical trials further underline a relationship between pain and fatigue. In RA, improvement in fatigue is associated with pain reduction following treatment with DMARDs[49]. However, a causal link between pain and fatigue is not proven, and fatigue potentially also enhances pain[152]. Similar to fatigue, pain constitutes a survival mechanism and is an 'alarm system' of ongoing or impending damage. Therefore, pain and fatigue could be two symptoms of a coordinated response of the body to chronic stressors with shared underlying mechanisms. It has also been suggested that pain is an activator rather than a consequence of sickness behaviour, leading to a state of hyperalgesia to enable the body to remain vigilant against an external threat. The alternative model stipulates that peripheral pain leads to the production of cytokines, specifically IL-1β, which then promotes sickness behaviour including fatigue[152].

Psychosocial factors. Adverse life events (whether in early life or adulthood), access to psychosocial support, relationship status, income and educational levels are associated with fatigue in chronic diseases[156]. Additionally, coping strategies and attitudes to illness have been linked with fatigue[157]. One example is learned helplessness, which predicts fatigue in recent-onset inflammatory polyarthritis (that is, symptom duration of ≤2 years)[158]. Tendency to catastrophize, avoidance and negative illness perception or belief are associated with fatigue in many chronic diseases[159,160]. The mechanisms linking these factors to the development or maintenance of fatigue are unclear. However, understanding which psychosocial factors are amenable to intervention and can contribute to effective self-management of fatigue is helpful in making treatment decisions[161]. Indeed, cognitive behavioural therapy has shown promising short-term and long-term benefits in fatigue management in patients with RA[3,161].

Access to data on premorbid fatigue would provide important insights into fatigue in inflammatory rheumatic diseases. Data on premorbid fatigue in patients with inflammatory rheumatic diseases are rarely available. However, premorbid fatigue is an important predictor of cancer-related fatigue[22], an observation that supports the notion that genetic or environmental factors pre-dispose individuals to fatigue.

The study of psychosocial factors in fatigue can be challenging and considered by some researchers to be less exciting or scientific or by some patients even to trivialize the seriousness of fatigue. Stigma around psychological factors can also contribute to prioritization of physical over mental aspects of health, which can influence agendas and resources in research. In care provision, the preference for treating physical over mental health issues can be enacted by both patients and clinicians (for example, in a consultation) and by health-care systems, which could prioritize the commissioning of services for physical health). However, psychological and social factors are not merely epiphenomena and emotions and feelings are no less 'real' than genes and molecules. The concept of 'self' consists of not only a 'neuro-biological self' but also a 'psychological self' that are intricately linked. For instance, a 2020 meta-analysis suggested that mindfulness is associated with the reduction of pro-inflammatory biomarkers such as IL-6 and TNF in blood and in saliva in patients with mood disorders[162]. Research into neurobiological changes associated with thoughts, emotions or feelings as potential therapeutic targets is increasing. However, such a neurobiological approach is not without potential pitfalls if the thoughts and emotions are the triggers of those neurobiological changes. As an illustration, to move one's right hand normally involves a conscious decision, leading to the activation of the left motor cortex, which sends signals to the muscles of the right hand. Electromagnetic stimulation of the left motor cortex also causes our right hand to move, but it is unlikely to cause one to 'make a conscious decision'. Therefore, critical issues in evaluating whether thoughts, emotions and feelings are amenable to therapeutic intervention include determining if they are a cause or consequence of the associated neurobiological changes. The evidence for cognitive-behavioural approaches in reducing fatigue suggests that cognitions can be helpfully re-framed using Socratic questioning to promote a change in beliefs and enhanced coping[161]. Such changed beliefs and enhanced coping lead to better knowledge of why the person thinks the way that they do and how that has been influencing their behaviours (for example, avoiding physical activity because they think it will make their fatigue worse and this thought frightens them). Such changed beliefs and enhanced coping also increase confidence and reactivation in everyday activities. The use of daily activity diaries for patients to monitor their energy expenditure and individualized goal setting lends weight to the argument that a 'personalized' and 'holistic' approach is likely to be needed to optimally manage fatigue[3]. Psychosocial factors in fatigue research deserve more attention but require a multidisciplinary and open-minded approach.

Putative models of fatigue

In this section, we present models of fatigue in inflammatory rheumatic diseases that take into consideration several important clinical observations that are among the most consistent findings (BOX 3). We first explore the 'mechanistic' model — that is, how the different mechanisms discussed in the previous sections might contribute to the pathogenesis of fatigue in inflammatory rheumatic disease. We then discuss the possible physiological and functional value of fatigue, which we term the 'conceptual' model of fatigue.

Learned helplessness
An attributional style whereby a person perceives that they have little control over the events in their life and so responds passively to the challenges that they face.

Mindfulness
The ability to be fully aware of one's thoughts, feelings and sensations without being overly reactive.

Socratic questioning
The technique of asking focused, probing, open-ended questions that encourage reflection.

Mechanistic model of fatigue

Both physical and psychological aspects are important considerations in fatigue research and have many interconnecting functional systems. Stressors elicit a coordinated response from several functional systems, even if the stressor was directed primarily to one system. For instance, a simple cut to the skin triggers responses from multiple systems: vasoconstriction and coagulation to stop blood loss, inflammatory cascades to prevent infection and nociception and SNS activation to alert and prepare the body for danger. In chronic inflammatory rheumatic diseases, multiple functional systems are affected. However, determining how these different functional systems contribute to fatigue is challenging.

As inflammation is the central pathological condition, it is probably the main initiator of fatigue through several interconnecting biological, psychological and physiological mechanisms. The relationship between inflammation and these various mechanisms is likely to be bi-directional. As the underlying inflammatory condition becomes chronic, additional mechanisms, such as neuroendocrine and psychological mechanisms, might become involved in an attempt to establish a new equilibrium. In individuals with fatigue, such maladaptive responses can perpetuate fatigue, and possibly suppress inflammation at the same time, providing a potential explanation for the observation of the inverse relationship between circulating pro-inflammatory cytokines and fatigue severity. At this chronic stage of disease, systemic inflammation might have a much lesser or even no role in the maintenance of fatigue. However, it is likely that an acute disease flare would disturb the established equilibrium, with systemic inflammation again contributing to fatigue.

As some patients experience minimal or no fatigue despite ongoing systemic inflammation[38], inflammation alone is therefore unlikely to be sufficient to cause fatigue. Furthermore, fatigue is prevalent in many chronic conditions in which evidence of an inflammatory basis is weak, and yet very similar predictors of fatigue have been identified, such as pain, depression, autonomic dysfunction, neuroendocrine disturbances and sleep disturbances[22,163]. Moreover, a study of fatigue across five chronic conditions (RA, heart failure, multiple sclerosis, chronic kidney disease and chronic obstructive pulmonary disease) found that the qualitative experience of fatigue is similar across these conditions[20]. In addition, the unpredictability and variability of fatigue experienced by many patients within short timeframes is also difficult to explain with a mechanistic model centred solely on inflammation. We hypothesize that inflammation is one of the many interconnecting mechanisms that contribute to the development of fatigue in response to external or internal stressors to the body (FIG. 1). Consistent with this model, circulating levels of the stress protein HSP-90α are elevated in patients with pSS and fatigue compared with patients with pSS without fatigue[149]. Furthermore, together with depression, plasma concentration of HSP-90α was an independent predictor of fatigue in patients with pSS in multi-regression analysis[149].

The ANS, the HPA axis and the immune system have important functions in the body's response to biological, physiological and psychosocial stressors and are probably the systems mainly involved in the initiation of a complex network of responses that contribute to fatigue. For instance, the ANS is adept at prompting rapid reactions to a threat and engaging the body in anticipatory actions, and therefore is likely to be an important contributor in the day-to-day (even hour-to-hour) variability of fatigue severity. By contrast, the HPA axis and aspects of the immune system are less flexible than the ANS[164], and therefore might contribute to other facets of fatigue.

Different subtypes of fatigue have been proposed, such as 'physical', 'mental' and 'motivational'. However, no formal definition for such fatigue subtypes exists. Furthermore, studies using fatigue questionnaires including different subscales to assess different facets of fatigue that are similar to the proposed fatigue subtypes (for example, the Multidimensional Fatigue Inventory, which has five subscales: general, physical, activity, motivational and mental) did not reveal any clear subsets of patients with different subtypes of fatigue[165–167]. Additional research is needed to determine if these fatigue subtypes exist in inflammatory rheumatic diseases. Our view is that different subtypes of fatigue are different facets of the same symptom, with the relative manifestations of each facet of fatigue depending on the relative contribution of the various mechanisms discussed in this Review (FIG. 3). For instance, autonomic dysfunction might contribute more to the 'physical' than to the 'mental' facet of fatigue, whereas depression might contribute more to the 'mental' and 'motivational' than the 'physical' facet of fatigue. Individual differences in how patients interpret and respond to fatigue complicate the identification of the underlying mechanisms.

Overall, fatigue is a phenomenon that is experienced physically and mentally and is driven by physiological, psychological, behavioural, socio-cultural and temporal factors. The relative contribution of these factors is dynamic and varies between individuals.

Conceptual model of fatigue

What is the physiological or functional relevance of fatigue in chronic conditions? Fundamentally, fatigue describes an inability to achieve the expected or maximal

Box 3 | **Important consideration for developing mechanistic and conceptual models of fatigue**

Below are some of the most consistent clinical observations that should be considered in the development of mechanistic and conceptual models of fatigue.

- Defining features of fatigue include a multifaceted, overwhelming, highly variable and unpredictable nature, and lack of improvement with rest[1].
- Shared predictors of fatigue exist across different diseases, with pain and depression often being the strongest predictors[21–23].
- There is an inconsistent relationship between systemic inflammation and fatigue — perhaps stronger correlation exists in the early stages and during acute flares, but poor, or even inverse, correlation exists with systemic inflammation in the chronic stages[14,25–27,35,38,46,47].
- Fatigue often persists despite the underlying inflammatory rheumatic disease being in remission[37,48].
- Some people with inflammatory rheumatic disease do not experience fatigue despite clear evidence of systemic inflammation[38,39].

levels of performance. Fatigue can be considered as part of the sickness behaviour response to inflammation, and chronic fatigue a consequence of maladaptive sickness behaviour. However, fatigue is not a condition that a person either has or not, but a continuum. Therefore, we believe that fatigue is a bio-psycho-physiological state reflecting the body's overall resource (energy) management strategy. These resources include the energy needed for physical activities and other bodily functions, cognition and emotions, providing an explanation for the multifaceted nature of fatigue. Interestingly, in the aforementioned qualitative metasynthesis of the experience of fatigue, the most prominent themes noted by participants were 'running out of batteries' and having a 'bad life' (defined as restrictions in their ability to engage in physical and social activities)[20], which could be considered consistent with our model in that patients with fatigue perceive a (relative) lack of energy or resources in the context of perceived sustained threat and they adopt a rationing approach to restrict energy use for essential activities at the expense of other activities such as leisure. Furthermore, resource management is part of a coordinated bio-psycho-physiological response to perceived current and anticipated stress. Therefore, fatigue can be accompanied by responses of multiple systems, such as altered ANS activity, altered pain sensitivity, changes to the immune system and changes to diurnal rhythm. Several factors could determine resource management strategies, including perceived current dangers or stressors, the anticipated dangers or stressors, assessment of the body's physiological state (interoception), outcomes from previous exposure to danger or stressors, and various other factors (such as genetic and environmental factors). In addition, perceptions and related behavioural responses are shaped by individual beliefs, expectations and cultural norms.

There are several non-mutually exclusive explanations for the persistence of fatigue despite remission of the underlying disease. First, clinical remission does not always equate to molecular remission. Furthermore, in the context of inflammatory rheumatic diseases, if immune tolerance has not been restored, the 'threat' remains. After all, resource management has as much to do with planning for the future (perceived threats) as for the present. Additionally, irreversible or semi-permanent changes might occur in some of the mechanistic pathways that mediate fatigue (for example, through epigenetic changes, changes in neural connections or depletion of certain proteins or other bioactive substances). Furthermore, other comorbidities that perpetuate fatigue can develop. For instance, chronic fatigue can lead to reduced cardiovascular fitness or sleep disorders. Finally, factors such as genetics, medical or life history, or other psychosocial factors can influence the susceptibility of an individual to fatigue. If fatigue reflects the body's resource management, genetics and past medical and life events might shape interoception and anticipatory danger perception. Psychologically, a patient's coping resources might reduce over time, which can affect their experiences of fatigue[168]. As well as fatigue and flares, other health and life circumstances can affect the coping strategy of the patient. Patients whose inflammatory rheumatic disease is stable can still be inconsistent in their ability to cope with their circumstances and distress can steadily increase. Rather than a gradual deterioration, a patient's ability to cope with their disease might not fully recover from a setback even though their symptoms have returned to the previous state. They might withdraw from social activities, discontinue regular exercise or increase their reliance on medication, all of which can exacerbate or maintain fatigue.

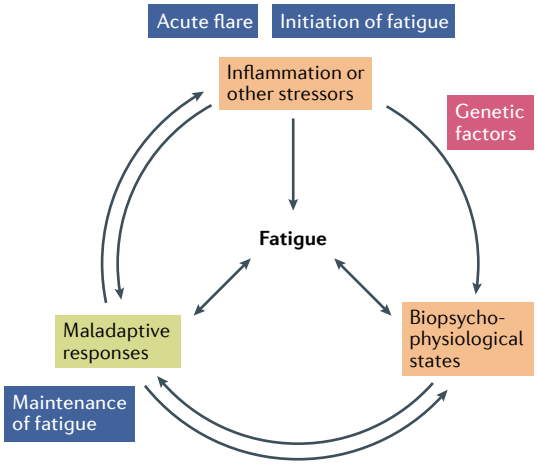


Fig. 3 | **Mechanistic model of fatigue.** Fatigue is driven by physiological, psychological, behavioural, socio-cultural and temporal factors. The relative contribution of these factors is dynamic and varies between individuals. Inflammation, the central pathological condition in inflammatory rheumatic diseases, is probably the main initiator of fatigue through several interconnecting biological, physiological and psychological mechanisms. As the underlying inflammatory condition becomes chronic, maladaptive responses might develop that perpetuate fatigue. During this chronic stage of disease, systemic inflammation might have a much smaller or even no role in the maintenance of fatigue. However, it is likely that an acute disease flare would disturb the established equilibrium, with systemic inflammation again contributing to fatigue. In addition, genetic factors might also influence the biological, physiological and psychological responses to stressors, which, in turn, can affect fatigue pathogenesis.

To summarize, fatigue might be a consequence of the body's resource management strategy favouring conservation over expenditure in response to a stressor; in the context of inflammatory rheumatic disease, the stressor could be inflammation or loss of immunological tolerance, but other factors might also contribute.

Conclusions

Fatigue is a symptom that is prevalent, disabling and difficult to manage for patients with inflammatory rheumatic diseases, as well as those with many other rheumatic and non-rheumatic conditions[5,169,170]. Consensus on how to define and measure fatigue is urgently needed, to provide a broad framework covering the various facets of fatigue. For example, fatigue could be described as a multifaceted phenomenon in which the biological, physiological, cognitive, motivational and emotional state of the body is affected, resulting in

impairment of an individual's ability to function in their normal capacity. Such a definition is still up for debate but could function as a starting point for future discussions. The mechanistic and conceptual models of fatigue presented in this Review could also provide a framework for future research into the mechanisms underlying this condition in the context of inflammatory rheumatic diseases and other diseases. Recommendations on the categories of data to be included and reported in fatigue research will facilitate harmonization of datasets for comparison and meta-analysis. Caution is needed in extrapolating findings based on experimental models of induced physiological fatigue unless they have been replicated in patients with pathological fatigue. This is because many induced fatigue models are transient and self-limiting, unlike fatigue in chronic conditions, which is often long-standing and not relieved by rest. Identification of objective biomarkers of fatigue should improve fatigue assessment and understanding of pathophysiology. Given the complexity of the underlying mechanisms of fatigue, future research should ideally involve multidisciplinary expertise to enable concurrent investigation of different mechanisms and confounding factors. Future studies investigating fatigue pathogenesis could help to identify targets for interventions across multiple chronic diseases. Optimal management of fatigue is likely to require a personalized and holistic approach.

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Detection of microvascular changes in systemic sclerosis and other rheumatic diseases

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Abstract | Morphological and functional analysis of the microcirculation are objective outcome measures that are recommended for use in the presence of clinical signs of altered peripheral blood flow (such as Raynaud phenomenon), which can occur in systemic sclerosis (SSc) and other autoimmune rheumatic diseases. Several advanced non-invasive tools are available for monitoring the microcirculation, including nailfold videocapillaroscopy, which is the best-studied and most commonly used method for distinguishing and quantifying microvascular morphological alterations in SSc. Nailfold videocapillaroscopy can also be used alongside laser Doppler techniques to assist in the early diagnosis and follow-up of patients with dermatomyositis or mixed connective tissue disease. Power Doppler ultrasonography, which has been used for many years to evaluate the vascularity of synovial tissue in rheumatoid arthritis, is another promising tool for the analysis of skin and nailbed capillary perfusion in other autoimmune rheumatic diseases. Other emerging methods include raster-scanning optoacoustic mesoscopy, which offers non-invasive high-resolution 3D visualization of capillaries and has been tested in psoriatic arthritis and SSc. The principle functions and operative characteristics of several non-invasive tools for analysing microvascular changes are outlined in this Review, and the clinical roles of validated or tested imaging methods are discussed for autoimmune rheumatic diseases.

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In the past decade, the science of imaging in rheumatology has undergone rapid and impressive progress and several safe, non-invasive advanced methodologies are now available for assessing the microcirculation in various autoimmune rheumatic diseases[1,2]. Both morphological (static) and functional (dynamic) analysis of the microvasculature of the fingers represent objective outcome measures that can be used in clinical practice. In fact, in the presence of subjective or objective clinical symptoms of altered microcirculation (such as Raynaud phenomenon), investigation of the microvasculature is recommended[3–5].

In systemic sclerosis (SSc) and several other autoimmune rheumatic diseases, including dermatomyositis, antisynthetase syndrome, antiphospholipid syndrome, mixed connective tissue disease (MCTD), systemic lupus erythematosus (SLE) and Sjögren syndrome, Raynaud phenomenon is secondary to the rheumatic disease and is associated with structural alterations to the microvasculature. In fact, in SSc (and some other autoimmune rheumatic diseases), microvascular injury or damage and endothelial cell dysfunction occur at an early stage of pathogenesis and are followed by cellular mechanisms that promote endothelial-to-myofibroblast transition[6]. Microvessels in the skin and visceral organs are progressively deleted as a consequence of the local immune or inflammatory reaction and substituted by regions of fibrosis and ischaemia, resulting in their irreversible damage or failure[6]. Thus, it is important to differentiate between secondary Raynaud phenomenon and primary Raynaud phenomenon, which is purely functional (no morphological microvascular changes); a feat that is possible at a very early stage of disease using non-invasive imaging techniques[2].

A classification system for patterns detectable by nailfold videocapillaroscopy (NVC) in SSc was published in 2000 (REF.[7]). Following this publication, the addition of nailfold capillary abnormalities and telangiectasias visible on the skin to the ACR classification criteria for SSc improved their sensitivity for identifying patients with limited cutaneous SSc and predicting disease progression from 34% to 89%[8]. Furthermore, the overall sensitivity of the ACR classification criteria for SSc increased from 67% to 99% with the addition of nailfold capillary abnormalities identified using a dermoscope[9]. In a 2008 study, the presence of microvascular damage (as detected by NVC) and SSc-specific autoantibodies at baseline could predict the development of definite SSc in 79.5% of

Key points

- Careful detection of the microvasculature can be performed using a variety of advanced imaging methods, which have also enabled new insights into the pathophysiology of several autoimmune rheumatic diseases.
- Nailfold videocapillaroscopy is the gold standard method for distinguishing between primary and secondary Raynaud phenomenon, through the identification of a 'scleroderma' pattern, and for quantifying differences in microvascular morphology.
- The presence of a 'scleroderma-like' nailfold videocapillaroscopy pattern supports the diagnosis of autoimmune rheumatic diseases such as dermatomyositis, mixed connective tissue disease and antisynthetase syndrome.
- Several non-invasive methods, including laser Doppler techniques, enable the detection and quantification of characteristic alterations in peripheral blood perfusion in a number of autoimmune rheumatic diseases.
- Imaging of the microcirculation is recommended at least twice a year for patients with persistent Raynaud phenomenon, and also for the follow-up of patients with selected autoimmune rheumatic diseases.
- Almost all the most common techniques for morphological and functional microvascular evaluation can be used in combinations and managed by rheumatologists, bringing diagnostic power to the rheumatologist's imaging armamentarium.

patients with Raynaud phenomenon who had progressed to SSc at follow-up (up to 20 years later)[10]. The presence of specific NVC abnormalities and autoantibodies in patients with Raynaud phenomenon were independent predictive factors for SSc and indicated a very high probability of developing definite SSc, whereas their absence ruled out this outcome[10]. Finally, in 2013, abnormal capillaroscopic findings were officially included in the ACR–EULAR classification criteria for SSc and contributed to their increased sensitivity and specificity compared with the 1980 ACR SSc classification criteria (0.91 and 0.92 versus the 0.75 and 0.72, respectively)[11].

In addition to a role in classification and diagnosis, images obtained by NVC or using dynamic tools such as the laser Doppler methods seem to facilitate the follow-up of patients with SSc, as well as assessment of the efficacy of targeted therapies[12]. Over the years, the combination of NVC and new tools for dynamic analysis of peripheral blood flow has also enabled new insights into the pathophysiology of some autoimmune rheumatic diseases[6]. In this Review, we describe the principle function of non-invasive tools for the detection of microvascular damage and discuss the clinical role of imaging methods that have been validated and tested in autoimmune rheumatic diseases, suggesting the optimal use for each tool in the diagnosis and follow-up of SSc, dermatomyositis, antisynthetase syndrome, antiphospholipid syndrome, MCTD, SLE and Sjögren syndrome.

Detecting microvascular changes

Morphological microvascular changes

The detection of changes to the morphology of microvessels started in the eighteenth century and, in the past few decades, has undergone an impressive progression in terms of the quality and reliability of the available tools. According to an international survey from 2017 on non-invasive techniques for assessing the microcirculation (both morphologically and functionally) in patients with Raynaud phenomenon, of all the techniques available, NVC was the one that was most commonly used in clinical and research settings by physicians, the majority of whom used this tool in their daily clinical practice[3]. In this section, we cover widefield stereomicroscopy, NVC, dermoscopy and USB video microscopy, as well as optoacoustic imaging (OAI), raster-scanning optoacoustic mesoscopy (RSOM) and optical coherence tomography (OCT). A comparison of the characteristics of different methods for the detection of the microvascular status of the nailfold bed is presented in TABLE 1.

Direct capillaroscopic analysis. The optical microscopy technique of nailfold capillaroscopy was developed almost 50 years ago and was the first well-established method for the assessment of the microvasculature of patients with primary or secondary Raynaud phenomenon or SSc[13]. From the outset, the skin site that was most often evaluated was the periungual region, where capillaries are parallel to the surface of the skin, enabling their full visualization[14]. Over time, a method of nailfold capillaroscopy was developed that combined the high magnification offered by widefield microscopy (FIG. 1a) with a computerized system and frame registration software to fuse together adjacent images, resulting in a panoramic mosaic of the entire nailfold bed[15,16].

The development of NVC by the addition of a manageable video camera to the system enabled the easy evaluation of the nailfold microvascular array and the rapid acquisition of high-quality images. NVC is currently the gold standard method for the detection of capillary status in patients with autoimmune rheumatic diseases who present with Raynaud phenomenon[3]. NVC is a non-invasive, safe and validated method for the detection of morphological microvascular abnormalities and for differentiating between Raynaud phenomenon secondary to autoimmune rheumatic diseases such as SSc, and primary Raynaud phenomenon (FIG. 1b)[17]. The standard methodology for NVC involves counting the number of nailfold capillaries in eight fingers, looking at two fields of view per finger, so that a mean value of capillaries can be reported; thus, the reported capillary number (or alterations) refers to a number of pooled concomitant observations.

In the 2020 classification system for NVC patterns in SSc, the abnormal morphological parameters specific to different stages of disease progression were categorized as 'early', 'active' and 'late' patterns (at a suggested magnification of ×200)[7]. The early NVC pattern in SSc is characterized by some dilated capillaries (<33%) that must include homogeneously dilated 'giant' capillaries (diameter >50 μm) and some microhaemorrhages (<33%), whereas the active NVC pattern is characterized by many giant capillaries (>66%), many microhaemorrhages and moderate capillary loss (<33%), and the late NVC pattern is characterized by the presence of several irregular non-specific capillary dilations (but no giant capillaries), severe capillary loss (>66%) with evident avascular areas and ramified or bushy capillaries (indicative of angiogenesis)[2] (FIG. 1c). Following the publication of the classification system, terminology for reporting NVC findings in autoimmune rheumatic diseases was clearly defined[18] and a scoring system for microvascular changes was also introduced[19]. Furthermore, in 2019, an externally validated, fast-track decision algorithm was

Telangiectasias
Also named spider veins, these are small, dilated blood vessels that can occur near the surface of the skin or mucous membranes.

Table 1 | **Characteristics of techniques and devices for microvascular analysis of the nailfold bed**

Characteristic	Widefield stereomicroscopy	NVC	Dermoscopy	RSOM	OAI
Count of capillary number	Yes	Yes	Yes	Cannot be tested	Yes
Evaluation of capillary shape	Yes	Yes	Yes	Yes	Yes
Evaluation of capillary width	Yes	Yes	No	Cannot be tested	Yes
Direct visualization of capillaries	Yes	Yes	Yes	No	No
Portable device	No	Yes	Yes	No	No
Automatic or semiautomatic capillary count	No	Yes	No	Cannot be tested	Cannot be tested
Image storage	Yes	Yes	No	Yes	Yes
Dedicated software	Yes	Yes	NA	Yes	Yes
Time needed for execution	Short	Short	Short	Medium-to-long	Medium-to-long
Practical device for daily clinical use	Yes	Yes	Yes	No	No
Cost	High	Moderate	Low	Very high	Very high

NA, not applicable; NVC, nailfold videocapillaroscopy; OAI, optoacoustic imaging; RSOM, raster-scanning optoacoustic mesoscopy.

developed by clinical experts to differentiate between 'non-scleroderma' and 'scleroderma' patterns on NVC images and showed excellent reliability when applied by capillaroscopists with different amounts of expertise compared with expert capillaroscopists[20]. This algorithm offers a decision tree designed by experts with the aim of enabling every capillaroscopist to be able to differentiate between different clinically relevant NVC patterns, and particularly between the scleroderma patterns and the non-scleroderma patterns.

Several semi-automated or automated systems have been developed in the past 20 years, mainly for aiding the detection of altered capillaries (abnormal shapes) during NVC image analysis (in the presence or absence of Raynaud phenomenon); however, some of these systems are excessively time consuming to use[21–24]. From a clinical and research perspective, it has been suggested that automated capillary analysis could facilitate large-scale prospective studies using NVC parameters as possible biomarkers of SSc-spectrum disorders[24]. In addition, as the capillary density is sometimes regarded as the most important parameter in nailfold microvascular analysis, several different approaches have been proposed to quickly assess capillary density, including a fully automated software for measuring the absolute nailfold capillary number in SSc NVC images in a few seconds[25–27]. Some of these validated automated systems are included in the software of commercial capillaroscopes and are used in the daily clinical setting, and large studies are ongoing in SSc to investigate the role of these systems further[25].

Other techniques that can be used include dermoscopy, which is a lower-cost alternative to NVC and seems to be a useful clinical tool for those without access to NVC. A dermoscope is a hand-held device (FIG. 1d), and dermoscopy has been mainly used to image SSc-related telangiectasias and to correlate their appearances on the skin with advanced NVC scleroderma patterns (active and late) in the same patient[28] (FIG. 1e). In a multi-centre study, NVC and dermoscopy images were acquired of the nailbeds of individuals with a range of capillary abnormalities to compare the intrarater and interrater reliability of both techniques[29]. The results showed that the agreement between the techniques was moderate, and suggested that although dermoscopy might be comparable with NVC, NVC images were more likely to be classified with a specific grade of severity and were usually classified with a more severe grade than matched dermoscopy images. The findings of a consensus-based evaluation confirmed the promise of dermoscopy for microvascular assessment, but showed that this method often results in non-interpretable and non-specific findings, suggesting that dermoscopy should be restricted to the initial fast screening of nailfold capillaries in the presence of Raynaud phenomenon if NVC is unavailable[30].

Another technique is USB video microscopy, in which a digital microscope is connected to a computer with a standard USB cable and used to record video images. This device could offer rheumatologists an easy-to-use, low-cost, hand-held tool for examining the microvessels at the nailfold bed and skin capillaries where appropriate (such as in psoriatic skin). In a pilot study, USB capillaroscopy could be used to differentiate between patients with SSc and healthy individuals on the basis of capillary width[31]. This finding further supported the potential of USB capillaroscopy as a low-cost, easily accessible clinical tool for the initial assessment of patients with Raynaud phenomenon. However, it is important to note that the resolution and magnification of USB capillaroscopy images are substantially reduced compared with NVC.

Other optical imaging techniques. In the past decade, OAI, also referred to as photoacoustic imaging, has been proposed for use in research into microvascular changes. OAI is a hybrid imaging technique that involves the ultrasonic detection of optical light absorption via the photoacoustic effect, a physical phenomenon in which absorbed light energy is converted into acoustic energy. OAI enables the non-invasive high-resolution 3D visualization of capillaries (for example, in psoriatic or SSc skin lesions), producing data on microvascular changes from which volumetric measurements can be extracted[32].

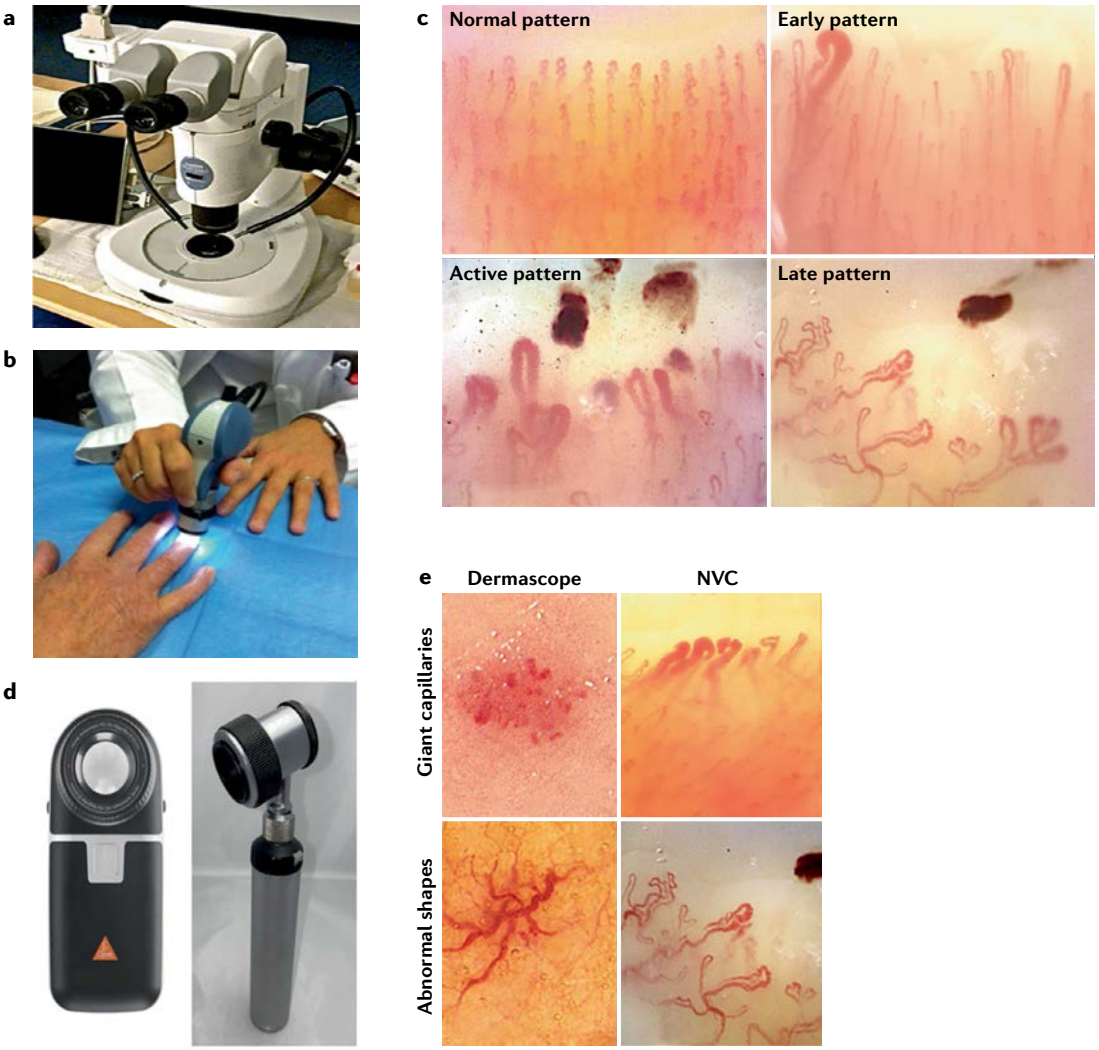

Fig. 1 | **Direct optical imaging of microvascular morphology. a** | A stereomicroscope that has been adapted for nailfold capillaroscopy. **b** | Nailfold videocapillaroscopy (NVC) device being used to analyse the nailfold bed. **c** | The classic 'normal' pattern and three sequential NVC scleroderma patterns ('early', 'active' and 'late'), showing giant (dilated) capillaries, microhaemorrhages (brown spots) and loss of capillaries (×200 magnification). In particular, a decrease in the number of capillaries is evident between the normal and early patterns, and continues up to the more advanced late NVC pattern, which mainly shows specific abnormal shapes, indicative of angiogenesis. **d** | Two different models of dermoscope. **e** | Capillaroscopic images obtained using a dermoscope (×40 magnification) or NVC (×200 magnification), showing giant capillaries in the skin (dermascope) and nailfold bed (NVC), and abnormal shapes indicative of angiogenesis in the skin (dermascope) and nailfold bed (NVC) in systemic sclerosis.

One type of OAI called photoacoustic mesoscopy (also named RSOM) uses widefield optical illumination and a single focused high-frequency ultrasonic transducer to produce a high spatial resolution (FIG. 2A). A 2020 study showed that RSOM is sensitive enough to visualize anatomical differences in the nailfold capillaries in patients with SSc and in healthy individuals[33] (FIG. 2B). In particular, RSOM could complement NVC in borderline cases to gain an alternative view of the microvasculature. Furthermore, a 2021 study showed that the combination of photoacoustic imaging and high-frequency ultrasonography to evaluate oxygenation and skin thickening could help to distinguish between patients with (early) SSc, individuals with primary Raynaud phenomenon and healthy individuals[34]. However, further investigations into the differentiation between primary and secondary Raynaud phenomenon and the grading of SSc progression are required before RSOM can be introduced to the clinic.

OCT is another technology that has been proposed for use in research into SSc[35]. OCT as an imaging method in which a low-intensity infrared laser is used to produce high-contrast images of skin (up to 2 mm deep) with a resolution power of 4–10 µm — this combination of features makes it possible to explore the most superficial layers of the skin and enables the high-speed acquisition of angiograms to visualize microvessels[35]. In a short time frame, OCT can thereby provide 'virtual biopsies' of the examined tissue (skin or nail) with high-resolution images of the microvascular array in patients with SSc[35,36]. To date, the response to therapy for nail disease in patients with psoriasis has been

evaluated by OCT, and OCT has been compared with NVC for analysis of the nailfold microvascular structure in patients with SSc[37]. However, most of these studies are still in the initial stages and more investigation is required. Interestingly, the authors of a 2020 study combined OCT with angiography (OCTA), with the aim of assessing the choroid in the eyes of patients with SSc from a microcirculatory and dynamic point of view[38]. The results showed a substantial impairment of the choroidal blood flow in patients with SSc, even in the absence of ophthalmological symptoms. Further OCTA analysis confirmed impairment of both retinal and choroidal micro-perfusion in patients with SSc, supporting the assumption of a spreading microvascular injury in SSc[39]. Interestingly, on the basis of these results, retinal and choroidal perfusion seem to be independently reduced in the early stages of SSc, suggesting a predictive and clinical value for OCTA in detecting early SSc that will need to be corroborated in larger studies[38,39].

Functional microvascular changes

Evaluation of peripheral blood flow is an important complementary analysis for investigating the functional status of the microvascular system. In this section, we cover infrared thermography (IRT), laser Doppler flowmetry (LDF), laser Doppler perfusion imaging (LDPI), laser speckle contrast analysis (LASCA), power Doppler ultrasonography (PDUS) and fluorescence optical imaging (FOI). A comparison of the characteristics of different methods for the detection of peripheral microcirculation in different body areas in patients with autoimmune rheumatic diseases is presented in TABLE 2.

IRT has been used for more than 50 years in various clinical settings to examine patients with autoimmune rheumatic diseases, including those with Raynaud phenomenon and SSc[40,41]. IRT is a non-invasive and easy method of indirectly evaluating peripheral blood flow with the aid of a camera that displays the temperature of the skin (FIG. 3a). By monitoring response to a change in temperature (cold challenge), IRT can be used to differentiate between healthy individuals and those with Raynaud phenomenon. Specifically, a difference in temperature between the fingertips and the dorsum of the hand is present in patients with an SSc-spectrum disorder (less perfusion in the fingertips and more perfusion at the dorsum of the hand), whereas this gradient is absent in healthy individuals, for whom the fingers and the dorsum of the hand are well perfused. IRT is not fully discriminatory between primary Raynaud phenomenon and secondary Raynaud phenomenon due to SSc; however, as an objective microvascular imaging tool, IRT does have the potential to overcome the diagnostic limitations of self-reported outcome assessments for Raynaud phenomenon and SSc[42]. Furthermore, abnormal thermography has been associated with the development of digital ulcers and disease severity in patients with SSc[43].

A more advanced method for evaluating peripheral blood flow at a single point on the skin is LDF, a non-invasive method that has been in use for at least 40 years[44]. LDF produces an index of skin perfusion by measuring the Doppler shift induced by the way that light is scattered by the flow of circulating red blood cells. LDF is usually used to estimate peripheral blood flow

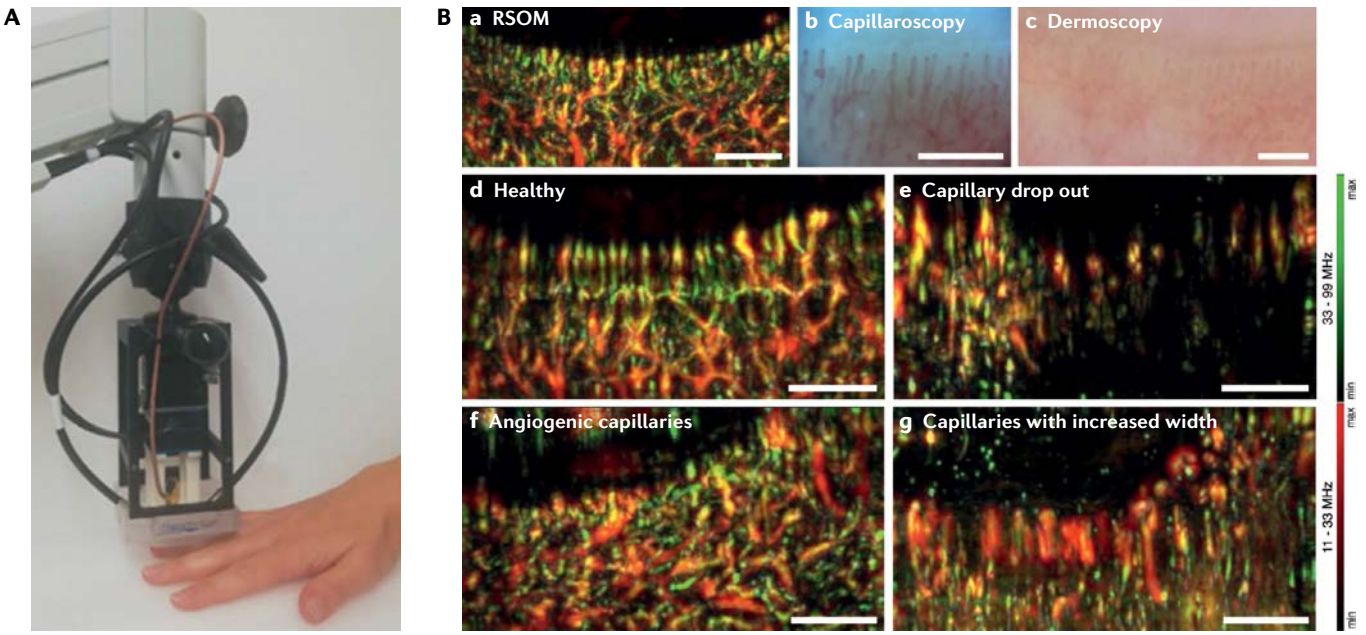

Fig. 2 | **Optoacoustic imaging of microvascular morphology.** **A** | A raster-scanning optoacoustic mesoscopy (RSOM) imaging system (RSOM Explorer C5, iThera Medical, Munich, Germany) that can be used to obtain optoacoustic images of the nailfold bed. **B** | An RSOM image of a healthy nailfold (panel **Ba**) differs considerably from optical images obtained by capillaroscopy (panel **Bb**) or low-magnification dermoscopy (panel **Bc**) (all scale bars 1 mm). RSOM imaging can show differences between a healthy nailfold (panel **Bd**) and nailfolds from patients with systemic sclerosis (panels **Be**–**Bg**), including capillary drop out, angiogenesis and increased width. In the RSOM images, green indicates high frequencies and smaller structures, red indicates low frequencies and larger structures, and yellow shows the merging of both frequencies. Part **A** adapted and part **B** reprinted with permission from Nitkunanantharajah et al.[33], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

Table 2 | **Characteristics of techniques and devices for the analysis of peripheral blood flow**

Characteristic	IRT	LDF	LDI	LASCA	PDUS	FOI
Visual colour grading	Yes	No	Yes	No	Yes	Yes
Software for quantification of blood flow	No	Yes	Yes	Yes	Yes	Yes
Determination of region of interest	No	No	Yes	Yes	Yes	Yes
Applicable to areas of the body other than the hands	Yes	Yes	Yes	Yes	Yes	No
Portable device	Yes	No	No	No	No	No
Contact required	No	Yes	Yes	No	Yes	Yes
Invasive method	No	No	No	No	No	Yes
Time needed for execution	Short	Medium	Medium	Short	Short	Medium
Practical for daily clinical use	Yes	No	Yes	Yes	Yes	No
Useful for clinical research	Yes	Yes	Yes	Yes	Yes	Yes
Cost	Low	Moderate	Moderate	Very high	High	High

FOI, fluorescence optical imaging; IRT, infrared thermography imaging; LASCA, laser speckle contrast analysis; LDF, laser Doppler flowmetry; LDI, laser Doppler imaging; PDUS, power Doppler ultrasonography.

by measuring basal finger temperature and the dilation aptitude of microvessels (to test their expansion capability) after the probe has been heated. Using LDF, patients with SSc were found to have a statistically significant reduction in fingertip blood flow compared with healthy individuals and patients with primary Raynaud phenomenon, both at baseline and after heating the probe[45]. In addition, patients with SSc with the late NVC pattern had a lower blood flow rate than patients with either an early or an active NVC pattern[45]. However, the authors of a 2020 systematic review have emphasized the preliminary validation status of LDF for the assessment of peripheral blood flow in patients with SSc, as well as highlighting regional heterogeneity of skin perfusion as a well-known factor contributing to the poor reproducibility of the technique[46].

LDPI (or simply LDI) was introduced almost 30 years ago and is another non-contact laser Doppler method; however, this technique enables the evaluation of blood flow over a larger area of the skin than LDF[44]. LDPI uses light detectors to pick up visible colour changes caused by the Doppler shift that are induced as light is scattered by moving blood cells. Several studies have suggested that LDPI can support the differential diagnosis between primary and secondary Raynaud phenomenon (present in patients with SSc)[46]. However, in practice, it has been recommended that combining LDPI with other imaging modalities (such as NVC or IRT) would be more efficient than LDPI alone[44].

More recently, high frame rate LASCA (also known as laser speckle contrast imaging) has been introduced for the evaluation of the peripheral blood flow of patients with SSc. This technique has the advantage of enabling the detection of superficial blood flow over large areas of skin in a fast, non-contact, high-resolution manner[47]. Technically, LASCA operates on the principle that when laser light illuminates a tissue, it creates a speckle pattern that is recorded by a charge-coupled device camera and then scrutinized by dedicated software (FIG. 3b). Whereas static areas show stationary speckle patterns, moving red blood cells cause the speckle pattern to fluctuate and appear blurred, and the intensity of the contrast (blurring) can be elaborated and quantified as perfusion units (FIG. 3c). In patients with SSc, a statistically significant negative correlation exists between the extent of nailfold microangiopathy (evaluated by NVC) and blood flow (evaluated by LASCA) in the same area of the finger[48]. Furthermore, a 2018 systematic review of the literature regarding the reliability of LASCA measurements reported good-to-excellent interrater agreement, with similar results in an external validation study[49]. In fact, LASCA is currently thought to be a good tool to use for evaluating the variation in peripheral blood perfusion during long-term follow-up, and can be used to safely monitor digital ulcer evolution during standard treatment in patients with SSc[50–52]. In addition, a 2020 study has shown that by evaluating peripheral blood perfusion with LASCA and the proximal–distal gradient in patients with SSc, it is possible to predict major vascular complications and 5-year mortality[53].

Another method that has been tested for analysing the finger microcirculation is PDUS, which provides images of Doppler amplitude (FIG. 4a,b). For several years, PDUS has been used to evaluate the vascularity of synovial tissue in symptomatic inflamed joints in diseases such as rheumatoid arthritis, and it can also be used to detect the effects of therapies on synovitis[54]. Looking at finger microcirculation, PDUS with a cold challenge is a useful and reliable method for diagnosing Raynaud phenomenon and for discriminating between primary and secondary Raynaud phenomenon[55]. The results of a study to evaluate microvascular involvement at the level of the fingers by using both PDUS and NVC in patients with SSc suggest that PDUS provides potentially complementary information to NVC[56], and further studies are ongoing at the academic division of Rheumatology at Genoa University into the use of ultra-high frequency PDUS to investigate peripheral blood flow in the fingers of patients with SSc with different NVC scleroderma patterns. In addition, high-frequency PDUS and OAI have been compared in a study to examine patients with SSc-associated Raynaud phenomenon and healthy individuals[57]. Interestingly, PDUS resulted in poor differentiation between patients with SSc and healthy individuals, whereas OAI demonstrated improved accuracy at baseline over PDUS, and the oxygenation levels derived using OAI enabled the identification of those individuals with Raynaud phenomenon. Last, indocyanine green (ICG)-enhanced FOI is a relatively unique and quantitative imaging method that has already been investigated for use in the diagnosis of rheumatoid arthritis and psoriatic arthritis and has shown good agreement with other imaging systems (including MRI and ultrasonography)[58]. In this technique, the light from a (near)-infrared spectrum device penetrates the tissue of the hands and stimulates the fluorophore ICG. However, the method requires the intravenous infusion of ICG as a contrast substance, and is thus invasive, although

Proximal–distal gradient
In healthy individuals, perfusion is typically high in the distal fingertips and low in the dorsum of the hands.

it rarely causes allergic reactions[59]. A 2017 study has suggested that FOI can be a helpful tool in the assessment and location of hypoperfused areas in the hands of patients with SSc, particularly when used in association with concomitant NVC findings[60] (FIG. 4c). However, in this study, FOI findings of reduced microcirculation were only associated with the late NVC scleroderma pattern. At present, FOI seems to have several limitations for the analysis of peripheral blood flow in the hands of patients with SSc compared with the validated and fast LASCA technique, and further research is needed.

Microvascular imaging in diagnosis

Systemic sclerosis

NVC patterns and Raynaud phenomenon. NVC can be used to distinguish between primary Raynaud phenomenon and Raynaud phenomenon secondary to SSc (and other autoimmune connective tissue diseases) by detecting morphological patterns of specific abnormalities[20]. In individuals with primary Raynaud phenomenon, NVC images are characterized by capillaries with a 'hairpin shape' morphology or non-specific abnormalities (such as tortuosity or crossing), similar to those visible in healthy individuals. Capillaries usually show morphological and structural homogeneity at a density of 10–12 capillaries per linear millimetre, capillary branches have a diameter of <20 μm and there is a lack of specific abnormalities (such as giant capillaries or a combination of loss of capillaries and neoangiogenesis)[20]. Therefore, only non-specific abnormalities in the density, dimensions or morphology of capillaries may be present for a diagnosis of primary Raynaud phenomenon. Conversely, specific abnormalities are required for the differential diagnosis of SSc in the presence of secondary Raynaud phenomenon, including giant capillaries (capillaries with a diameter of >50 μm) microhaemorrhages, progressive capillary loss and angiogenesis[61,62].

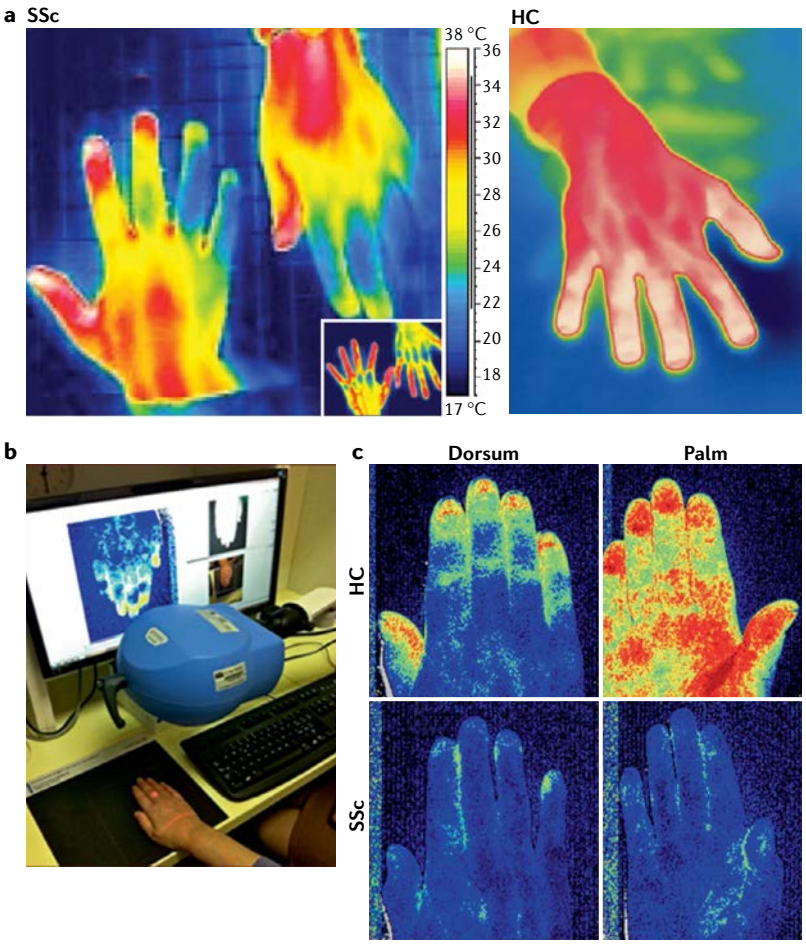

Fig. 3 | **Imaging of microvascular function. a** | Thermal imaging of cutaneous blood vessel function (blood flow) showing finger skin temperature (blue range of 20–24°C) after exposure to cold temperatures in a patient with systemic sclerosis (SSc) and secondary Raynaud phenomenon (left) and in a healthy individual (right; labelled HC). The small inset shows the fingers of the same patient with SSc before the cold test. **b** | A laser speckle contrast analysis (LASCA) device being used to examine the hand. **c** | Examples of LASCA images from a healthy individual (labelled HC) and a patient with SSc. Blue indicates less perfusion and red indicates more perfusion. The dorsum and palm of the hands show a reduction in blood flow in the patient with SSc. Part **a** adapted from Cutolo et al.[2], Springer Nature Limited.

The predictive value of NVC in SSc. In patients with SSc, the early, active and late NVC scleroderma patterns (FIG. 1c) might have prognostic value[63–65]. NVC analysis can be used to provide a simple prognostic index for digital trophic lesions in SSc that is suitable for day-to-day clinical use[66]. For this scoring system, analysis was limited to the mean score for capillary loss calculated over eight NVC fields-of-view (one field per finger over eight fingers). A concomitant early pilot study was the first to demonstrate an association between NVC patterns at baseline and future severe, peripheral vascular and lung involvement in patients with SSc, with stronger odds ratios associated with worsening NVC scleroderma patterns[67]. Another study reported that capillary diameter is an independent predictor of the occurrence of SSc-associated Raynaud phenomenon; the results showed that it was unlikely for individuals with primary Raynaud phenomenon to progress to SSc if their average capillary diameter on NVC images remained below 30 μm for a mean time of 3 years[68]. These results suggest that NVC qualitative and/or quantitative analysis at least twice a year should be essential for the follow-up of individuals with Raynaud phenomenon in order to detect very early morphological signs associated with autoimmune rheumatic diseases.

In a large international prospective cohort study (the CAP study), in which detailed NVC evaluation was carried out on 623 patients with SSc, the mean number of capillaries per millimetre in the middle finger of the dominant hand and the number of digital ulcers at enrolment were identified as major independent risk factors and predictors of the development of new digital ulcers[69]. Furthermore, in a 2020 systematic review that investigated if NVC could be an outcome measure in future algorithms for SSc-related interstitial lung disease (SSc-ILD), cross-sectional and longitudinal studies reported that SSc-ILD is inversely associated with a decreased number of capillaries on NVC[64]. In one of the longitudinal studies, the active and late NVC scleroderma patterns in particular were associated with the presence of SSc-ILD[70]. Similarly, in a cross-sectional study, incident SSc-ILD was linked with progressive

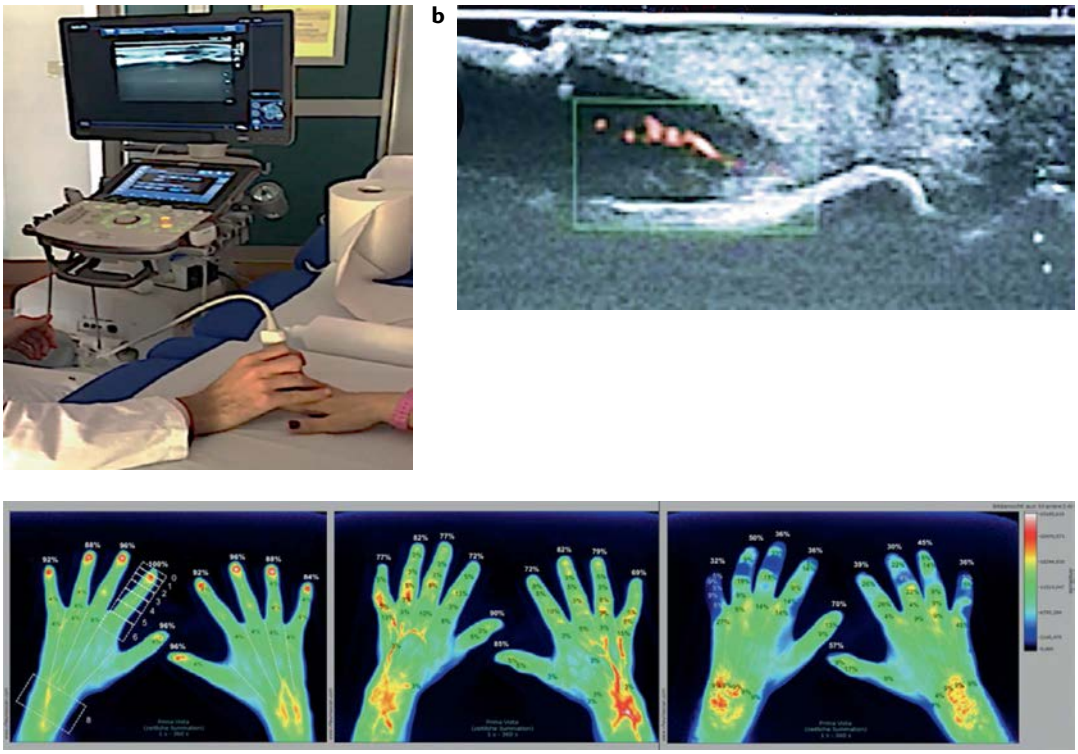

Fig. 4 | **Alternative imaging tools for assessing peripheral blood flow. a** | An ultrasound scanner with power Doppler equipped with a high-frequency device (33 MHz) can be used for the detection of nailbed capillary perfusion by holding the transducer sagittal to each finger. **b** | Image of capillary vascularization obtained by power Doppler ultrasonography at the nailbed area (red spots inside the green rectangle). The perfusion is always evaluated as the resistive index. **c** | Hand regions of initial enhancement for indocyanine green-enhanced fluorescence optical imaging in a healthy individual (left), a patient with limited cutaneous systemic sclerosis (centre) and a patient with diffuse cutaneous systemic sclerosis (right). Part **c** adapted from Friedrich et al.[60], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

loss of capillaries and with the development of active or late NVC scleroderma patterns, suggesting that NVC might represent an outcome measure that could be used for screening algorithms for incident or progressive SSc-ILD[71]. Furthermore, a 2020 study showed that a lower mean number of capillaries measured by NVC at baseline was associated with higher disease activity after 6 months of follow-up in patients with SSc, as well as with reduced survival[72].

Combining morphological and functional analysis. Several investigations have demonstrated that microvascular involvement in SSc, assessed either qualitatively by NVC patterns or semi-quantitatively by NVC scoring, negatively correlates with skin blood perfusion[45]. As discussed in the section on functional microvascular changes, microvascular blood flow can be assessed and quantified using a variety of direct and indirect functional methods. For example, a promising NVC system has been developed that simultaneously evaluates both nailfold capillary structure and finger blood flow automatically[73]. However, larger studies that include patients with early SSc and long-term follow-up are required, and the sensitivity of blood flow measures needs to be compared with other physiological measurement techniques (such as IRT, LDI or LASCA) to calibrate and validate the dynamic challenges reported in initial investigations[73].

Interestingly, in a 2003 study, IRT and LDI images of patients with Raynaud phenomenon were taken in three regions of interest from each hand: the dorsum, the tip of the middle finger and the 'gradient' between these regions[40]. IRT and LDI results correlated poorly in this study and it was concluded that one functional technique for the analysis of blood flow cannot substitute for the other. In fact, one possible explanation for this poor correlation is that LDI is more sensitive to blood flow variations than IRT[40].

The results of a 2020 study that used a combination of IRT and NVC in the diagnostic algorithm for Raynaud phenomenon suggest that IRT is useful for distinguishing healthy individuals from patients with Raynaud phenomenon, whereas NVC is useful for differentiating primary Raynaud phenomenon from the secondary form[74]. In addition, the authors concluded that IRT could be used to detect which fingers are the most affected by the disease and determine where to focus the NVC analysis. Furthermore, a pilot study has been conducted to investigate the possible relationship between ^{99m}Tc-pertechnetate hand perfusion scintigraphy (HPS) and NVC findings in SSc[75]. The results suggested that the use of ^{99m}Tc-pertechnetate HPS could improve the evaluation of vascular damage in patients with SSc and that both methods could be integrated for microvascular assessment; however, a direct relationship between NVC and ^{99m}Tc-pertechnetate HPS findings was lacking.

Dermatomyositis

The importance of endothelial injury, perivascular inflammation and capillary loss, together with abnormal NVC findings, seem to be common in all idiopathic inflammatory myopathies[76]. At baseline, an early severe microangiopathy is often present in patients with dermatomyositis, characterized by the appearance of some major capillaroscopic abnormalities, including giant capillaries, some microhaemorrhages, severe capillary loss and angiogenesis[77,78]. When present at the same time, these abnormalities are described as a 'scleroderma-like' pattern (FIG. 5a), as they are all associated with SSc microangiopathy, but the pattern presented (for example, in dermatomyositis) is not classifiable as any of the specific NVC scleroderma patterns (early, active or late). Data from a cohort of patients with dermatomyositis with a mean disease duration of 4 years at their first NVC assessment, showed that dermatomyositis-related microangiopathy might be characterized by more stable microvascular changes over time than the progressive changes detectable by NVC in patients with SSc[79]. In addition, positivity for anti-Jo1 antibodies did not affect the NVC pattern in this cohort of patients with dermatomyositis.

Antisynthetase syndrome

Antisynthetase syndrome is a heterogeneous autoimmune disease that is characterized by the classic clinical triad of arthritis, myositis and ILD. In addition, individuals with this condition are frequently positive for anti-Jo1 antibodies[80] and can have Raynaud phenomenon or altered microcirculation, fever and 'mechanic's hands', as well as abnormal NVC findings[81]. The results of a multi-centre study in which NVC findings were evaluated in a large population of patients with antisynthetase syndrome reported the characteristics and frequency of the scleroderma-like pattern in such patients, and its correlation with the clinical features of the disease[82]. The most prevalent NVC finding seems to be the presence of angiogenesis, whereas the full scleroderma-like pattern was observed in 35.3% of patients and was associated with the presence of anti-Jo1 antibodies (an association also observed in patients with dermatomyositis).

Antiphospholipid syndrome

In patients with antiphospholipid syndrome, the occurrence of small-vessel occlusions (thrombotic microangiopathy) and/or alterations in loop diameter on NVC images has been frequently described[83,84]. In patients with antiphospholipid syndrome who had microhaemorrhages on NVC analysis, anti-cardiolipin antibody positivity and anti-β2 glycoprotein 1 (β2GP1) antibody positivity were associated with thrombotic events. However, although IgA anti-β2GP1 antibodies were detected in patients with antiphospholipid syndrome who had NVC-detectable microangiopathy, they lacked any statistically significant association with thrombotic complications, unlike their IgG and IgM counterparts[85]. The most common NVC pattern in antiphospholipid syndrome seems to be the presence of parallel microhaemorrhages arranged perpendicularly to the nailfold bed (a 'comb-like' pattern) (FIG. 5b), which are associated with the presence of anti-cardiolipin antibodies[86]. The association between the presence of the comb-like NVC pattern and a diagnosis of active antiphospholipid syndrome was confirmed in a large study in 2020 that included 384 patients ($P < 0.001$)[87].

Connective tissue diseases

Raynaud phenomenon is the most common presenting symptom in patients with overlap syndromes such as undifferentiated connective tissue disease (UCTD) and MCTD. NVC enables the detection of early microvascular abnormalities that are essential for a preclinical differential diagnosis of secondary Raynaud phenomenon in these patients. In a study of 447 patients with autoimmune connective tissue diseases, 186 patients had Raynaud phenomenon, and a possible NVC scleroderma pattern was identified in 18.3% of patients with UCTD and 26.9% of patients with dermatomyositis[88]. In fact, in patients with UCTD, MCTD or dermatomyositis, the most common NVC pattern is the scleroderma-like pattern[77,78,89]. In MCTD, the presence of the capillaroscopic scleroderma-like pattern can provide valid support for a differential diagnosis from other autoimmune rheumatic diseases. The results of a 2019 study following ten patients with MCTD suggest that nailfold microvascular damage does not seem to progress during 3 years of follow-up[89]. These patients with MCTD (70% showed the scleroderma-like pattern) had statistically significantly lower numbers of dilated or giant capillaries, and higher numbers of total and normal capillaries than age-matched and disease duration-matched patients with SSc at the initial NVC assessment[89]. The authors of the study concluded that the identification of a validated or specific NVC pattern (as opposed to the non-specific scleroderma-like pattern) does not yet seem to be possible for MCTD[89]. More work is needed to determine precisely which capillaroscopic pattern might predict the evolution of MCTD into another connective tissue disease.

a 'Scleroderma-like' pattern

b 'Comb-like' pattern

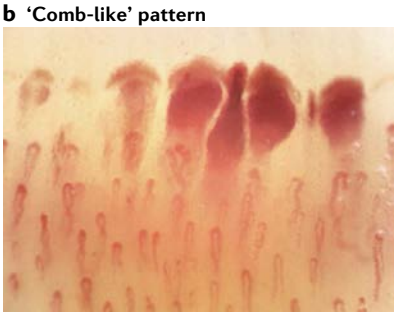

Fig. 5 | **Nailfold capillaroscopic patterns in rheumatic diseases other than systemic sclerosis. a** | Example of a 'scleroderma-like' nailfold videocapillaroscopy (NVC) pattern seen in diseases such as dermatomyositis, showing 'giant' capillaries, some microhaemorrhages, severe capillary loss and angiogenesis (abnormal shapes). These abnormalities are also all individually signals of one or more of the 'early', 'active' and 'late' NVC scleroderma patterns; however, in the 'scleroderma-like' pattern these signals are mixed together at the same time. **b** | The most frequent NVC pattern observed in patients with active antiphospholipid syndrome is the presence of parallel microhaemorrhages arranged perpendicularly to the nailfold bed in a 'comb-like' pattern.

Systemic lupus erythematosus

A 2018 systematic review of 40 studies reported that several NVC findings are more prevalent in patients with SLE than in healthy individuals, including tortuous capillaries, haemorrhages and abnormal capillaries[90]. The semi-quantitatively determined nailfold capillaroscopic (NFC) score was higher in patients with SLE than in healthy individuals, and disease activity correlated with the NFC score in at least seven studies. Furthermore, the NFC score correlated with non-specific abnormal morphologies in one study and with microhaemorrhages in a further study, whereas frequent episodes of Raynaud phenomenon and gangrene correlated with the presence of dilated capillaries. A possible correlation between the presence of anti-SSA/Ro antibodies and a reduced number of capillaries was also noted[90]. By contrast, a 2020 systematic review of nailfold capillaroscopy findings in childhood-onset SLE (cSLE) identified only six studies and reported inconclusive results[91]. In this analysis, no difference was found between patients with cSLE and healthy individuals regarding capillary density. However, a 2021 study on patients with cSLE reported that giant capillaries, abnormal capillary morphology and capillary haemorrhages were all present at NVC, as has already been shown for adults with SLE[92]. It is important to note that studies in children are a special case, and experience in performing NVC on children is slowly increasing[92]. Looking beyond NVC, a study has been carried out to evaluate peripheral blood perfusion by LASCA in various cutaneous regions of the hands and face in patients with SLE and patients with primary Raynaud phenomenon[93]. Individuals with SLE or Raynaud phenomenon had statistically significantly lower peripheral perfusion levels than healthy individuals in three hand areas (the fingertips, palms and periungual region), and a positive correlation was found between a lower density of capillaries (as evaluated by NVC) and blood perfusion in patients with SLE[93].

Sjögren syndrome

The presence of NVC abnormalities in patients with Sjögren syndrome seems to be related to the hypothesis that individuals with such abnormalities might actually have secondary Sjögren syndrome associated with other autoimmune conditions[94]. An investigation that included 61 consecutive patients with primary Sjögren syndrome showed that the scleroderma-like NVC pattern was present in a small but significant proportion of these patients (7 patients, 11.5%), and that 6 of them had secondary Raynaud phenomenon, suggesting a systemic involvement[94]. However, in a study in which NVC and LDI were used to evaluate patients with autoimmune rheumatic diseases, no specific pattern seemed to be evident for secondary Sjögren syndrome[95]. Nevertheless, the results obtained by the combined use of NVC and LDI were useful for the detection of secondary Raynaud phenomenon and for distinguishing if the reduced blood flow was linked to the presence of an autoimmune rheumatic disease. In general, only a small number of studies have investigated the role of NVC in Sjögren syndrome, and a 2020 systematic review confirmed that, at present, no specific NVC pattern has been identified for primary Sjögren syndrome[96].

Microvascular imaging in follow-up

Assessment of peripheral blood flow (for example, using LDI or LASCA), together with analysis of morphological status of capillaries (using NVC), might be useful for the evaluation of the effects of specific therapeutic agents, as has been proposed for both psoriasis and SSc[12]. Vascular modifications are an important feature in psoriatic skin lesions in patients with psoriatic arthritis, and skin psoriatic inflammation can be monitored by LASCA (increased blood flow compared with the non-involved skin) (FIG. 6a) and by NVC (to measure the number of skin capillaries in the same psoriatic lesion) (FIG. 6b). The effects of therapies on microvascular involvement in psoriatic lesions can also be monitored by LASCA (FIG. 6c,d). A previous study that used LDI to study plaque psoriasis revealed that the cutaneous perfusion within homogenous-appearing psoriatic lesions is actually very heterogeneous[97]. A further study gained insight into the relationship between skin perfusion and changes in biomarkers in skin tissue samples by evaluating the therapeutic effects of calcipotriol-betamethasone dipropionate ointment on the microvasculature in psoriatic lesions and on the expression of psoriasis-related biomarkers, including IL-17 and CD31 (REF.[98]). The perfusion intensity, as evaluated by LDI, decreased in all skin lesions during therapy, and the expression levels of all investigated psoriasis-related biomarkers in the treated lesions reduced to the same levels as those in uninvolved skin in those patients who had a good response to the treatment[98].

Notably, NVC has been used to monitor responses to treatment in patients with SSc. One of the earliest studies showed that the long-term treatment of patients with SSc with an endothelin 1 receptor antagonist (bosentan) in combination with the vasodilator iloprost reduced the progression of NVC damage over a 3-year follow-up period[99]. A further study evaluated the long-term effects of endothelin 1 antagonism on both peripheral blood perfusion (evaluated by LDF) and capillary morphology (evaluated by NVC) in patients with SSc[100]. The results showed, by using concomitant morphological and functional microvascular analysis, an increase in capillary number and dilation capacity, as well as fingertip blood perfusion. Similar results for NVC qualitative analysis (scleroderma patterns) were observed in a study of patients with SSc who were treated with bosentan and the phosphodiesterase inhibitor sildenafil, in which the frequency at which the advanced NVC scleroderma patterns (active and late) occurred reduced after 6 months of treatment[101]. A further study quantified the absolute nailfold capillary number per linear millimetre on NVC images and detected fingertip blood flow by LDF in patients with SSc during long-term therapy with bosentan and iloprost[102]. In this study, patients with SSc who had received >4 years of combined therapy showed a progressive recovery in both microvascular morphology and function, as well as improved clinical outcomes, independent of disease severity, suggesting that the combined analysis (morphological and functional) might increase the precision of evaluation of the treatment's effect. Interestingly, a pilot study has also used NVC evaluation to show that two treatment courses of rituximab in patients with early diffuse cutaneous SSc

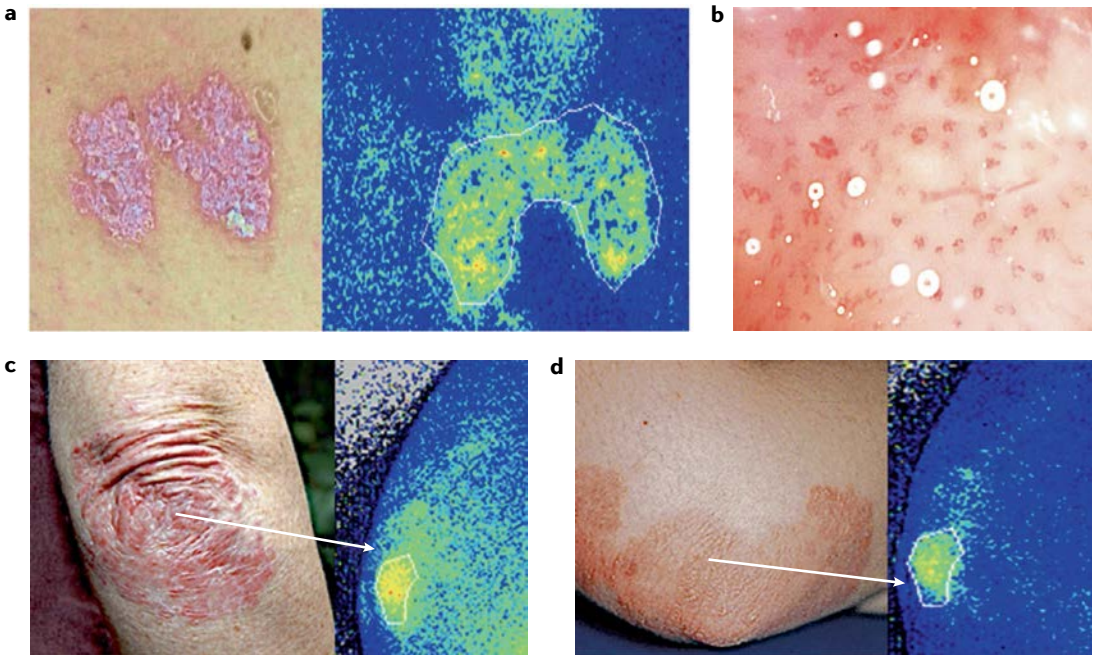

Fig. 6 | **Combined microvascular morphology and functional blood flow analysis.** Laser speckle contrast analysis (LASCA) and nailfold videocapillaroscopy (NVC) can be combined to analyse the blood flow and capillary status in the skin. **a** | Images of the skin of a patient with psoriatic arthritis (PsA), showing a skin psoriatic lesion (left) and the same lesion evaluated by LASCA (right), revealing increased blood flow (green and yellow) compared with the non-lesional skin (blue). **b** | NVC of the skin capillaries in the same psoriatic lesion (×200 magnification). **c** | Images of the skin of a patient with PsA before therapy, showing the affected elbow (left) and the LASCA analysis (right). **d** | The same elbow of a patient with PsA after 8 weeks of targeted treatment showing skin lesion improvement (left) and a reduction in the blood flow after therapy (right).

who are receiving stable background methotrexate therapy might be able to stabilize and halt the progression of microvascular damage over 12 months, particularly the loss of capillaries[103].

Looking specifically at peripheral blood flow, a 2018 study that used LDF to evaluate microvascular function in patients with Raynaud phenomenon secondary to SSc showed an increase in peripheral blood flow after 3 days of iloprost infusion, but the increase faded within a week of the final infusion[104]. In addition, LASCA has been tested for use as a tool to safely monitor the progress of digital ulcers in patients with SSc by assessing blood perfusion at the level of the skin lesion during standard treatment[105], and as a method of monitoring the kinetics of blood flow in patients with SSc following ischaemia or during cold challenge as a secondary end point in clinical trials for treatments such as phosphodiesterase inhibitors[106,107]. Overall, imaging of the microcirculation offers an important non-invasive and safe complementary tool for use in diagnosis and monitoring the progression of several autoimmune rheumatic diseases, and the diffusion of imaging technologies will enable larger inter-centre investigations to be carried out.

Conclusions

The availability of safe and validated methods for morphological analysis of the microvasculature (static imaging), as well as those for analysing peripheral blood flow (dynamic imaging), is of fundamental importance in supporting the diagnosis of several autoimmune rheumatic diseases.

Direct nailfold capillaroscopy (particularly NVC) is one of the most frequently used techniques in both an everyday clinical setting and in research to detect microvascular damage in patients with autoimmune rheumatic diseases. The low proportion of studies using other indirect methodologies, such as OAI, RSOM and OCT, suggests that these techniques are currently mostly used as research tools in specialist SSc centres. Among the most advanced methods of evaluating peripheral blood flow in autoimmune rheumatic diseases (mainly in SSc), LDI (or LDPI) is the most commonly used method in clinical practice in specialist centres, followed by LASCA, which is increasingly being used. By contrast, PDUS and FOI are still currently employed mainly as research tools to evaluate the finger blood flow in SSc. The quantitative evaluation of the images obtained (such as the capillary count on NVC images or perfusion units in LASCA and LDI analysis), which is often handled by the rheumatologists themselves, together with the evaluation of the disease status, seems to be of great usefulness in detecting the severity of microvascular damage at baseline and in evaluating changes during follow-up, including the effects of targeted therapies. In conclusion, the most popular imaging methods for the detection of microvascular changes and their related scoring systems represent objective and validated complementary outcome measures for several autoimmune rheumatic diseases, and particularly for SSc.

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Targeting interferon-γ in hyperinflammation: opportunities and challenges

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Abstract | Interferon-γ (IFNγ) is a pleiotropic cytokine with multiple effects on the inflammatory response and on innate and adaptive immunity. Overproduction of IFNγ underlies several, potentially fatal, hyperinflammatory or immune-mediated diseases. Several data from animal models and/or from translational research in patients point to a role of IFNγ in hyperinflammatory diseases, such as primary haemophagocytic lymphohistiocytosis, various forms of secondary haemophagocytic lymphohistiocytosis, including macrophage activation syndrome, and cytokine release syndrome, all of which are often managed by rheumatologists or in consultation with rheumatologists. Given the effects of IFNγ on B cells and T follicular helper cells, a role for IFNγ in systemic lupus erythematosus pathogenesis is emerging. To improve our understanding of the role of IFNγ in human disease, IFNγ-related biomarkers that are relevant for the management of hyperinflammatory diseases are progressively being identified and studied, especially because circulating levels of IFNγ do not always reflect its overproduction in tissue. These biomarkers include STAT1 (specifically the phosphorylated form), neopterin and the chemokine CXCL9. IFNγ-neutralizing agents have shown efficacy in the treatment of primary haemophagocytic lymphohistiocytosis in clinical trials and initial promising results have been obtained in various forms of secondary haemophagocytic lymphohistiocytosis, including macrophage activation syndrome. In clinical practice, there is a growing body of evidence supporting the usefulness of circulating CXCL9 levels as a biomarker reflecting IFNγ production.

Autoinflammatory diseases are characterized by unexplained episodes of inflammation in which an innate immune response is activated in the absence of triggers or by trivial triggers, such as cold exposure[1]. An increasing body of evidence points to a major role of IL-1β overproduction (through inflammasome activation) or of increased type I interferon production and signalling in different forms of autoinflammatory diseases[2]. Hyperinflammation refers to the excessive or disproportionate activation of inflammation, involving innate and/or adaptive immunity, in response to a 'reasonable' stimulus (for example, viral infection), eventually leading to host tissue damage. Haemophagocytic lymphohistiocytosis (HLH) in its various forms is a typical example of a hyperinflammatory disease that is classified within the umbrella term 'cytokine storm syndrome'[3].

Interferon-γ (IFNγ; also known as type II interferon or immune interferon) is a cytokine with multiple effects on innate and adaptive immunity. IFNγ has been implicated in several, potentially fatal, diseases. In this Review, we discuss the involvement of IFNγ in diseases for which there are sufficient data from animal models and translational research in humans to suggest a pathogenetic role for this immune modulator. These diseases include hyperinflammatory diseases, such as primary HLH (pHLH), various forms of secondary HLH (sHLH), including macrophage activation syndrome, and cytokine release syndrome, as well as other immune-mediated diseases, such as systemic lupus erythematosus (SLE). Furthermore, we discuss currently available biomarkers of IFNγ production, which might be important in patient management, as circulating IFNγ levels do not always reflect the extent of IFNγ overproduction in tissue. Finally, we summarize promising preliminary clinical data regarding the use of IFNγ neutralizing agents for the treatment of various forms of sHLH, including macrophage activation syndrome.

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Key points

- Hyperinflammation is characterized by excessive systemic inflammation resulting from an exaggerated immune response to physiological stimuli, which eventually leads to tissue damage.
- Haemaphagocytic lymphohistiocytoses (HLHs) are hyperinflammatory diseases and are considered canonical cytokine release syndromes.
- Studies of interferon-γ (IFNγ) biology have shed light on the role of this immune modulator in various hyperinflammatory diseases, such as the different forms of HLH, and also in other immune-mediated diseases.
- IFNγ hyperproduction is a feature of the different forms of HLH, and IFNγ neutralization decreases disease severity and reverts lethality in mouse models of HLH.
- As serum IFNγ levels do not always reflect IFNγ overproduction in tissue in patients, efforts are underway to identify biomarkers that are easily measurable in blood and reflect tissue IFNγ activity.
- Therapeutic IFNγ neutralization shows promising results in early clinical trials in patients with various forms of HLH.

Interferon-γ biology

IFNγ is produced predominantly by activated T helper 1 (T_H1) cells and natural killer (NK) cells, and, to a lesser extent, by macrophages, dendritic cells (DCs) and B cells[4,5]. T-bet, a transcription factor whose expression is induced by IFNγ and IL-12, is the master regulator of T_H1 cell responses. T-bet regulates chromatin remodelling at the *IFNG* locus (which encodes IFNγ), resulting in upregulated expression of IFNγ, and represses the differentiation of T_H2 cells and T_H17 cells[6]. In NK cells, IFNγ expression is induced by type I interferons (which include IFNα and IFNβ) through STAT4-mediated signalling[7]. $CD8^+$ T cells also produce IFNγ[4]. Production of IFNγ by NK cells and $CD8^+$ T cells is typically also stimulated by the combination of IL-12 and IL-18 (REF.[8]). Importantly, neither IL-12 nor IL-18 alone are sufficient to induce IFNγ production by NK cells[8,9].

IFNγ signalling. IFNγ is an homodimer that signals via the IFNγ receptor (IFNGR), which is a heterodimer consisting of two subunits, the high-affinity subunit IFNGR1, which is required for ligand binding and signal transduction, and the low-affinity subunit IFNGR2, which is required primarily for signal transduction[10] (FIG. 1). IFNGR1 and IFNGR2 are constitutively associated with the tyrosine kinases Janus kinase 1 (JAK1) and JAK2, respectively.

When IFNγ binds to IFNGR1, IFNGR2 subunit is recruited, and the intracellular carboxyl termini of both receptor subunits are phosphorylated by JAK1 and JAK2 (REF.[10]) (FIG. 1). This phosphorylation induces a conformational change in the receptors, creating binding sites for signal transducer and activator of transcription (STAT) proteins, primarily STAT1 (REF.[10]). JAK1 and JAK2 then phosphorylate Tyr701 of STAT1, leading to STAT1 homodimerization and translocation to the nucleus, where these homodimers bind to γ-activation site sequences in the promoters of target genes[10]. Major response genes transactivated via this signalling pathway include the transcription factors IFN-regulatory factor 1 (IRF1) and IRF5 (REF.[11]). IFNGR is present on a wide range of immune and non-immune cells, including $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, plasmacytoid DCs, macrophages, platelets, eosinophils, endothelial cells, epithelial cells, hepatocytes, fibroblasts and keratinocytes[10] (FIG. 2).

IFNγ in innate and adaptive immunity. IFNγ is the main activator of macrophage polarization to a pro-inflammatory (M1) phenotype[12]. IFNγ activates the transcription of several hundred interferon-stimulated genes, including IRF1 and IRF5. IRF1 is expressed at low levels in resting macrophages but IFNγ induces high IRF1 expression in M1 polarized macrophages, where it translocates to the nucleus and induces the expression of pro-inflammatory genes and the production of reactive nitrogen and oxygen intermediates that are crucial for inhibition of intracellular pathogens[12,13]. M1 macrophages also express high levels of IRF5, which stimulates the expression of pro-inflammatory cytokines and suppresses production of the anti-inflammatory cytokine IL-10 (REF.[11]).

IFNγ is also a central player in other macrophage functions, including promoting antimicrobial activity via upregulation of microbicidal products and enhancing phagocytic ability[14]. IFNγ is involved in granuloma formation, as shown by its presence in granuloma-containing lymph nodes and by inhibition of granuloma formation by anti-IFNγ antibodies[15]. IFNγ also upregulates production of chemokines and adhesion molecules, such as intercellular adhesion molecule 1 and vascular cell adhesion molecule 1, directing lymphocytes to sites of inflammation[16]. IFNγ induces expression of the three CXC receptor 3 (CXCR3) ligands, CXCL9, CXCL10 and CXCL11, in activated macrophages, DCs and non-haematopoietic cells, such as endothelial cells and fibroblasts[17]. These chemokines, in turn, attract effector T cells and memory B cells to sites of inflammation[17].

IFNγ influences the early phases of the adaptive immune response by promoting DC maturation and T cell differentiation, with an important role in inducing and driving T_H1 cell responses and suppressing T_H2 and T_H17 cell responses[18,19]. IFNγ plays a crucial role in orchestrating humoral immunity by regulating T follicular helper (T_{FH}) cell formation and maintenance[20]. IFNγ overproduction leads to the formation of germinal centres[20], specialized structures in secondary lymphoid organs where B cells proliferate, differentiate and undergo selection. Transient expression of T-bet during T_{FH} cell differentiation imprints these cells to produce IFNγ[21]. IFNγ also acts on B cells, inducing expression of T-bet, which favours the expression of B cell lymphoma-6 (BCL-6) protein, the master regulator of the germinal centre reaction, creating a positive feedback loop that reinforces T_{FH} cell differentiation[22] and promotes immunoglobulin class switching in B cells[23].

IFNγ in disease

Given the pleiotropic effects of IFNγ in immune and inflammatory responses, it is not surprising that IFNγ is involved in multiple diseases. Below, we summarize the data pointing to a pathogenetic role of IFNγ in classic hyperinflammatory diseases, including several forms of HLH, in cytokine release syndrome, in lung disease associated with systemic juvenile idiopathic arthritis (sJIA),

and in other immune-mediated conditions, such as Blau syndrome and SLE (TABLE 1; BOX 1).

pHLH, sHLH and MAS. The term HLH refers to clinical syndromes of hyperinflammation associated with excessive activation and expansion of T cells and macrophages, and with hypercytokinaemia[24]. Symptoms include persistent fever, splenomegaly, cytopenias, liver dysfunction and coagulation abnormalities with hypofibrinogenaemia and hypertriglyceridaemia. Hyperferritinaemia is the characteristic laboratory sign in HLH. Tissue haemophagocytosis, typically in bone marrow, and increased levels of soluble IL-2 receptor reflect macrophage and T cell activation, respectively. Central nervous system (CNS) involvement and liver failure are common. HLH occurs most often in children but is increasingly recognized in adults and ultimately leads to multiple organ failure and death if untreated[25].

HLH comprises primary and secondary forms: pHLH is a constellation of genetically inherited errors of immunity, mainly autosomal recessive diseases, all of which are caused by mutations in genes whose products are involved in granule-mediated NK and T cell cytotoxicity[24]. Signs and symptoms of pHLH are defined in the HLH-2004 diagnostic criteria[26] (TABLE 2). Following immunochemotherapy, the only curative treatment is haematopoietic stem cell transplantation (HSCT).

Acquired forms of HLH are referred to as sHLH and are clinically indistinguishable from pHLH. sHLH can be associated with a variety of infections, such as those caused by herpes viruses, *Leishmania donovani* and H1N1 influenza virus. sHLH can also be observed in association with malignancies, most often lymphoma, or with rheumatic diseases. Finally, sHLH can also occur in the absence of any evident underlying disease[27]. When associated with rheumatic diseases, sHLH is referred to as MAS[28]. MAS most frequently presents in sJIA and its adult counterpart, adult-onset Still's disease (AOSD)[29], but is also observed in SLE and Kawasaki disease, albeit less frequently[29]. Symptoms and clinical manifestations of MAS are similar to those of other forms of sHLH[28], with some peculiarities owing to the inflammatory nature of the underlying diseases. These differences are captured by the MAS classification criteria[30]. As chronic inflammation in sJIA is associated with an increase in platelet count and fibrinogen levels, threshold values for these parameters are indeed higher than those of the HLH-2004 pHLH criteria (TABLE 2).

Both pHLH and sHLH represent an unmet medical need and a major diagnostic and treatment challenge for managing physicians. Despite substantial progress during past decades, particularly with the introduction of immunochemotherapy, mortality associated with HLH and MAS remains high. The 5-year probability of survival is approximately 60% in patients with pHLH

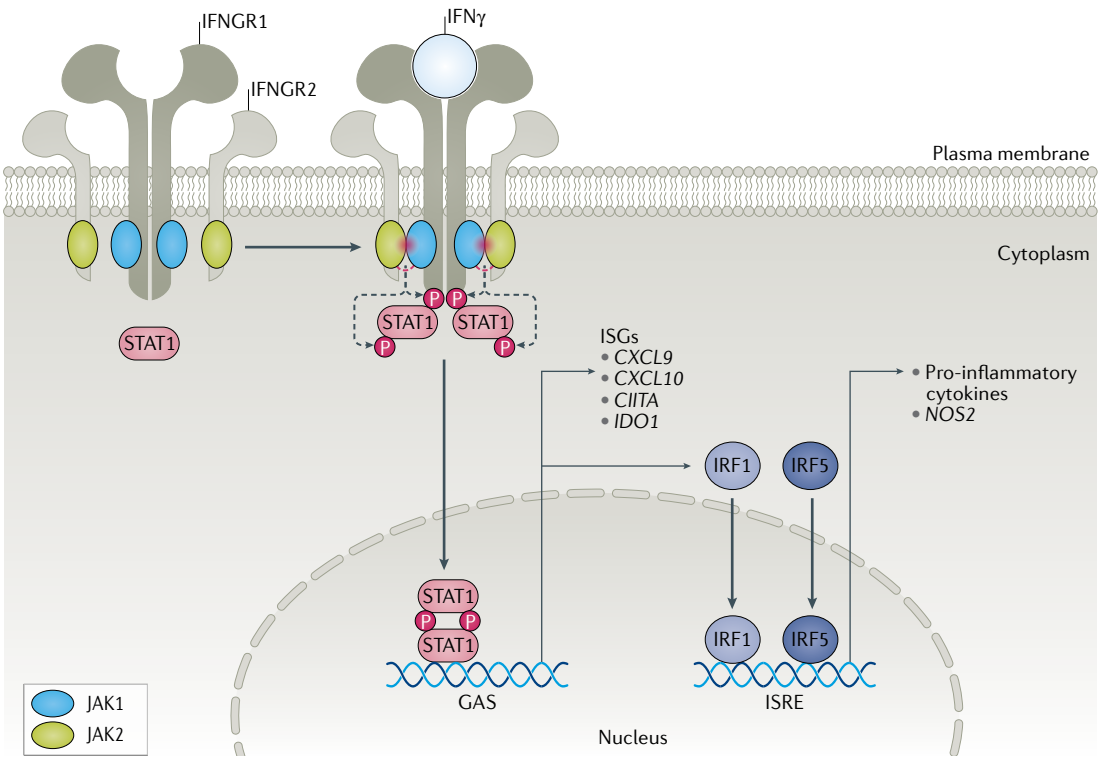

Fig. 1 | **The IFNγ signalling pathway.** Binding of interferon-γ (IFNγ) to the receptor subunit IFNGR1 leads to engagement of IFNGR2, which activates Janus kinase 1 (JAK1) and JAK2 to undergo autophosphorylation, and to phosphorylate both IFNGR subunits. This leads to recruitment of signal transducer and activator of transcription 1 (STAT1), which binds to IFNGR1 and is phosphorylated in the C terminus by JAK1 and JAK2. Phosphorylated STAT1 monomers form homodimers and translocate to the nucleus, where they bind to IFNγ-activation site (GAS) elements in IFN-stimulated genes (ISGs) and activate their transcription. These target genes include *IRF1* and *IRF5*, which function as transcription activators or repressors of various target genes by binding to an interferon-stimulated response element (ISRE) in their promoters.

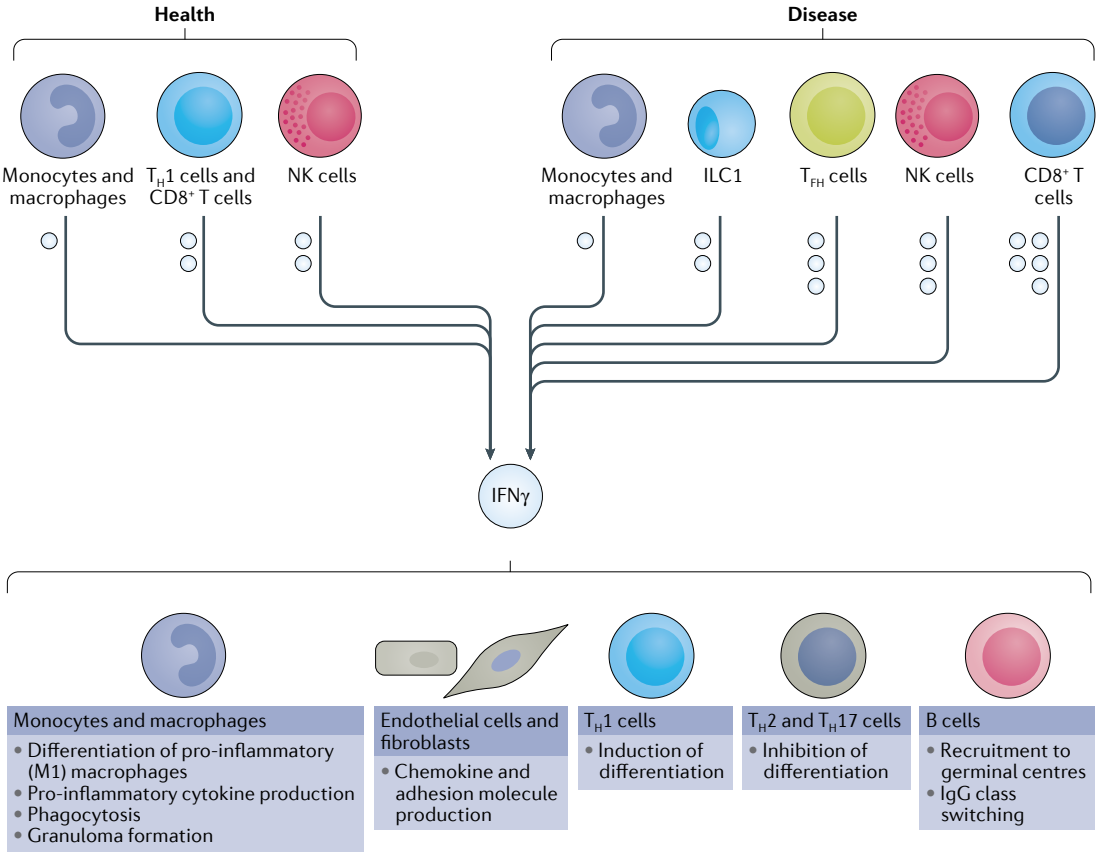

Fig. 2 | **Sources and roles of IFNγ in health and disease.** In health, interferon-γ (IFNγ) is produced by multiple immune cells, including activated T helper 1 (T_H1) cells and CD8⁺ T cells, as well as natural killer (NK) cells, monocytes and macrophages. IFNγ is important for innate and adaptive immune responses to various challenges. In disease states in which the production of IFNγ is increased, additional cell types are involved in the production of IFNγ, including CD65^{bright} NK cells, T follicular helper (T_{FH}) cells and type 1 innate lymphoid cells (ILC1s). The number of circles beside each arrow corresponds roughly to the relative amount of IFNγ released by each cell type. IFNγ exerts multiple effects on immune responses, including innate immunity through its effects on monocytes and macrophages, adaptive immunity through its effects on T cells and B cells, and the inflammatory process through its effects on non-immune cell types, such as endothelial cells and fibroblasts. IgG, immunoglobulin G.

who are receiving first-line immunochemotherapy[31]. The 5-year survival probability in patients with sHLH is variable depending on the patients' background disease and age and is lower in adults (approximately 60%) than in children (70–90%).

IFNγ levels in pHLH, sHLH and MAS. Serum IFNγ levels are elevated in patients with pHLH, sHLH or MAS and correlate with several disease activity parameters[32–37]. In a large-scale prospective study of 756 patients with fever, patients diagnosed with HLH had markedly elevated circulating IFNγ levels, suggesting that increased IFNγ is a feature of HLH-associated hyperinflammation but not of other febrile illnesses[36]. In patients with pHLH or sHLH, IFNγ levels were elevated and correlated with disease parameters, such as the levels of alanine aminotransferase (ALT), aspartate aminotransferase, lactate dehydrogenase (LDH) and ferritin, and the extent of neutropenia, thrombocytopenia and hypofibrinogenaemia[38]. IFNγ levels have also been correlated with increases in liver enzyme levels in pHLH[34]. With regard to infection-associated sHLH, high circulating levels of IFNγ have been found in patients with Epstein–Barr virus (EBV)-associated sHLH and in those with non-EBV-associated sHLH, and IFNγ levels correlated with the extent of neutropenia, thrombocytopenia and elevations in ferritin, LDH and ALT levels[33,39]. In patients with MAS, circulating levels of IFNγ and of IFNγ-induced chemokines, but not of IL-1β or IL-6, were markedly elevated and correlated with ferritin levels[40]. In active sJIA without MAS, the circulating levels of IFNγ and IFNγ-induced chemokines were not elevated, demonstrating that activation of the IFNγ pathway only occurs during MAS[40]. These observations show that circulating IFNγ levels are consistently elevated in HLH and are related to disease activity parameters.

Animal models of pHLH. Studies in mice have provided substantial evidence that IFNγ overproduction has a central role in pHLH pathogenesis. Viral infection of mice deficient in perforin, an important cytotoxic component of the innate immune response, triggers the production of IFNγ, mainly by virus-specific CD8⁺ T cells, which drives increased production of pro-inflammatory

cytokines (TNF, IL-6 and IL-18) and results in all features of human HLH[41], as well as lethality. Administration of an anti-IFNγ antibody reverses lethality in these mice[41,42]. Similarly, viral infections trigger all features of human HLH and result in increased circulating IFNγ levels in mice deficient for other genes associated with pHLH, such as syntaxin 11 or Rab27a[43,44], and administration of an anti-IFNγ antibody improved signs of disease and survival in Rab27a-deficient mice[44]. In wild-type mice, prolonged IFNγ administration directly induces macrophage haemophagocytosis, leading to a severe consumptive anaemia[45]. Incidentally, lymphocytic choriomeningitis virus was used as an infection trigger in these studies, and IFNγ was found to have a smaller role than TNF in the immunopathology of the HLH-like disease in perforin-knockout mice when mouse cytomegalovirus (CMV) was used as a trigger[46]. Studies in mouse models of pHLH have also implicated other pathways that might be upstream of IFNγ overproduction in HLH pathogenesis, including an IL-2-dependent signalling pathway, which is involved particularly in immunological features (that is, expansion and activation of $CD8^+$ T cells and $CD4^+$ T cells), and an IL-33–IL1RL1-dependent pathway[47,48]. Altogether, these data demonstrate that IFNγ is elevated in mouse models of pHLH and that IFNγ neutralization markedly improves clinical features and reverses lethality in several models. Pathways complementary to IFNγ hyperproduction are also relevant in mouse models of pHLH. In fact, HLH features can present in the absence of increased IFNγ production in humans, supporting the involvement of other pathways in pHLH pathogenesis. Indeed, some patients who lack IFNGRs and, consequently, IFNγ activity, develop, albeit rarely, a clinical phenotype that mimics HLH[47,49].

Animal models of sHLH. Mouse models of sHLH have focused primarily on recapitulation of infection-associated sHLH. A mouse model of sHLH, in which infection is mimicked by repeated stimulation of TLR9 with CpG DNA, shows the salient features of human sHLH and increased IFNγ production[50]. In this model, various features of sHLH, such as anaemia, thrombocytopenia, splenomegaly and liver inflammation, are reduced or absent in IFNγ-knockout mice[50]. IFNγ administration is required for these IFNγ-knockout mice to develop sHLH, showing that cooperation between TLR9 and IFNγ-dependent signalling is necessary for the development of sHLH-like disease; this cooperation involves the expansion and activation of myeloid cells[51]. Furthermore, IFNγ tissue levels strongly correlated with the extent of systemic hypercytokinaemia, and IFNγ neutralization led to

Table 1 | **Involvement of IFNγ overproduction in the pathogenesis of immune diseases**

Disease (classification)	Symptoms	Blood IFNγ levels	Blood biomarkers of IFNγ activity	Site of IFNγ overproduction[a]	Effects of IFNγ deficiency or neutralization in mouse models
HLH (hyperinflammatory)					
pHLH	Fever, splenomegaly, cytopenia, liver damage, activation of the coagulation cascade, and hyperferritinaemia	Elevated[32,34–36,38]	CXCL9 and neopterin	Liver[35]	Reverses lethality in mouse models; improvement in cytopenia, splenomegaly, macrophage infiltration of the liver, BM histiocytosis, CNS involvement, hyperferritinaemia and hypercytokinaemia[41,44]
sHLH		Elevated[33,35–37,39]	CXCL9 and neopterin[39,96,97]	Liver[35,37]	Mouse models of sHLH not lethal[39,50,51]; improvement in cytopenia, hyperferritinaemia, hypercytokinaemia and liver inflammation
MAS		Elevated[37,40]	CXCL9, pSTAT1 and neopterin[37,40,95,99,101,105–109]	Liver[35,37] and lymph node[139]	Reverses lethality in mouse models; improvement in hyperferritinaemia, liver damage, hypofibrinogenaemia and hypercytokinaemia[57,58,60]
Cytokine release syndrome (hyperinflammatory)	Fever, hypovolaemic shock, respiratory failure and hyperferritinaemia	Elevated[70,71]	NA	NA	Elevated levels of IFNγ and CXCL9
SLE (autoimmune)	Anti-dsDNA antibodies, cytopenia, nephritis, lymphadenopathy, splenomegaly and hypocomplementaemia	Elevated[84,86]	CXCL9[b], neopterin[b] and pSTAT1 (REFS[90,102])	NA	Reverses lethality in mouse models; improvement in proteinuria, glomerular inflammation and anti-dsDNA antibody titre[80,83]
Blau syndrome (inflammatory granulomatous)	Arthritis, uveitis and dermatitis	NA	Neopterin[103]	Lymph node, synovium and skin[75]	Inhibits development of uveitis[74]

BM, bone marrow; CNS, central nervous system; dsDNA, double-stranded DNA; HLH, haemophagocytic lymphohistiocytosis; MAS, macrophage activation syndrome; NA, not available; pHLH, primary HLH; pSTAT1, phosphorylated signal transducer and activator of transcription 1; sHLH, secondary HLH; SLE, systemic lupus erythematosus. [a]Or overexpression of interferon-γ (IFNγ)-induced genes or proteins. Refers to the site in which IFNγ overproduction has been detected in tissue biopsy samples from patients; it is likely that IFNγ overproduction occurs in other tissues that have not been investigated by biopsy. [b]Also in the urine of patients with active nephritis.

Box 1 | **IFNγ involvement in the pathogenesis of hyperinflammatory or immune-mediated diseases**

The involvement of interferon-γ (IFNγ; encoded by *IFNG*) in the pathogenesis of various hyperinflammatory and other immune-mediated diseases (such as haemophagocytic lymphohistiocytosis, macrophage activation syndrome, cytokine release syndrome, systemic lupus erythematosus and Blau syndrome) has been investigated in vivo in mice. These studies involve IFNγ overexpression in wild-type mice or IFNγ neutralization or *IFNG* deletion in relevant disease models. The different cell types and/or organs that are affected and the symptoms that are observed with IFNγ modulation are as follows:

Liver and spleen

- Hepatic dysfunction (ranging in severity from transaminitis to liver failure)
- Splenomegaly
- Liver haemophagocytosis

Bone marrow

- Haemophagocytosis
- Histiocytic and T cell infiltrates

Blood

- Hyperferritinaemia
- Hypercytokinaemia
- Cytopenias (thrombocytopenia and neutropenia)
- Hypofibrinogenaemia (resulting from disseminated intravascular coagulation)
- Anti-double-stranded DNA antibodies

Lymph nodes

- Germinal centre formation (induced by T follicular helper cell accumulation) and class switching to immunoglobulin G in B cells
- Autoantibody production, including anti-double-stranded DNA antibodies

Other affected sites

- Granuloma formation
- Lung inflammation

substantial reduction of pro-inflammatory cytokine and CXCL9 levels and improved disease parameters[39].

Somewhat different observations were reported in another model of infection-associated sHLH, BALB/c mice infected with mouse CMV. In this model, some HLH-like features are triggered by high viral load and are more severe in IFNγ-knockout mice than in wild-type mice[52]. This increased severity is associated with markedly higher viral counts and is reversed by antiviral treatment but not by therapeutic depletion of T cells[52,53]. Therefore, the HLH-like features seem to be a consequence of disseminated infection rather than hyperinflammation caused by hyperactivated immune responses. In contrast to hyperinflammation induced by an artificial trigger of infection, such as CpG DNA, in this mouse CMV-BALB/c model, the pathogenetic effects of disseminated infection are not clearly distinguishable from the immunological consequences of immune response hyperactivation.

Animal models of MAS. Transgenic mice engineered to overexpress IL-6 (IL-6TG mice) develop a chronic inflammatory condition that is similar to sJIA and characterized by pathologically high levels of IL-6 (REFS[54,55]). When challenged with TLR ligands, which mimic bacterial or viral infection, IL-6TG mice show increased cytokine production and mortality[56]. IL-6TG mice challenged with lipopolysaccharide develop features typical of human MAS[56]. This mouse model replicates the events leading to the development of MAS in patients with active sJIA, which is often triggered by infections. IL-6TG mice with MAS-like disease show substantial upregulation of the IFNγ pathway, with increased levels of IFNγ mRNA and phosphorylated STAT1 in liver and spleen, and mRNA of the IFNγ-inducible chemokine CXCL9 in the liver, spleen and blood[57]. Administration of an anti-IFNγ antibody improved survival and resulted in reduced serum levels of ferritin, fibrinogen, ALT and pro-inflammatory cytokines, including IL-1β, IL-6, TNF and CXCL9 (REF.[57]).

Overproduction of IL-18 has been proposed to contribute to MAS development, based on the observations that very high IL-18 levels are a risk factor for the development of MAS in patients with sJIA and that serum IL-18 levels increase even further in active MAS[58,59]. TLR9 stimulation induces more severe HLH-like disease in mice deficient for the endogenous IL-18 inhibitor IL-18-binding protein (IL-18BP) than in wild-type mice. IL-18 seems to act upstream of IFNγ, as HLH signs and symptoms in these mice are blocked by anti-IFNγ antibody administration[60]. Overproduction of IL-18 and perforin deficiency seem to act synergistically: whereas HLH features emerge only after a viral trigger in perforin-deficient mice, hyperinflammation associated with $CD8^+$ T cell expansion develops spontaneously when perforin-deficient mice are crossed with IL-18 transgenic mice, overproducing IL-18. Both HLH symptoms and $CD8^+$ T cell expansion are improved by IFNγ blockade[61], consistent with IFNγ acting as a downstream mediator.

Together, the observations in mouse models established against a background of high production of IL-6 or IL-18, which are characteristic of sJIA and AOSD, point to IFNγ as a potential mediator in MAS in the context of sJIA and AOSD.

Incomplete or relapsing MAS. Although MAS in patients with sJIA usually presents with acute onset and full-blown disease, there is a subset of patients who do not fully meet the criteria for sJIA-associated MAS, who are considered to have chronic incomplete MAS[62]. Many of these patients have predominantly liver involvement. The prominent histopathological feature is portal and sinusoidal infiltrates, consisting predominantly of $CD8^+$ T cells that overexpress IFNγ and of highly activated Kupffer cells showing haemophagocytosis[35,37]. The liver biopsy samples of these patients show increased levels of phosphorylated STAT1 and highly increased expression of IFNγ-induced genes, whereas the expression of other canonical pro-inflammatory cytokines, such as IL-6 and TNF, are not increased[37]. The increased expression levels of IFNγ and IFNγ-inducible proteins within tissues affected by the disease are consistent with data in animal models showing that IFNγ neutralization or genetic deletion of *IFNG* substantially decreases inflammatory infiltrates in the liver[39,50,57].

MAS and lung disease in sJIA. MAS has recently been linked to a seemingly new life-threatening chronic pulmonary disease in patients with sJIA, termed sJIA lung

disease (sJIA-LD)[63,64]. The vast majority of patients with sJIA-LD have recurrent episodes of MAS, against a background of sJIA with predominant systemic features and high serum IL-18 levels[63].

Lung histopathology revealed extensive interstitial lymphocytic infiltrates, as well as alveolar inflammation with macrophage accumulation, and features resembling pulmonary alveolar proteinosis (PAP), a disorder of alveolar macrophage dysfunction[63,64]. Patients with sJIA-LD lack genetic, serological or functional evidence of granulocyte–macrophage colony-stimulating factor pathway dysfunction, which is a typical feature of familial or autoimmune PAP[63]. By contrast, sJIA-LD usually presents with prominent septal expansion caused by lymphocytic infiltration and with fibrosis with evidence of remodelling[63,64]. The bronchoalveolar lavage fluid from these patients typically contains elevated levels of IL-18, CXCL9 and CXCL10. Transcriptional profiling of lung tissue biopsy samples revealed upregulated expression of IFNγ-response and T cell-activation gene networks[63].

The association of MAS with sJIA-LD suggests that the cytokine milieu driving macrophage dysfunction in MAS might also promote alveolar macrophage dysfunction. Consistent with the hypothesis that IFNγ is involved in sJIA-LD pathogenesis, mice with T cell-restricted overexpression of T-bet (which drives IFNγ overproduction) show bone marrow macrophage dysfunction that results in haemophagocytosis and, of note, alveolar macrophage dysfunction with development of PAP-like lung pathology and marked lymphocytic interstitial infiltrates[65]. Furthermore, in the TLR9 repeated stimulation model, lung inflammation and injury and reprogramming of alveolar macrophage phenotype were present, were more pronounced following experimental triggering of recurrent episodes and were decreased in mice with macrophages that were insensitive to IFNγ[66].

Cytokine release syndrome. Cytokine release syndrome has been described after infusion of antibody-based anti-cell therapies (for example, blinatumomab) and is the most frequent serious complication of T cell-engaging immunotherapeutic agents (such as chimeric antigen receptor T cells), occurring in up to 70% of patients. Cytokine release syndrome is characterized by high fever, hypotension, hypoxia and respiratory distress[67,68]. Organ function impairment, including liver and renal failure, as well as CNS dysfunction, can occur and rapidly progresses to multiple organ failure and death. Laboratory abnormalities of this syndrome include cytopenias, coagulopathy with hypofibrinogenaemia, and very often hyperferritinaemia. Animal models of cytokine release syndrome are characterized by a rapid rise in blood IFNγ levels[69]. Similar elevations of IFNγ levels in the context of a cytokine expression pattern resembling that of HLH and MAS are present in humans with cytokine release syndrome and correlate with disease severity[70,71]. As the current treatment approach of administering IL-6 inhibitors is far from effective in all patients, alternative therapies are needed[70].

Blau syndrome. Blau syndrome is a rare systemic inflammatory disease characterized by early onset granulomatous inflammation in joints, eyes and skin and is associated with gain-of-function variants in nucleotide-binding oligomerization domain 2 (*NOD2*). Blau syndrome is considered the monogenic equivalent of idiopathic sarcoidosis[72]. In pluripotent stem cells from patients with Blau syndrome, IFNγ acts as a priming signal for subsequent pro-inflammatory stimuli by upregulating *NOD2* expression, inducing nuclear factor-κB activation and leading to increased production of pro-inflammatory cytokines[73]. In a mouse model of Blau syndrome involving ocular injection of the bacterial cell wall component muramyl dipeptide, NOD2-dependent ocular inflammation (that is, uveitis) is accompanied by a substantial elevation of IFNγ levels in the eyes, and IFNγ deficiency inhibits uveitis development[74]. Granulomas from patients with Blau syndrome show IFNγ overexpression in macrophages and T cells[75]. Current treatment of Blau syndrome includes glucocorticoids with inhibitors of TNF or IL-1, which are only partially effective, and the incidence of visual loss remains high[76].

Systemic lupus erythematosus. SLE is the prototypical autoimmune disease and is characterized by antibodies directed against self nuclear antigens. In SLE, nucleic acid-containing immune complexes accumulate in tissues and trigger inflammation and damage in multiple organs, including skin, heart, CNS and kidneys, with glomerulonephritis being a leading cause of death.

Table 2 | **Clinical and laboratory features of hyperinflammation that raise suspicion of HLH or MAS**

Features of HLH or MAS	Criteria for primary HLH[a]	Criteria for MAS in sJIA[b]
Fever	Presence of fever	Presence of fever
Splenomegaly	Splenomegaly	Not used
Hyperferritinaemia	Ferritin ≥500 µg/l	Ferritin ≥684 ng/ml
Bone marrow involvement: leukopenia, neutropenia, anaemia and thrombocytopenia	Cytopenia (affecting ≥2 lineages) Haemoglobin <90 g/l Platelets $<100\times10^9$/l Neutrophils $<1.0\times10^9$/l	Platelets $\leq181\times10^9$/l
Haemophagocytosis	Haemophagocytosis in bone marrow, spleen or lymph nodes	Not used
Liver involvement: elevated ALT, AST, LDH and triglycerides	Triglycerides ≥265 mg/dl	AST >48 U/l; triglycerides >156 mg/dl
Activation of the coagulation cascade: elevated D-dimers and reduced fibrinogen	Fibrinogen ≤150 mg/dl	Fibrinogen ≤360 mg/dl
T cell activation	Soluble CD25 ≥2,400 U/ml	Not used
Defective cytotoxicity	Low or absent natural killer cell activity	Not used

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase. [a]The diagnosis of primary haemophagocytic lymphohistiocytosis (HLH) is established according to the criteria of the Histocyte Society if either a molecular diagnosis consistent with HLH is made or five of the eight criteria listed in this column are fulfilled[26]. [b]A febrile patient with known or suspected systemic juvenile idiopathic arthritis (sJIA) is classified as having macrophage activation syndrome (MAS) if the following criteria established by the EULAR, ACR and Paediatric Rheumatology International Trials Organisation (PRINTO) are met: ferritin ≥684 ng/ml and two of the criteria listed in this column are met[30].

Although attention has been given mainly to the involvement of type I interferons, several observations implicate IFNγ in SLE pathogenesis. Transgenic mice overexpressing IFNγ in the skin develop anti-double-stranded DNA (anti-dsDNA) and anti-histone antibodies and immune complex-mediated glomerulonephritis[77]. IFNγ signalling is also elevated in lupus-prone (NZB x NZW)F_1 mice[78]. Administering IFNγ to these mice accelerates the development of glomerulonephritis and reduces survival[79,80]. Conversely, treatment with anti-IFNγ antibodies improves survival[79]. Subsequent studies in IFNγ-knockout or IFNGR-knockout mice confirmed that deletion of either of these genes protects against early death and glomerulonephritis and results in a marked reduction in serum anti-dsDNA antibodies and anti-histone antibodies[81,82]. Of note, these studies revealed that genetic modification resulted in better outcomes than administration of anti-IFNγ antibodies[79,80], suggesting that incomplete IFNγ neutralization and/or timing of these antibody treatments might affect efficacy. More recent studies using B cell-specific deletion of *IFNGR* demonstrated that IFNγ acts on T cells and B cells to promote the spontaneous generation of germinal centres, where autoreactive B cells expand and undergo immunoglobulin class switch. This deletion also caused the delayed appearance of anti-nuclear antibodies, proteinuria and glomerular deposition of IgG molecules and a decrease in glomerular inflammatory infiltrates[22,83].

Elevated *IFNG* expression and serum IFNγ levels have been observed in blood from patients with SLE[84–86]. Increased IFNγ levels are detectable years before the onset of SLE, and before the onset of autoantibody positivity and increased type I score, suggesting a crucial early role for IFNγ in SLE pathogenesis[87]. The IFN signature observed in blood of patients with SLE, which was previously attributed to IFNα activity, has a complex regulation, with other interferons, including IFNγ, playing a role in this signature[88]. Interestingly, in patients with SLE, the expression of genes induced by IFNγ fluctuates over time, whereas that of genes regulated mainly by IFNα remains stable[88]. Although IFNα and IFNγ signalling pathways share STAT1, their regulation in vivo in disease states only partially overlaps. To add another layer of complexity, IFNα and IFNγ regulate each other's expression: for example, in mouse models of viral infection, IFNα is crucial for the production of IFNγ[89].

Patients with active proliferative nephritis have a profound elevation in IFNγ-regulated chemokines in kidney tissue and urine[90]. The cells responsible for the production of IFNγ and these chemokines seem to be kidney-infiltrating $CD8^+$ T cells, NK cells and myeloid cells[90]. These observations in mice and humans point to a potential early role of IFNγ in the pathogenesis of abnormal T cell and B cell immunity in SLE. An involvement of other targetable pathways is also suggested by the lack of considerably improved outcomes in clinical trials targeting type I interferons only[91,92].

Biomarkers of IFNγ activity

Data in animal models indicate that despite high *IFNG* expression and IFNγ levels in tissues (such as liver and spleen), circulating IFNγ levels might be low[39]. In the TLR9 stimulation model of sHLH, when a systemically administered anti-IFNγ antibody binds to this cytokine in tissues and the cytokine–antibody complex drains into the blood, blood IFNγ levels are >100-fold higher than those measured in the absence of the anti-IFNγ antibody[39]. This result demonstrates that only a marginal portion of the high IFNγ amounts produced in tissues reach the systemic circulation. This limited IFNγ release into the blood might be because tissue IFNγ binds to cells expressing IFNGRs and/or to extracellular matrix and heparan sulfate[93]. IFNγ has a half-life of 1–2 min in blood[93,94], which, combined with its retention in tissues, might limit its detection in blood. Indeed, a sizeable proportion of patients with HLH or MAS or with SLE do not have detectable levels of IFNγ in serum despite active disease, suggesting that blood IFNγ levels do not reflect tissue production. Therefore, circulating IFNγ levels are not a suitable biomarker for activity and severity of IFNγ-mediated diseases, and surrogates that reflect IFNγ production have been suggested as potential biomarkers. These biomarkers include phosphorylated STAT1, which is induced by the activation of the IFNGR; neopterin, a pteridine derivative released by IFNγ-activated macrophages and DCs; and CXCL9, a chemokine specifically induced by IFNγ.

STAT1. Levels of phosphorylated STAT1 in monocytes of patients with sHLH and MAS are substantially higher than those of patients with active sJIA[95]. However, in patients receiving glucocorticoids before sampling, pSTAT1 is markedly lower than in untreated patients[95]. Given that pSTAT1 is also linked to type I IFN signalling, the direct effect of glucocorticoids on STAT1 protein expression and phosphorylation[96], and the need for freshly isolated cells and the challenging methodology to accurately measure phosphorylated STAT1 levels means that STAT1 is not an ideal biomarker for identifying patients with sHLH or MAS and monitoring disease activity.

Neopterin. Although assessed in only a few studies, serum neopterin levels are elevated in pHLH and EBV-associated sHLH, and correlate with ferritin levels[97,98]. Neopterin levels are also elevated in MAS, correlate with increased ferritin, aspartate aminotransferase and LDH levels, and are higher than in patients with active sJIA who do not have MAS[99–101]. Urinary neopterin levels are elevated in patients with active SLE and correlate with plasma levels of C3 and C4 (REF.[102]). Neopterin was also reported to be markedly elevated in one patient with active Blau syndrome[103]. Based on these studies, it seems that neopterin is a promising biomarker of IFNγ activity, although additional research is needed as studies are limited and the correlation with IFNγ activity in patients and animals remains to be confirmed.

CXCL9. Whereas CXCL10 and CXCL11 production is induced not only by IFNγ but also by type I interferons and TNF, CXCL9 production is induced predominantly by IFNγ[17,104]. In mouse models of HLH, CXCL9 levels are increased in blood and tissues, and therapeutic IFNγ neutralization counters this increase[39,57,60]. Serum CXCL9

levels are elevated in patients with any form of HLH, including infection-associated sHLH and MAS. CXCL9 levels decline with disease improvement or remission and correlate strictly with parameters of MAS severity, including hyperferritinaemia, thrombocytopenia and ALT and LDH levels[37,40,101,105–109].

CXCL9 is stable, is easily measurable in serum at nanogram concentrations, has a clear association with pathogenetic pathways and correlates with disease severity and response to treatment. CXCL9 is being progressively used as a biomarker for IFNγ activity, measuring disease activity and monitoring response to treatment in HLH and MAS. Of note, monocytes from patients with HLH or MAS are hyperresponsive to IFNγ ex vivo compared with control monocytes[95]. This hyperresponsiveness might be due to in vivo pre-exposure to IFNγ or to other cytokines, which affect the responsiveness of these monocytes to IFNγ. Gene expression profiling of monocytes and macrophages from patients with sJIA or MAS revealed marked overexpression of IFNGRs and tripartite motif 8 protein (TRIM8), which is implicated in the potentiation of IFNγ signalling[110]. Downregulation of TRIM8 expression markedly decreases IFNγ-induced phosphorylation of STAT1 (REF.[110]). These observations suggest that overexpression of IFNGRs and TRIM8 are important contributors to increased responsiveness to IFNγ of monocytes and macrophages from patients with MAS[110]. As a downstream factor, CXCL9 levels reflect not only IFNγ production but also the degree of activation of IFNγ-induced signalling pathways. This close link to IFNγ signalling might explain the strict correlations of CXCL9 levels with parameters of disease in patients with MAS.

CXCL9 is elevated in conditions other than HLH or MAS. For example, in patients with cytokine release syndrome after treatment with chimeric antigen receptor T cells, circulating CXCL9 levels are increased and correlate with disease severity[71]. Furthermore, CXCL9 levels are increased in patients with SLE and correlate with disease severity and response to treatment[111,112]. Levels of circulating CXCL9 are higher in patients with lupus nephritis than in patients with SLE without renal involvement (E.M., unpublished observations), and urinary CXCL9 levels are increased in patients with proliferative lupus nephritis[90].

Therapeutic targeting of IFNγ

The three IFNγ-targeting agents that have been formally tested in clinical trials in humans are discussed below (TABLE 3).

Primary HLH. In a phase II/III trial, the anti-IFNγ antibody emapalumab improved clinical and laboratory parameters of disease activity in patients with pHLH, in whom, in most cases, conventional HLH immunochemotherapy had failed[113]. The improvement in disease parameters paralleled the temporal pattern of decrease in serum CXCL9 levels. In logistic regression analysis, the decrease in CXCL9 levels correlated with clinical response to emapalumab[113]. In patients in whom immunochemotherapy was ineffective, the estimated 12-month survival probability was approximately 73%, comparing favourably with the survival probability in treatment-naïve patients receiving conventional therapy[113]. Emapalumab is approved by the FDA for the treatment of patients with pHLH for whom conventional therapy was ineffective.

Secondary HLH. Initial experiences of treating various forms of sHLH with emapalumab are being reported. One patient with refractory EBV-associated sHLH was successfully treated with emapalumab, despite having severe pre-existing comorbidities, including multiple life-threatening infections[114]. In two patients with severe recurrent sHLH associated with gain-of-function variants in *NLRC4*, who had highly elevated IFNγ and CXCL9 levels, emapalumab treatment achieved disease control, facilitating tapering of conventional therapies[115]. In a patient with recurrent sHLH in the context of chronic autoinflammation linked to a pathogenetic variant in *CDC42*, emapalumab treatment led to resolution of HLH, allowing successful HSCT[116], whereas three other patients who did not receive emapalumab died with refractory HLH[116]. sHLH associated with pathogenetic variants in *NLRC4* and *CDC42* is characterized by activation of the NLRC4 inflammasome, which causes a marked increase in IL-18 production that in turn promotes IFNγ production[115,116].

In a phase II study of emapalumab treatment in patients with sJIA complicated by MAS, in whom high-dose glucocorticoid treatment was ineffective, preliminary analysis of the first nine patients revealed a prompt improvement of all clinical and laboratory parameters in parallel with IFNγ neutralization, which was demonstrated by a decrease in serum CXCL9 levels[117]. In a patient with AOSD whose MAS was unresponsive to high-dose glucocorticoids and anakinra, emapalumab treatment produced a prompt and sustained response, allowing rapid tapering of glucocorticoids[118].

These observations with emapalumab, together with evidence from studies in animal models and observational studies in humans, suggest that IFNγ neutralization represents a valid therapeutic approach to various forms of sHLH.

Systemic lupus erythematosus. In a phase Ib study in patients with SLE with long disease duration (6–12 years) with or without nephritis, administration of the anti-IFNγ antibody AMG-811 did not show effects on comprehensive clinical score, proteinuria, complement levels or anti-dsDNA antibody titre[119]. Serum levels of CXCL10, an interferon-induced chemokine, were used as a surrogate of IFNγ activity. However, CXCL10 production is regulated by both IFNγ and IFNα and thus does not specifically indicate levels of biologically active IFNγ[119]. In addition, an interferon transcriptional signature, based on the levels of genes induced by IFNγ in cells of healthy volunteers in vitro and downregulated in healthy individuals receiving AMG-811, was used to assess the activity of IFNγ. No baseline comparison of this signature between patients with SLE and healthy volunteers, or between patients with or without lupus nephritis, was performed. At the highest dose, the effect of AMG-811 on CXCL10 levels and this unvalidated interferon transcriptional signature

was modest and transient[119]. These modest results are difficult to interpret, as they might be due to the biomarkers chosen, which might not specifically reflect IFNγ activity, and/or to incomplete neutralization of IFNγ. A pharmacodynamics analysis of all patients treated with AMG 811 concluded that, compared with the highest dose (60 mg) administered monthly (a total of three doses), multiple monthly higher dosing (up to 180 mg monthly) might provide more effective CXCL10 suppression[120].

Table 3 | **Clinical trials investigating IFNγ neutralization therapies**

Study design and treatment duration	Patient population	Response	Infection reported as serious adverse events and outcome	Pharmacodynamics data or outcome of IFNγ neutralization	Refs
Crohn's disease: fontolizumab (humanized anti-IFNγ monoclonal antibody)					
Phase I/II, randomized, placebo-controlled, dose-escalating safety and tolerability study Treatment duration up to 85 days (up to 4 doses)	Adults with moderate-to-severe Crohn's disease ($n = 35$)	Not evaluable	No serious infection	Not available	140
Phase II, randomized, double-blind, placebo-controlled safety and efficacy study Treatment duration 56 days (3 doses)	Adults with moderate-to-severe Crohn's disease ($n = 90$)	Significant improvement in clinical response rate (69% and 67% in the two fontolizumab dose groups versus 32% in placebo) at day 56 ($P < 0.05$ for both groups); higher rate of response in patients with increased CRP	1 serious infection (condyloma acuminata), resolved; among grade 2 infections, 1 herpes zoster infection	Not available	135
Phase II, randomized, double-blind, placebo-controlled, safety and efficacy study Treatment duration up to 85 days (4 doses)	Adults ($n = 161$) with moderate-to-severe Crohn's disease	Significant improvement in clinical response rate at day 85 (58% and 53% in fontolizumab groups versus 38% in placebo group; $P < 0.05$ for both groups)	1 serious infection (peritonsillar cellulitis)	Not available	136
Systemic lupus erythematosus: AMG 811 (fully human (IgG1) anti-IFNγ monoclonal antibody)					
Phase I, single-dose escalation trial to assess safety and immunological effect of IFNγ inhibition	Adults with mild-to-moderate SLE ($n = 18$)	Not evaluable	1 infection (pyelonephritis), resolved	Modest decrease in CXCL10 levels; no IFNγ-related biomarkers measured	141
Phase Ib, randomized, placebo-controlled, multiple dose escalation study to assess safety, pharmacokinetics and pharmacodynamics Treatment duration 56 days (3 doses)	Adults with SLE with ($n = 21$) or without ($n = 20$) nephritis	No improvement in SELENA-SLEDAI scores, proteinuria, complement levels or anti-dsDNA antibodies	1 infection (*Salmonella* gastroenteritis) that evolved to disseminated infection, resolved	Dose-related decrease in CXCL10 levels without normalization; dose-related reduction in a blood-based IFNγ blockade signature (less pronounced in patients with nephritis)	119,120
HLH: emapalumab (fully human (IgG1) anti-IFNγ monoclonal antibody)					
Phase II/III, open-label, single-arm study Treatment duration 8 weeks	Children with pHLH who did not respond to conventional treatment ($n = 27$) or were treatment-naïve ($n = 7$)	Overall response rate 65% at 8 weeks; complete response in 21%; partial response in 32%; HLH improvement in 12%	Severe infections in 8 patients, which all resolved except for 1 case of fatal septic shock after the second emapalumab infusion; disseminated histoplasmosis and necrotizing fasciitis in 1 patient each (reported as related to emapalumab treatment), resolved	CXCL9 established as a biomarker for IFNγ activity; rapid, marked decrease in CXCL9 levels after emapalumab treatment; low CXCL9 levels at end of treatment associated with response	113
Phase II, open-label, single-arm study Treatment duration 8 weeks	Children with sJIA and MAS who did not respond to high-dose glucocorticoids ($n = 14$)	Preliminary analysis of the first 9 patients showed 100% complete response rate at 8 weeks	Mouse CMV reactivation in 1 patient	Rapid, marked decrease in CXCL9 levels after emapalumab treatment	117

CMV, cytomegalovirus; dsDNA, double-stranded DNA; HLH, haemophagocytic lymphohistiocytosis; IFNγ, interferon-γ; IgG1, immunoglobulin G1; MAS, macrophage activation syndrome; pHLH, primary HLH; SELENA, Safety of Estrogens in Lupus Erythematosus National Assessment; sJIA, systemic juvenile idiopathic arthritis; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

Based on the growing body of evidence in animals suggesting a role for IFNγ in SLE pathogenesis, further investigation of anti-IFNγ treatments in SLE is warranted. The insufficient clinical responses in trials might be due to incomplete neutralization of IFNγ and/or improper timing of antibody administration vis-à-vis duration of disease.

Challenges in targeting IFNγ

The well-known role of IFNγ in defence against infections must be taken into account when targeting IFNγ in patients. Studies of IFNγ-deficient mice and, more importantly, humans with defective IFNγ activity (that is, individuals with genetic IFNGR deficiency and patients with anti-IFNγ antibodies) provide robust data supporting the protective role of IFNγ against selected pathogens.

In mice, IFNγ has a major role in the immune response to *Salmonella* infection and, consistent with the role of IFNγ in granuloma formation, in protection from *Mycobacterium tuberculosis* infection[121–124]. These infections have a fatal course in IFNγ-deficient mice. IFNγ is also essential for inducing protective anti-microsporidial immunity[125]. In an acute model of primary mouse CMV infection in BALB/c mice, which are highly susceptible to this virus, genetic ablation of *IFNG* led to markedly increased viral load, which is associated with higher infection severity[53,126].

Similarly, individuals with defective IFNγ activity have increased susceptibility to both typical and atypical mycobacteria, and increased risk of *Varicella zoster* reactivation[127–130]. *Salmonella, Herpes simplex* virus, *Varicella zoster* virus, respiratory syncytial virus and parainfluenza virus type 3 infections have also been reported in individuals with defective IFNγ activity[131,132]. Of note, these individuals do not have increased susceptibility to extracellular bacteria and generally have limited symptoms when infected with exanthematous viruses, including chicken-pox and measles viruses[132]. Although animal data suggest that IFNγ might protect against mouse CMV reactivation[121,122], only two cases of mouse CMV reactivation or infection have been reported in individuals with defective IFNγ activity[133,134]. Together, these data allow the risk of developing infections during therapeutic IFNγ neutralization to be predicted.

Observations in clinical trials of anti-IFNγ antibodies are consistent with data reported in animals and individuals lacking IFNγ activity (TABLE 3). Isolated cases of *Herpes zoster* reactivation were reported in clinical studies with the anti-IFNγ antibody fontolizumab in 286 patients with Crohn's disease and one case in the phase I trial of emapalumab[135–137]. Among 42 patients who received AMG-811 in the phase Ib trial in patients with SLE, one case of *Salmonella* enteritis, which evolved to disseminated salmonellosis, was reported, with recovery following treatment[118]. Other infections reported during these studies that are not considered to be related to anti-IFNγ therapy include peritonsillar cellulitis[136].

Among the 34 patients with pHLH in the phase III trial with emapalumab, one case of disseminated histoplasmosis was reported, which resolved after conventional treatment (whereas emapalumab was still measurable in blood), and one case of necrotizing fasciitis was reported as being related to emapalumab treatment[113]. Of note, the majority of patients enrolled in this trial were severely immunosuppressed and first-line therapies were ineffective[113].

In the phase II trial of emapalumab treatment in patients with sJIA and MAS, one case of mouse CMV reactivation (positive by PCR but with no symptoms) was reported as a serious adverse event possibly related to emapalumab treatment[117]. In addition, three patients were transiently positive (by PCR) for mouse CMV and for adenovirus, parainfluenza virus and urinary BK virus (one patient each), all in the absence of symptoms[117]. By contrast, a child with refractory EBV-induced sHLH was successfully treated with emapalumab, despite multiple pre-existing severe, life-threatening concurrent infections, including viraemias (EBV, mouse CMV and adenovirus), sepsis (*Escherichia coli)* and fungaemia (*Trichosporon* spp.), which all resolved with antimicrobial medications during IFNγ blockade[114]. In a patient with adenosine deaminase-severe combined immunodeficiency complicated by disseminated BCGitis (caused by *Mycobacterium bovis*) and sHLH, emapalumab treatment allowed for control of HLH disease and successful haploidentical HSCT, with no mycobacterial reactivation[138].

In these trials, evidence of infections that are known to occur more frequently with IFNγ deficiency or blockade was an exclusion criterion, allowing risk mitigation. Two patients developed *Salmonella* or *Histoplasma capsulatum* infections (one each), which were successfully managed with antimicrobial therapy[117,119]. No tuberculosis has been reported. Serious infections that do not occur more frequently with IFNγ deficiency or blockade have also been reported in patients, typically in those exposed to multiple immunosuppressive and/or chemotherapeutic agents.

Conclusions

Accumulating evidence shows that IFNγ overproduction has a role in the pathogenesis of hyperinflammatory diseases, including pHLH, various forms of sHLH, cytokine release syndrome, the canonical autoimmune disease SLE and the rare monogenic granulomatous disease Blau syndrome. Following the convincing efficacy data of IFNγ neutralization in patients with pHLH, anti-IFNγ therapy also shows promising results in various forms of sHLH, including MAS, which are routinely managed by rheumatologists. Although analysis of data from the phase II trial of emapalumab treatment in patients with sJIA and MAS awaits completion, the available evidence supports evaluation of anti-IFNγ therapies in other forms of sHLH, both in children and in adults, including infection-associated sHLH without an identifiable trigger and/or underlying condition. An in-depth evaluation of the therapeutic potential of IFNγ neutralization in SLE is also required, given the unmet need, results from animal model studies and observational data in patients with SLE. Studies of IFNγ-targeted treatments might be facilitated by measurement of circulating CXCL9 and

other potential biomarkers that reflect IFNγ activity, which should aid inpatient diagnosis and stratification, tailoring treatment choice and dose, and monitoring response to therapy. Thanks to data from mice and humans with deficient IFNγ activity, infection risk with anti-IFNγ therapies seems to be mitigated by screening for infections that are known to occur more frequently with IFNγ neutralization. In the rare reported cases of these infections, they have been successfully managed with antimicrobial therapy. We anticipate that further advances in the understanding of IFNγ biology and its pathogenetic role will benefit the identification of additional diseases that are therapeutically targetable with anti-IFNγ approaches.

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The non-coding RNA interactome in joint health and disease

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Abstract | Non-coding RNAs have distinct regulatory roles in the pathogenesis of joint diseases including osteoarthritis (OA) and rheumatoid arthritis (RA). As the amount of high-throughput profiling studies and mechanistic investigations of microRNAs, long non-coding RNAs and circular RNAs in joint tissues and biofluids has increased, data have emerged that suggest complex interactions among non-coding RNAs that are often overlooked as critical regulators of gene expression. Identifying these non-coding RNAs and their interactions is useful for understanding both joint health and disease. Non-coding RNAs regulate signalling pathways and biological processes that are important for normal joint development but, when dysregulated, can contribute to disease. The specific expression profiles of non-coding RNAs in various disease states support their roles as promising candidate biomarkers, mediators of pathogenic mechanisms and potential therapeutic targets. This Review synthesizes literature published in the past 2 years on the role of non-coding RNAs in OA and RA with a focus on inflammation, cell death, cell proliferation and extracellular matrix dysregulation. Research to date makes it apparent that 'non-coding' does not mean 'non-essential' and that non-coding RNAs are important parts of a complex interactome that underlies OA and RA.

Non-coding RNAs constitute 99% of total cellular RNA content and, alongside DNA methylation and histone modification, represent one of three major epigenetic mechanisms that contribute to health and disease[1]. Although non-coding RNAs are encoded in DNA and transcribed to RNA, they are not translated to protein; however, this does not negate their important role in regulating cellular processes. The precise mechanism of action is dependent on the class of non-coding RNA — short non-coding RNAs (such as microRNAs (miRNAs)), long non-coding RNAs (lncRNAs) or circular RNAs (circRNAs) — although all types ultimately function to regulate the expression of specific gene targets[1]. As such, non-coding RNAs are essential for establishing and maintaining homeostatic balance in biological systems, including regulating the signalling pathways and biological processes that govern joint development[2]. Deregulation of this balance contributes to the pathogenesis of joint diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA)[3,4].

Non-coding RNAs are found in almost all joint tissues and biofluids across different species, demonstrating their biological importance[5]. In joint disease, non-coding RNAs have been explored as potential biomarkers, mediators of pathogenesis and therapeutic targets. Adding to the complexity of these epigenetic regulators, the different classes of non-coding RNAs can provide redundancy by targeting the same genes, work in concert by targeting the same pathways and directly interact to regulate gene expression[6]. Although this putative 'interactome' of non-coding RNAs has yet to be comprehensively explored in joint health and disease, its elucidation is improving with the use of technologies for high-throughput profiling and integrative computational analysis.

To demonstrate that 'non-coding' does not mean 'non-essential', in this Review we discuss literature published in the past 2 years on non-coding RNAs in OA and RA. We first describe the classes of non-coding RNAs and their mechanisms of action, followed by the role of non-coding RNAs in osteogenesis and chondrogenesis, two vital biological processes in joint development. We next review non-coding RNAs in OA and RA joint tissues and biofluids, and their roles in inflammation, cell death, cell proliferation and extracellular matrix (ECM) dysregulation. Finally, we discuss the therapeutic potential of non-coding RNAs in OA and RA and the deep-dive efforts that will be required in the future to unravel the complex interactions among non-coding RNAs.

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Key points

- An increasing body of literature on non-coding RNAs in joint health and disease has revealed important regulatory functions that indicate that 'non-coding' does not equate to 'non-essential'.
- Non-coding RNAs, including microRNAs, long non-coding RNAs and circular RNAs, can directly interact and have co-regulatory functions.
- In osteoarthritis and rheumatoid arthritis, non-coding RNAs are important contributors to pathogenesis and serve as potential biomarkers and therapeutic targets.
- With the emergence of data from high-throughput studies, detailed reporting and accurate annotation of results are required to integrate individual studies and enable interrogation of the non-coding RNA interactome.
- An expanded understanding of the non-coding RNA interactome could reveal essential regulatory mechanisms and novel therapeutic opportunities for osteoarthritis, rheumatoid arthritis and other related joint diseases.

Classes of non-coding RNAs

Non-coding RNAs are classified on the basis of their biogenesis, length and mechanism of action (FIG. 1a). Following transcription, non-coding RNAs are processed to form short, long or circular non-coding RNAs with unique secondary and tertiary structures. The first class are short non-coding RNAs, which are fewer than 200 nucleotides in length. This class includes miRNAs, small nucleolar RNAs (snoRNAs), small nuclear RNAs, Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), transfer RNAs (tRNAs), tRNA-derived fragments (tRFs) and Y RNA fragments (YRFs)[5]. The most frequently studied of the short non-coding RNAs are miRNAs. miRNA biogenesis begins with a primary miRNA transcript from the intron, exon or intergenic region of the host gene, followed by processing within the nucleus to produce a precursor miRNA. After export to the cytoplasm, the precursor miRNA undergoes cleavage to produce the mature miRNA. Mature miRNAs are single stranded, 18–24 nucleotides in length and function to inhibit target gene expression through mRNA degradation or repression of translation[4] (FIG. 1b).

The second class of non-coding RNAs are lncRNAs, which are greater than 200 nucleotides in length. Similar to short non-coding RNAs, lncRNAs function to modulate mRNA stability and translation in the cytoplasm through multiple mechanisms, including the post-translational modification of target molecules[7] (FIG. 1c). Circular forms of lncRNAs, or circRNAs, comprise 1–5 introns or exons and form a covalently closed loop structure that functions as an miRNA sponge, protein sponge or scaffold for translation[5,6] (FIG. 1d). miRNA sponging depends on the presence of miRNA response elements within lncRNAs and circRNAs that can specifically bind and sequester miRNAs, thereby blocking their activity. This sponging is a type of competing endogenous RNA activity and is a mechanism through which the different classes of non-coding RNAs can directly interact. circRNAs primarily function as competing endogenous RNAs, binding to miRNA response elements and reducing the quantity of miRNAs available to target mRNA, thereby promoting mRNA stability or protein expression[6]. In this Review, we focus on miRNAs, lncRNAs and circRNAs, as these types of non-coding RNA have been explored the most in OA and RA to date[3,8].

Non-coding RNAs in joint development

Healthy joint development is dependent on precise regulation of the signalling pathways that govern osteogenesis and chondrogenesis, among other processes, and if these become dysregulated, joint pathologies can result. miRNAs, lncRNAs, circRNAs and even piRNAs are differentially expressed during the early stages of osteogenic and chondrogenic differentiation in human bone marrow-derived mesenchymal stromal cells and/or bone marrow-derived mesenchymal stem cells (BMSCs), suggesting that non-coding RNAs might affect these processes[9,10]. Non-coding RNAs can also regulate important signalling pathways, including the Wnt–β-catenin and Hedgehog signalling pathways, which are essential for tissue induction, patterning, growth and morphogenesis[11]. For example, overexpression of miR-378 in transgenic mice results in abnormal bone formation and quality, as well as compromised osteogenic differentiation in both mouse and human BMSCs[12]. Interestingly, miR-378 targets two Wnt family members, Wnt6 and Wnt10a, thereby attenuating Wnt–β-catenin signalling[12]. These results suggest that miRNAs might be upstream regulators of certain developmental signalling pathways, which has implications for bone health.

Chondrogenesis is essential for endochondral and intramembranous ossification and for tissue homeostasis, and is also subject to regulation by non-coding RNAs. The Indian Hedgehog signalling pathway is well known to regulate chondrogenesis during normal development[13]. The gene encoding Indian Hedgehog contains two putative sites at which miR-1 can bind and inhibit its activity, resulting in increased expression of type II collagen and aggrecan and decreased expression of type X collagen and matrix metalloproteinase 13 in mouse thorax chondrocytes[14]. These results suggest that miR-1 induces an anabolic effect in chondrocytes through inhibition of the Indian Hedgehog pathway, which is consistent with previous findings that aberrant activation of the Indian Hedgehog pathway can have catabolic effects on cartilage[15]. Furthermore, the transcription factor SOX9, which is critical for mesenchymal condensation prior to chondrogenesis, is targeted by miR-30a to inhibit chondrogenic differentiation in human BMSCs[16], again demonstrating a direct

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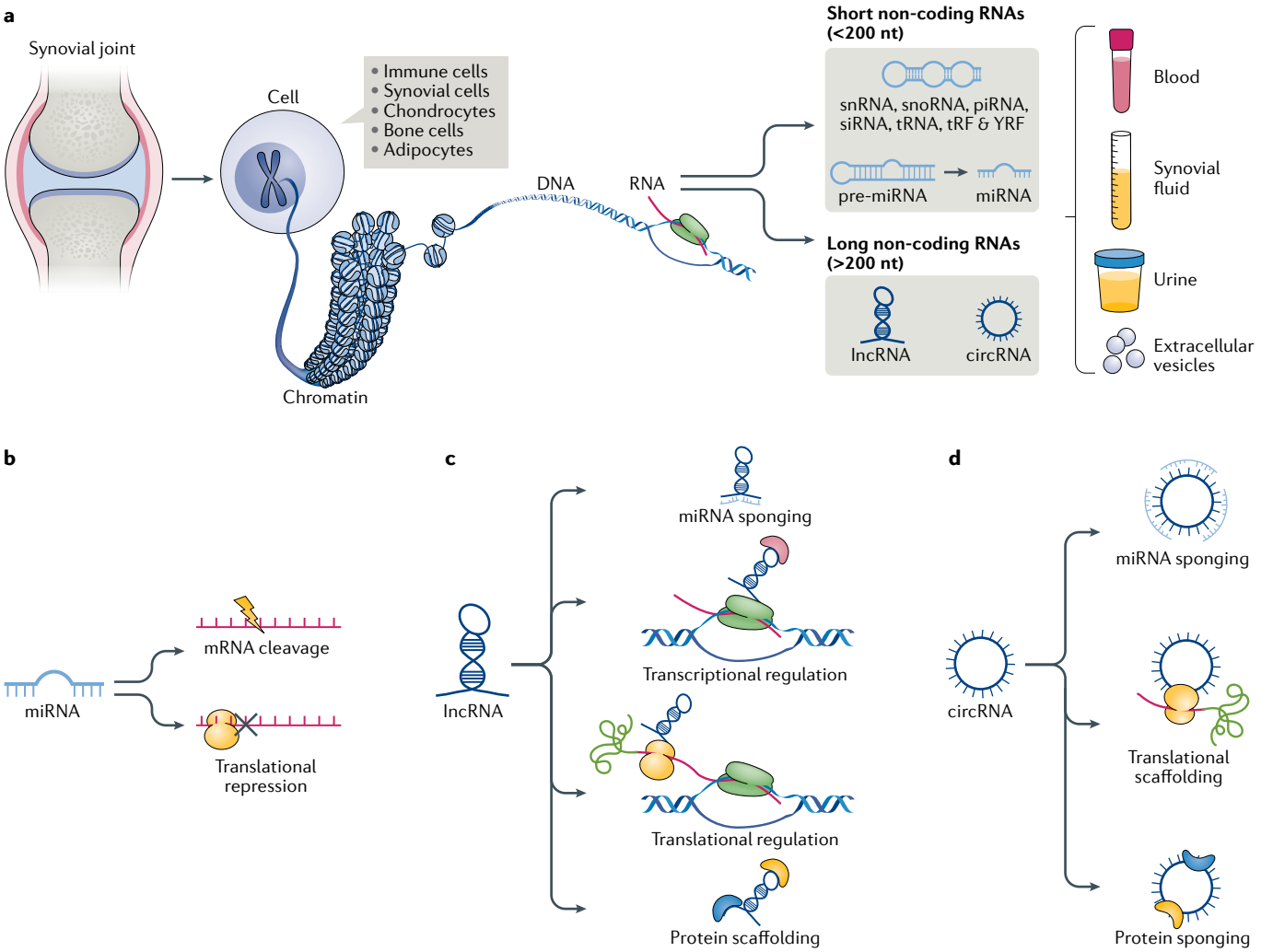

Fig. 1 | **Biogenesis and function of microRNAs, long non-coding RNAs and circular RNAs.** Within the synovial joint, several cell types can be a source of non-coding RNAs that are transcribed from DNA (part **a**). Non-coding RNAs can function within the producing cell or in a target cell, and are secreted into biofluids as free molecules or within extracellular vesicles. Potential functions for microRNAs (miRNAs) include mRNA cleavage and translational repression (part **b**); for long non-coding RNAs (lncRNAs) include transcriptional regulation, translational regulation, protein scaffolding and miRNA sponging (part **c**); and for circular RNAs (circRNAs) include miRNA sponging, protein sponging and translational scaffolding (part **d**). nt, nucleotides; piRNA, Piwi-interacting RNA; siRNA, small interfering RNA; snRNA, small nuclear RNA; snoRNA, small nucleolar RNA; tRF, tRNA-derived fragment; tRNA, transfer RNA; YRF, Y RNA fragment.

regulatory role for miRNAs in established mechanisms that govern chondrogenesis.

Looking at interactions between classes of non-coding RNAs in osteogenesis and chondrogenesis, evidence exists of competing endogenous RNA activity. The lncRNA LINC00707 sponges miR-145 in human BMSCs and increases the expression of lipoprotein receptor-related protein 5, a co-receptor for Wnt proteins, thereby promoting osteogenic differentiation[17]. Similarly, the lncRNA ADAMTS9-AS2 sponges miR-942-5p in human BMSCs and increases expression of the transcription factor SCRG1, thereby promoting chondrogenic differentiation[18]. circRNAs have also emerged as novel orchestrators of signalling pathways that govern osteogenesis[19]. Relevant to development, an axis has been identified whereby circRNA_0079201 sponges miR-140-3p in human chondrocytes and increases expression of the transcription factor SMAD2, thereby suppressing cell proliferation, hypertrophy and endochondral ossification[20]. Taken together, these examples illustrate an important role for non-coding RNAs in governing signalling pathways and biological processes in joint development that have implications for joint health and disease.

Non-coding RNAs in OA and RA

Expression in joint tissues

Strong evidence exists to support cell-specific and tissue-specific expression patterns of non-coding RNAs in OA and RA[21,22]. Two studies used microarrays to compare cartilage from patients with OA and healthy individuals and identified 58 and 70 differentially expressed miRNAs, respectively[23,24]. Beyond miRNAs, a diverse range of non-coding RNAs have been identified

in primary human OA chondrocytes and cartilage, including lncRNA MFI2-AS1, lncRNA LOXL1-AS1, tRF-3003a and U3 snoRNA[25–28]. In primary human synoviocytes and synovial tissue in OA and RA, reports have focused on lncRNAs such as MALAT1, NEAT1 and PVT1 (REFS[29–31]). MALAT1 expression crosses tissue types, being increased in both the synovium[29] and subchondral bone[32] of patients with OA compared with healthy individuals. Similarly, NEAT1 expression crosses both tissue types and diseases, being increased in OA cartilage[30] and in RA synovium[33]. These examples demonstrate a non-coding RNA expression pattern that is not only tissue specific, but potentially also disease specific, and support the need for further studies focused on profiling non-coding RNAs in other tissues and cells implicated in OA and RA, including bone[34,35], adipose tissue[36], meniscus[37] and macrophages[38,39]. Furthermore, this profiling should take into account unique patient endotypes[40,41] and apply appropriate inclusion and exclusion criteria during the selection of participants to facilitate the interpretation of findings and integration with other studies.

Sequencing is the gold standard approach for identifying tissue-specific non-coding RNAs, their targets and their interactions. In a 2019 study that compared lesioned with preserved cartilage from patients with knee or hip OA, RNA sequencing was used to identify 142 miRNAs and 2,387 mRNAs that were prioritized into a regulatory network comprising 62 miRNAs that targeted 238 mRNAs[42], which showed joint-specific expression patterns. Similarly, 1,068 mRNAs, 21 miRNAs and 395 miRNA–mRNA pairs were identified in synovial tissue from patients with knee OA using RNA sequencing[43]. Given the large number of candidate non-coding RNAs identified through sequencing, a deeper dive into the biological relevance of prioritized candidates is required through validation studies. In synovium, canonical correlation analysis of RNA sequencing and small RNA sequencing data has been used to identify miRNA–mRNA co-expression patterns[44]. Specifically, five miRNAs and four genes were predicted to be associated with pain in knee OA, suggesting their potential utility as biomarkers.

Although obtaining tissue samples by biopsy might be considered too invasive for use in biomarker detection, evidence from RA suggests that the amount of non-coding RNAs in the circulation might differ from that found in tissues. For example, the amount of miR-22 is increased in plasma from patients with RA compared with that from healthy individuals and is associated with disease activity in RA[45,46], but is decreased in synovial tissue from patients with RA compared with synovium from healthy individuals[47]. It is unclear whether this tissue-specific difference in miR-22 expression is due to sponging (as is the case for miR-145-5p, which is sponged by the lncRNA PVT1 in RA synovium to produce lower concentrations in the synovium than in the serum[31]) or other mechanisms of non-coding RNA regulation. Nevertheless, these data suggest that, in addition to tissue-specific expression patterns, biofluid-specific patterns of non-coding RNA expression must also be considered.

Expression in biofluids

Non-coding RNAs can be secreted by cells either as free RNA molecules or encapsulated into extracellular vesicles such as exosomes and can be identified in biofluids including blood, urine and synovial fluid[36,48,49] (FIG. 1). Given their association with disease activity, non-coding RNAs are thought to represent excellent candidate biomarkers[50]. Non-coding RNA classes are broadly altered in plasma from patients with RA, in which sets of miRNAs and tRFs are enriched and sets of YRFs are depleted compared with healthy individuals[46]. Such non-coding RNA class shifts might be caused by broad changes in RNA processing mechanisms, such as the upregulation of Dicer and Drosha in RA peripheral blood mononuclear cells (PBMCs)[51], which are a major source of non-coding RNAs in plasma. Surprisingly, non-coding RNAs of microbial origin have also been detected in human plasma; the abundance of microbial small RNAs and specific microbial tRFs were inversely associated with disease activity in two separate cohorts of patients with RA and also predicted response to therapy, suggesting that they might be useful as biomarkers[52].

Non-coding RNA profiling in OA biofluids has focused on miRNAs in the circulation because samples are accessible by minimally invasive blood draw (TABLE 1). Approaches used include real-time PCR[53,54], real-time PCR miRNA arrays[55], miRNA microarrays[35,38] and, most recently, miRNA sequencing of serum[56], plasma[57] and plasma-isolated extracellular vesicles[58]. miRNA sequencing is of particular interest as it enables the discovery of novel miRNAs that are potentially unique to a disease stage or phenotype[57,59]. Fewer reports have described miRNA profiles in urine or synovial fluid than in blood[60,61]. Real-time PCR miRNA arrays were used to interrogate synovial fluid samples taken before and 6 months after high tibial osteotomy in six patients with knee OA at Kellgren–Lawrence grade II or III[60]. Three miRNAs were identified as being differentially expressed at the two time points and, following validation by real-time PCR in 22 additional patients, increased miR-30c-5p was found to correlate with reduced postoperative pain[60]. Looking beyond miRNAs, lncRNAs and circRNAs are also dysregulated in OA biofluids[62]. For example, the expression of lncRNAs CAIF, LUADT1 and SNHG9 are decreased in OA synovial fluid[63–65], whereas CTBP1-AS2, MCM3AP-AS1 and CASC2 are increased[66–68], although the utility of these lncRNAs as biomarkers requires further research.

To date, no consistent profile of non-coding RNAs has been identified and validated in biofluids across OA or RA studies. Among the challenges faced by researchers are differences across studies in the joints characterized, the profiling platforms used, the biofluids profiled and how the patient groups are defined, all of which make it difficult to directly compare findings (TABLE 1). Going forward, panels of non-coding RNAs (potentially from multiple classes) could prove to be more reliable as biomarkers than an individual entity or class owing to the variable expression and interactions of individual non-coding RNAs. In addition to their roles as biomarkers, non-coding RNAs in biofluids might also function as systemic regulators of disease in

Table 1 | **Circulating microRNAs with potential for use as biomarkers in OA and RA**

Disease	Platform	Biofluid	Number of patients	Number of controls (type)	Differentially expressed miRNAs	Ref.
Knee OA	Real-time PCR	Plasma	150	150 (healthy individuals, traumatic amputation or meniscus injury)	Reduced in OA: miR-200c-3p, miR-100-5p and miR-1826	53
Knee OA	Real-time PCR	Serum	10	10 (trauma)	Reduced in OA: let-7e	54
Hip OA	Real-time PCR	Serum	28	2 (femoral neck fracture)	Increased in OA: miR-146a-5p	169
RA	Real-time PCR	Plasma	125	30 (healthy individuals)	Reduced in RA: miR-155	170
RA	Real-time PCR	Serum	20	20 (healthy individuals)	Increased in RA: miR-138	171
RA	Real-time PCR	Blood	90	30 (healthy individuals)	Increased in RA: miR-155, miR-150, miR-146a, miR-146b, miR-125a-5p and miR-223	172
RA	Real-time PCR	Serum	18	76 (SLE, SSc or MCTD)	Increased in RA: miR-145 and miR-181a	173
Knee OA	Real-time PCR array	Serum	114 (high pain relief 1 year post-TKR)	22 (low pain relief 1 year post TKR)	Increased in low pain relief group: miR-146a-5p, miR-145-5p and miR-130b-3p	55
Knee OA	Microarray	Blood	5	5 (healthy individuals)	Decreased in OA: miR-582-5p and miR-424-5p	35
RA	Microarray	Blood	5 (early RA)	5 (healthy individuals), 5 (CPP^+ healthy individuals)	Increased in RA: miR-361-5p	174
RA	Microarray	Serum	9 (divided into 3 pools)	15 (healthy individuals; divided into 5 pools)	Increased in RA: miR-187-5p, miR-4532 and miR-4516; decreased in RA: miR-125a-3p, miR-575, miR-191-3p, miR-6865-3p, miR-197-3p, miR-6886-3p, miR-1237-3p and miR-4436b-5p	175
RA	Microarray	Serum exosomes	22 (in clinical remission)	20 (not in clinical remission)	Increased in clinical remission group: miR-1915-3p and miR-6511-5p	176
Knee OA	Next-generation sequencing	Serum	10	10 (healthy individuals)	Increased in OA: miR-146a-5p and miR-186-5p	56
Knee OA	Next-generation sequencing	Plasma	41 (early-stage OA)	50 (late-stage OA)	Increased in early-stage OA: miR-335-3p, miR-199a-5p, miR-671-3p, miR-1260b, miR-191-3p, miR-335-5p and miR-543	57
Knee, hip or hand OA	Next-generation sequencing	Plasma extracellular vesicles	23	23 (healthy individuals)	None	58
RA	Next-generation sequencing	Plasma	167	91 (healthy individuals)	Increased in RA: miR-22-3p, miR-24-3p, miR-96-5p, miR-134-5p, miR-140-3p and miR-627-5p	45

Includes articles published between 2019 and 2021. CPP, cyclic citrullinated peptide antibody; MCTD, mixed connective tissue disease; miRNA, microRNA; OA, osteoarthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TKR, total knee replacement.

distal joints. Two studies from 2020 found alterations in the concentrations of circulating miRNAs that directly target single-nucleotide polymorphisms in *CXCR4* and *ADAMTS5* loci[69,70], both of which are related to OA risk. These results point to non-coding RNAs as circulating epigenetic factors that regulate risk loci in arthritis as an exciting new avenue for future research.

Role in pathogenesis

As important regulators of gene expression, non-coding RNAs can be expected to have pleiotropic effects in polygenic diseases such as OA and RA. Data suggest that non-coding RNAs can have both beneficial (such as maintaining tissue homeostasis) and detrimental (such as inducing tissue destruction) effects on the joints[4,22]. In fact, miRNAs regulate a diverse range of cellular processes (including inflammation[71–74], apoptosis[75–78], ECM dysregulation[79,80], chondrocyte differentiation[81], oxidative stress[82] and autophagy[83–85]), signalling pathways (including transforming growth factor-β[86,87], fibroblast growth factor (FGF)[88], Wnt–β-catenin[89–91] and Hedgehog[92]) and mediators (including the transcription factors FOXM1, SOX5, SOX6 and SOX9, and oestrogen receptor-α[93–98]) that are relevant for OA and RA. Similarly, lncRNAs (TABLE 2) and circRNAs (TABLE 3) can have multi-target regulatory effects on cell phenotype and tissue homeostasis and have the potential to mediate pathogenic mechanisms in OA and RA[6,62,99].

Table 2 | **Long non-coding RNAs of mechanistic importance in OA and RA**

lncRNA	Change in expression	Mechanism	Effects	Ref.
CTBP1-AS2	Increased in OA synovial fluid	Increased methylation of *miR-130a*	Promotes proliferation in OA chondrocytes	66
XIST	Increased in OA cartilage	Binds *TIMP3* promoter and accelerates methylation	Increases collagen degradation in OA chondrocytes	106
LINC01534	Increased in OA cartilage	Sponges miR-140-5p	Promotes ECM degradation (decreases aggrecan, type II collagen and increases MMP3, MMP9 and MMP13) and increases pro-inflammatory mediators (NO, PGE_2, IL-6, IL-8 and TNF) in IL-1β-treated chondrocytes	138
H19	Increased in OA cartilage	Sponges miR-140-5p	Increases apoptosis, reduces cell proliferation, increases ECM degradation (increases MMP1 and MMP13 and decreases type II collagen) and increases ECM calcification in chondrocytes	137
	Increased in RA FLSs and synovium	Sponges miR-103a, which negatively regulates *IL15* and *DKK1*	Increases inflammation and joint destruction in mice with CAIA	177
NEAT1	Increased in OA cartilage	Sponges miR-377-3p, which negatively regulates *ITGA6*	Reduces cell proliferation and increases apoptosis, ECM degradation and inflammation in IL-1β-treated chondrocytes	30
	Increased in RA FLSs and synovium	Sponges miR-410-3p, which negatively regulates *YY1*	Increases cell proliferation and TNF and MMP9 expression and decreases apoptosis in RA FLSs	33
	Increased in RA PBMC exosomes	Sponges miR-23a, which negatively regulates the MDM2–SIRT6 axis	Increases FLS proliferation and inflammation	135
	Increased in RA PBMCs and T_H17 cells	Reduces ubiquitylation of STAT3	Increases T_H17 cell differentiation and disease severity in mice with CIA	114
PINT	Decreased in RA FLSs and synovium	Sponges miR-155-5p, which negatively regulates *SOCS1*	Increases cell proliferation, invasion and pro-inflammatory cytokine production in RA FLSs	178
PVT1	Increased in RA synovium	Sponges miR-145-5p	Increases cell proliferation and pro-inflammatory cytokine production and decreases apoptosis in RA FLSs	31
	Increased in RA synovium	Sponges miR-543, which negatively regulates *SCUBE2*	Increases cell proliferation and IL-1β expression and decreases apoptosis in RA FLSs	179

Includes articles published between 2019 and 2021. CAIA, collagen antibody-induced arthritis; CIA, collagen-induced arthritis; ECM, extracellular matrix; FLS, fibroblast-like synoviocyte; lncRNA, long non-coding RNA; MDM2, E3 ubiquitin-protein ligase MDM2; MMP, matrix metalloproteinase; NO, nitric oxide; OA, osteoarthritis; PBMC, peripheral blood mononuclear cell; PGE_2, prostaglandin E_2; RA, rheumatoid arthritis; SIRT6, sirtuin 6; STAT3, signal transducer and activator of transcription 3; T_H17 cell, T helper 17 cell.

For example, lncRNAs can function through regulation of histone methylation[100], targeting single-nucleotide polymorphisms[101] and miRNA sponging[30,102] to regulate cellular processes as diverse as apoptosis[103,104], cell proliferation[103] and ECM degradation[102,105,106]. In the following sections, we curate literature published in the past 2 years on non-coding RNAs in inflammation, cell death, cell proliferation and ECM dysregulation in OA and RA. Overall, although further research is required to elucidate the interrelated effects of non-coding RNAs on the pathogenesis of OA and RA, existing evidence suggests that there could be merit to therapeutically targeting non-coding RNAs in these diseases.

Inflammation. A variety of signalling molecules, including non-coding RNAs, can induce and regulate joint inflammation. Cytokines such as IL-1β, TNF and IL-6 are often used as markers to gauge inflammatory responses in chondrocytes and fibroblast-like synoviocytes (FLSs). Amounts of these three cytokines were reduced in mouse primary chondrocytes by an increase in miR-410-3p[107], and in supernatant from lipopolysaccharide-treated human chondrocytes by a decrease in miR-20a[108]; both outcomes were mediated by nuclear factor-κB (NF-κB) signalling. NF-κB can regulate the expression of miRNAs, but miRNAs can also regulate the expression of NF-κB; for example, an increase in miR-382-3p leads to a decrease in phosphorylated NF-κB in IL-1β-stimulated human OA chondrocytes[109]. Furthermore, miR-140-5p can reduce human chondrocyte senescence[110] and can work synergistically with miR-146a to reduce NF-κB phosphorylation and the production of pro-inflammatory cytokines in OA chondrocytes[111]. These studies suggest that miRNAs regulate inflammatory responses through mechanisms that include canonical signalling pathways (such as NF-κB) and cytokines (such as IL-1β, IL-6 and TNF) in OA, and similar results have been reported in RA[47]. lncRNAs are also important mediators of inflammation in human OA chondrocytes. The lncRNAs PACER, CILinc01 and CILinc02 all show rapid and transient induction in response to IL-1β and other pro-inflammatory stimuli, indicating important regulatory roles[112].

In RA, non-coding RNAs in circulating immune cells, synovial immune cells and FLSs contribute to excess inflammation. T helper 17 (T_H17) cells that produce cytokines such as IL-17 and IL-22 stimulate inflammatory responses from FLSs and macrophages in RA to further promote synovial inflammation[113]. In RA PBMCs, the lncRNA NEAT1 (which is present in increased amounts compared with healthy individuals)

targets signal transducer and activator of transcription 3, causing decreased ubiquitylation-mediated degradation and leading to an increase in T_H17 cell differentiation[114]. Similarly, a lack of the miRNA let-7g-5p in patients with RA promotes the differentiation of naive $CD4^+$ T cells into T_H17 cells, whereas the treatment of mice with collagen-induced arthritis (CIA) with let-7g-5p mimics decreases the number of T_H17 cells in the blood and spleen, leading to reduced synovial hyperplasia, pannus formation and cartilage destruction[115]. Macrophages with a pro-inflammatory phenotype (M1-like) are also enriched in active RA synovium[116]. An increase in miR-155 in monocytes from patients with RA impairs monocyte differentiation into an inflammation-resolving phenotype (M2-like)[117] and, in RA synovial tissue and fluid, an increase in miR-221-3p leads to decreased IL-10 production (via direct targeting of Janus kinase 3) in M2-like macrophages and acts synergistically with miR-155-5p to increase the production of IL-12 (which is specific to M1-like macrophages)[118]. Given the role of inflammation in OA and RA, understanding the contribution of non-coding RNAs to its underlying mechanisms could provide new insights.

Cell death and cell proliferation. Abnormal cell death and cell proliferation in joint tissues create hallmark features of OA (such as cartilage degeneration) and RA (such as synovial hyperplasia). Studies have reported the effects of a variety of miRNAs on chondrocyte apoptosis. For example, increased expression of miR-33b-3p, miR-9-5p or miR-27a decreased chondrocyte apoptosis[119–121], whereas increased expression of miR-486-5p, miR-363-3p or miR-455-3p increased chondrocyte apoptosis[75,122,123]. The mechanisms through which unique miRNAs affect cell death and cell proliferation can often converge onto a single pathway, such as the phosphoinositide 3-kinase (PI3K)–AKT signalling pathway[124–131]. Beneficial effects produced by miRNAs through regulation of the PI3K–AKT pathway include a reduction in apoptosis and cartilage degeneration caused by an increase in miR-455-3p[124], the promotion of chondrocyte proliferation and reduced apoptosis caused by a decrease in miR-34a[125] and a reduction in chondrocyte apoptosis and inflammation caused by an increase in miR-128-3p[126]. Conversely, increased amounts of miR-155, miR-1236 or miR-103 all promote chondrocyte apoptosis by targeting PI3K[127–129].

Table 3 | **Circular RNAs of mechanistic importance in OA and RA**

circRNA	Change in expression	Mechanism	Effects	Ref.
circ_0136474	Increased in OA cartilage	Sponges miR-127-5p, which negatively regulates *MMP13*	Suppresses cell proliferation and increases apoptosis in OA chondrocytes	180
circ_0009119	Decreased in OA cartilage	Sponges miR-26a, which negatively regulates *PTEN*	Protects OA chondrocytes from IL-1β-induced apoptosis	181
circ_0001722 (circCDK14)	Decreased in OA cartilage	Sponges miR-125a-5p, which negatively regulates *SMAD2*	Regulates ECM metabolism (decreases MMP3 and MMP13; increases SOX9 and type II collagen), inhibits apoptosis and promotes cell proliferation in chondrocytes	182
circ_0023404 (circRNF121)	Increased in OA cartilage	LEF1 increases circRNF121 expression, which sponges miR-665, which negatively regulates *MYD88*	Regulates degradation of ECM (increases MMP13 and ADAMTS5; decreases type II collagen and aggrecan), apoptosis and cell proliferation in chondrocytes	163
circ_0000284 (circHIPK3)	Increased in OA cartilage	Sponges miR-124, which negatively regulates *SOX8*	Inhibits apoptosis in chondrocytes	183
circVCAN	Increased in OA cartilage	Inhibits activation of NF-κB signalling pathway	Increases cell proliferation and decreases apoptosis in OA chondrocytes	184
circ_0008956 (circUBE2G1)	Increased in OA cartilage	Sponges miR-373, which negatively regulates *HIF1A*	circUBE2G1 inhibition reduces the effects of LPS in OA chondrocyte viability and apoptosis	185
circPSM3	Increased in OA cartilage	Sponges miRNA-296-5p	Inhibits cell proliferation and differentiation in OA chondrocytes	186
circCDR1as	Increased in OA cartilage	Sponges miR-641, which negatively regulates *FGF2*	Regulates ECM metabolism (increases MMP13 and IL-6; decreases type II collagen)	187
circTMBIM6	Increased in OA cartilage	Sponges miR-27a, which negatively regulates *MMP13*	Regulates ECM degradation (increases MMP13)	188
circ_0000448 (circGCN1L1)	Increased in OA TMJ synovium	Sponges miR-330-3p, which negatively regulates *TNF*	Increases chondrocyte apoptosis and ECM metabolism (increases MMP3, MMP13 and ADAMTS4; decreases type II collagen), and increases synoviocyte hyperplasia and inflammation	189
circ_0088036	Increased in RA FLSs	Sponges miR-140-3p, which negatively regulates *SIRT1*	Increases FLS proliferation and migration	190
circ_09505	Increased in RA PBMCs	Sponges miR-6089, which negatively regulates *AKT1*	Increases macrophage proliferation and cell-cycle progression	191

Includes articles published between 2019 and 2021. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; circRNA, circular RNA; ECM, extracellular matrix; FLS, fibroblast-like synoviocyte; LEF1, lymphoid enhancer-binding factor 1; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; OA, osteoarthritis; PBMC, peripheral blood mononuclear cell; RA, rheumatoid arthritis; SOX9, transcription factor SOX9; TMJ, temporomandibular joint.

Other pathways, such as NF-κB[132], can also mediate the effect of non-coding RNAs on chondrocyte apoptosis. In addition, lncRNAs and circRNAs can interact with miRNAs to regulate cell death and cell proliferation. For example, lncRNA CTBP1-AS2 is upregulated in OA synovial fluid and regulates the expression of miR-130a through methylation in OA chondrocytes, but not in healthy chondrocytes, to promote cell proliferation[66]. New evidence to support the importance of non-coding RNAs in regulating cell death and proliferation is also continuing to emerge[133] (TABLE 3).

In RA, expression of miR-483-3p, which is thought to be oncogenic in several human cancers, is increased; in FLSs, this miRNA directly targets *IGF1* mRNA (which encodes insulin-like growth factor 1; IGF1) to impair apoptosis and induce tumour-like proliferation[134]. Expression of the lncRNA NEAT1 is also increased in human RA synovial tissue[33] and PBMCs[114]. Delivery of NEAT1 to RA FLSs via plasma exosomes isolated from humans and mice caused sponging of miR-23a and miR-410-3p and thereby increased the expression of their targets (including E3 ubiquitin-protein ligase MDM2 and transcriptional repressor protein YY1), leading to a decrease in apoptosis and an increase in FLS proliferation and inflammation[33,135]. Furthermore, the reduction of NEAT1 via siRNA can reduce the severity of CIA in mice[114]. These results illustrate the possibility of targeting non-coding RNAs to modulate cell death and cell proliferation.

Extracellular matrix dysregulation. miRNAs, lncRNAs, circRNAs and even snoRNAs have been implicated in ECM dysregulation in joint tissues such as cartilage, synovium and bone. IL-1β is widely used to induce cellular responses that mimic pathological conditions including inflammation[109], apoptosis[127] and cartilage degradation[97]. Researchers often use IL-1β to stimulate a response in chondrocytes in vitro that is subsequently rescued or exacerbated by manipulating a non-coding RNA. In cultured human chondrocytes, increased miR-377-3p expression reversed IL-1β-induced upregulation of inflammatory markers, cartilage degradation markers and chondrocyte apoptosis[30]. In a different experimental model, human chondrocytes transfected with an miR-613 agonist for 48 hours prior to administration of IL-1β had reduced markers of inflammation, apoptosis and cartilage degradation compared with IL-1β-treated cells[136]. A tentative role for the NEAT1–miR-377-3p–*ITGA6* axis has been described in IL-1β-treated chondrocytes, in which NEAT1 might function as an miR-377-3p sponge, thereby upregulating *ITGA6* expression to affect inflammatory responses, apoptosis and ECM degradation[30]. Among other notable lncRNAs, XIST increases collagen degradation in OA chondrocytes via increasing *TIMP3* promoter methylation through the recruitment of a DNA methyltransferase[106]. Furthermore, in OA cartilage, expression of the lncRNAs H19 and LINC01534 is increased and ECM degradation is promoted through their individual binding to miR-140-5p[137,138], an miRNA that is well characterized in OA for its cartilage matrix remodelling effects[139]. Roles for circRNAs (TABLE 3) and snoRNAs[28,140,141] have also been described in ECM metabolism. For example, impaired expression of U3 snoRNA, SNORD26 or SNORD96A alters the protein translation capacity of chondrocytes, chondrocyte differentiation, pro-inflammatory pathways and the expression of markers of OA[28,141].

Notably, ECM dysregulation can actually facilitate intercellular communication by making otherwise dense cartilage and bone matrices more permeable to extracellular vesicles that carry non-coding RNAs. Evidence from RA suggests that both chondrocytes and osteoblasts can respond to miRNAs carried by FLS-derived exosomes. In one study, exosomes from FLSs carrying miR-106b induced chondrocyte apoptosis and reduced proliferation by directly targeting *PDK4* mRNA[142]. In another study, exosomes from FLSs carrying miR-486-5p were phagocytosed by osteoblasts and promoted their differentiation and the expression of ECM markers such as type I collagen[143]. Given that inflamed and hyperplastic synovium is a common feature of RA, exosomes secreted by FLSs could serve as messengers to induce damage in surrounding joint tissues.

Therapeutic potential

Non-coding RNAs represent promising therapeutic targets in OA and RA because their activity can be modulated via small molecules and biological delivery systems (such as exosomes[144–146]) to reduce features of disease, and even pain, in experimental models of arthritis[147,148]. The cargo of these delivery systems can include non-coding RNA mimics (known as agomirs), non-coding RNA inhibitors (known as antagomirs) and other molecules, such as transcription factors. For example, in mouse cells in vitro, the lncRNA MM2P promotes macrophage polarization towards an M2-like phenotype and stimulates the release of exosomes containing SOX9 mRNA and protein from these cells, which can induce the production of ECM components in cultured chondrocytes[149]. As these exosomes contain a functional transcription factor (SOX9) that is known to promote chondrocyte anabolism, they represent a potential therapeutic strategy to restore cartilage homeostasis.

An important consideration in harnessing the potential of non-coding RNAs as possible therapeutics is the route of administration, whether systemic or intra-articular, so that the therapy can reach the intended target tissues. Systemic administration of non-coding RNAs can be achieved by intravenous injection. Using this method, an miR-365 agomir decreased disease severity in mice with CIA, potentially through downregulation of IGF1 (REF.[78]). Similarly, following intravenous injection, the concentration of an miR-26a mimic was increased in articular cartilage and could reduce disease severity in rats with CIA through the downregulation of connective tissue growth factor (CTGF)[150]. Intravenous injection of exosomes has also been explored. BMSC-derived exosomes enriched with miR-320a, which directly downregulates C-X-C chemokine ligand 9, could decrease disease severity in mice with CIA following intravenous injection[151]. Furthermore, BMSC-secreted exosomes enriched with miR-192-5p could be found in the synovial tissue of rats with CIA after intravenous injection, and could decrease disease

severity, potentially through downregulation of the signalling molecule RAC2 (REF.[152]). These data suggest that non-coding RNAs were able to traffic to the joints in rats and mice with CIA, where they reduced synovial inflammation, cartilage damage and bone erosion. However, because a single non-coding RNA can have multiple gene targets, local modulation (such as direct injection of miRNA agomirs and antagomirs into the joint) is also being explored to avoid unwanted systemic effects. In mice with CIA, intra-articular delivery of miR-146a-5p agomir decreased disease activity, synovial hyperplasia, the invasiveness of the pannus and cartilage erosion, potentially through downregulation of CTGF[153]. Similarly in rats with CIA, intra-articular delivery of miR-141-3p agomir improved disease outcomes via direct binding of the transcription factor FOXC1, which functions as an oncogene to promote tumour and RA FLS proliferation[154]. Intra-articular delivery of exosomes is also possible, and exosomes can even be engineered to target cells of interest. For example, the fusion of chondrocyte-affinity peptide to lysosome-associated membrane glycoprotein 2b molecules on the surface of exosomes promoted the trafficking and fusion of the exosomes to chondrocytes, the efficient delivery of miR-140 and the mitigation of disease progression in a rat surgical model of OA[155].

Alternative strategies for non-coding RNA modulation continue to emerge. For example, transplantation of cartilage pellets derived from human BMSCs that overexpress beneficial miRNAs (such as miR-27b) inhibited hypertrophic chondrocyte differentiation during cartilage defect repair[156]. Similarly, intra-articular injection of human umbilical cord-derived mesenchymal stem cells transfected to overexpress miR-140 had protective effects in a rat model of OA[157]. A biodegradable delivery system for an miR-365 antagomir based on non-pathogenic yeast cell wall particles has been developed that could resist degradation in the gastrointestinal system following oral administration, and which reduced features of disease in a mouse surgical model of OA[102]. Furthermore, cationic liposomes (lipoplexes) have been used for the intra-articular administration of miR-17-5p to reduce synovial immune cell infiltration, inflammation and bone erosion in mice with CIA[72]. As an alternative means of suppressing miRNA function, tough decoy RNAs have been developed, wherein vectors expressing miRNA target sites bind and reduce specific miRNA activity in cells; a tough decoy for miR-195-5p reduced its activity and the occurrence of hypertrophy in cultured chondrocytes[158]. Moreover, miRNA agomirs and antagomirs can be directly modified to improve their therapeutic properties. To improve specific binding, locking the conformation of antagomirs (known as locked nucleic acids) is effective, and intra-articular delivery of locked nucleic acid antagomirs for miR-181a-5p and miR-34a-5p could reduce disease severity in experimental models of OA[159,160]. To improve stability and delivery, non-coding RNAs can be conjugated to other molecules such as atelocollagen; intra-articular administration of an miR-9a-5p agomir–atelocollagen complex could effectively reduce disease severity in rats with CIA[161]. Additional delivery mechanisms and considerations for achieving clinical translation of antisense oligonucleotide-based therapies for OA have been reviewed in detail elsewhere[104,162].

Studying the interactions among non-coding RNAs, including regulators and effectors of circRNA–miRNA–mRNA axes, could also reveal new avenues for targeted treatment. One molecular mechanism proposed as a prospective therapeutic target for OA is the circRNF121–miR-665–*MYD88* axis, which is regulated by the transcription factor LEF1 (REF.[163]). In human chondrocytes, LEF1 increases the expression of circRNF121, which functions as a sponge for miR-665, thereby indirectly targeting *MYD88*. As such, modulation of miR-665 and circRNF121 could alter *MYD88* expression to promote chondrocyte apoptosis, proliferation and ECM degradation, both in vitro in human chondrocytes and in vivo in a rat model of OA. Furthermore, this axis was shown to activate the NF-κB signalling pathway[163]. Although the data suggest that miR-665 could be targeted to mitigate the detrimental effects of circRNF121, it is evident that the upstream regulator (LEF1), circRNA (circRNF121), miRNA (miR-665), gene target (*MYD88*) and downstream pathway (NF-κB) could all be potential targets. These data illustrate the importance of considering the non-coding RNA interactome for therapeutic targeting, as one or more of these factors might need to be modulated to improve disease outcomes (BOX 1).

On the basis of the current literature, outstanding questions remain to be answered before targeting of non-coding RNAs can be translated into a therapeutic strategy to improve patient care. First, the appropriate target must be identified, whether it is the non-coding RNA, its upstream regulator or the downstream mediator. Second, the appropriate tissue or tissues must be identified for targeting, as non-coding RNAs are known to have tissue-specific effects. Third, the appropriate

Box 1 | **The non-coding RNA interactome in gene expression regulation**

The regulation of gene expression is tightly controlled. Non-coding RNAs have important roles in this process, operating through direct mechanisms (such as degradation of gene transcripts) and indirect mechanisms (such as inhibition of other non-coding RNAs). Together, these mechanisms comprise the non-coding RNA interactome, which can be thought of as the complete set of the molecular interactions of non-coding RNAs. Emerging literature suggests that interactions among non-coding RNA entities and classes are common and have considerable implications for joint diseases. High-throughput profiling is a useful approach for beginning to unravel the non-coding RNA interactome. For example, researchers have used three publicly available microarray datasets for synovium from patients with rheumatoid arthritis (RA) to demonstrate potential direct regulation of interconnected gene targets by specific long non-coding RNAs (lncRNAs), wherein the lncRNAs NEAT1 and FAM30A were predicted to interact with major RA hub genes[192]. To explore interactions across non-coding RNA classes, circular RNA (circRNA)–microRNA (miRNA) networks have been constructed for synovium[193], cartilage[194] and chondrocytes[133,195] from patients with osteoarthritis (OA). For example, researchers have used RNA sequencing to identify OA-related circRNAs in cartilage, followed by bioinformatics analyses to discover 166,394 circRNA–miRNA–mRNA axes[194]. LncRNA–miRNA networks have also been explored in OA. In human knee cartilage, publicly available RNA sequencing data have been mined to identify differentially expressed lncRNAs and mRNAs that contribute to an integrated network of competing endogenous RNAs, including 10 lncRNAs, 69 miRNAs and 72 mRNAs[196]. These individual profiling studies are an important first step towards understanding the non-coding RNA interactome, but need to be followed by efforts to integrate findings across studies so that candidates for further validation and potential therapeutic targeting can be prioritized.

Table 4 | **Use of bioinformatics and computational biology tools in non-coding RNA research**

Challenge	Approach	Resources
Analysing high-throughput profiling data for non-coding RNAs in both health and disease contexts	A search tool can be used to discover novel non-coding RNA sequences in deep sequencing data	miRDeep2
Ensuring proper and consistent naming of all non-coding RNA entities so that data can be accurately integrated across studies	Several databases are helpful to ensure the correct use of primary names and identifiers	miRbase for microRNAs; DIANA-lncBase for long non-coding RNAs; circBase and circAtlas for circular RNAs; and Hugo Gene Nomenclature for gene names
Elucidating the potential functions of non-coding RNAs	Multiple tools can be used for target gene prediction, including for novel microRNA sequences	TargetScan; mirDIP; miRDB; and miRanda
Interpreting predicted target genes	Pathway prediction tools can be used to create functional groups with biological relevance (such as signalling cascades)	The Gene Ontology Resource for gene enrichment analysis; pathDIP for integrated pathway enrichment analysis; and integrated web portals, such as Enrichr, for access to diverse types of computational annotation and overrepresentation analysis
Combining non-coding RNA datasets to promote integrative computational analyses	Public repositories can be used to access and deposit high-throughput data	The NCBI Gene Expression Omnibus repository; the NCBI Sequence Read Archive; the NCBI Database of Genotypes and Phenotypes; and the EMBL-EBI European Nucleotide Archive

EMBL-EBI, European Molecular Biology Laboratory European Bioinformatics Institute; NCBI, National Center for Biotechnology Information.

delivery mechanism must be identified, including the vehicle (such as exosomes) and route of administration. For example, intra-articular delivery might offer benefits over systemic administration by providing local modulation and thereby reducing unwanted off-target effects. The answers to these questions and others (such as the best dosage to use) might be patient-specific, and tailored RNA-based therapeutics might need to be administered in a phenotype-dependent manner to achieve precision medicine in OA and RA. The utility of RNA-based therapeutics has now achieved global recognition through RNA vaccines, which were first described over two decades ago[164], and it is therefore reasonable to expect bolstered research efforts into RNA-based therapeutics, which should include non-coding RNAs.

Future directions

Unravelling the complex interactions among non-coding RNAs is becoming an important goal; however, the comprehensive high-throughput profiling of joint tissues and biofluids that will be necessary to achieve this aim comes with its own set of challenges (TABLE 4). Although microarrays are useful for profiling a pre-selected subset of known candidate RNAs, this technique is limited by factors such as the appropriate selection of an endogenous reference, which can vary by tissue type[165]. Increasingly, next-generation sequencing technologies are being used to achieve unbiased and quantitative measures of all varieties of non-coding RNAs. For example, next-generation sequencing can be used to identify the direct binding of miRNAs to target genes through RNA-immunoprecipitation and high-throughput sequencing (RIP sequencing), as has been described in human articular chondrocytes[166]. This approach enables the validation of predicted gene targets that are commonly obtained using prediction tools (TABLE 4). Furthermore, applying sequencing technology to fundamental processes in model systems has the potential to uncover important mechanisms that underlie disease. For example, combinations of RNA sequencing and small non-coding RNA sequencing have been applied to explore chondrogenesis and metabolism in human BMSCs[167], inflammatory cytokine responses in mouse induced pluripotent stem cells[168] and cartilage ageing in horse chondrocytes[140].

Among the limitations of unbiased discovery of non-coding RNAs is that researchers often focus on just one or two molecules for further investigation. How and why these molecules are chosen can be unclear, as other non-coding RNA entities that could have promising roles in joint pathobiology are often not investigated further. Notably, very few non-coding RNA studies in OA and RA include comparisons with other studies or meta-analyses with other available non-coding RNA datasets in order to validate, expand and build a comprehensive interactome of these important epigenetic regulators. However, efforts are ongoing around the world to curate comprehensive databases of published evidence to help researchers to investigate the complex interactions between non-coding RNAs, genes and proteins (TABLE 4). To this end, it is critical that nomenclature and annotations for the non-coding RNAs identified in studies are systematically reported. For example, investigators are encouraged to report the strands of miRNAs (3p or 5p) to ensure accurate integration of their data with other datasets and analyses. Furthermore, reporting of the clinical annotation of samples involved in non-coding RNA studies is required to enable correlation with molecular and clinical phenotypes. Finally, to improve the quality of basic and translational research by applying integrative analytical and machine learning techniques, well-annotated high-throughput data must be made available in the correct format (for example, the raw sequencing datasets) in online repositories.

Conclusions

A substantial surge has occurred in the number of published articles related to non-coding RNAs in OA and RA in the past few years, mostly for miRNAs, lncRNAs and circRNAs, and to a lesser degree for snoRNAs,

tRFs and other non-coding RNAs. This increased research output has been possible because of advances in next-generation sequencing technology and the availability of computational and analytical tools for data mining. Some of the non-coding RNAs that have been identified using these methods, as well as their regulatory interactome, could have crucial roles in joint health and disease, affecting biological processes and functioning as biomarkers, mediators of pathogenesis and potential therapeutic targets. Although these discoveries are promising, a concerted effort is required to validate, integrate and translate findings from current studies to harness the full potential of non-coding RNAs in OA and RA.

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

Competing interests
S.A.A. and M.K. declare that they have filed a US Provisional Patent Application no. 63/033,463 titled "Circulating MicroRNAs in Knee Osteoarthritis and Uses Thereof". The other authors declare no competing interests.

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