ANTIPHOSPHOLIPID SYNDROME

End of the road for direct oral anticoagulants in thrombotic APS?

> rivaroxaban did not show noninferiority to dose-adjusted VKAs for preventing recurrent thrombosis in patients with APS

Controversy has surrounded the possible use of direct oral anticoagulants (DOACs) as secondary thromboprophylaxis in patients with antiphospholipid syndrome (APS) following conflicting results as to their efficacy in preventing thrombotic events and reports of serious adverse events in patients taking DOACs. The results of a new head-to-head trial failed to demonstrate noninferiority of the DOAC rivaroxaban compared with dose-adjusted vitamin K antagonists (VKAs) in preventing recurrent thrombosis, as well as showing an increased risk of stroke with rivaroxaban, adding to mounting evidence against the use of DOACs in patients with APS.

Standard-of-care secondary thromboprophylaxis with dose-adjusted VKAs such as warfarin has several drawbacks, including adverse interactions with food and other drugs, the need for careful monitoring to keep patients within a desired range of the international normalized ratio (INR; a measure of clotting time) and an increased risk of bleeding. By specifically targeting one part of the coagulation cascade, DOACs are designed to provide predictable anticoagulation that can be delivered at a consistent dose and that does not require continuous monitoring.

Credit: Marko Zamurovic/Alamy Stock Photo

Rivaroxaban directly inhibits activated factor X and is approved for use in the prevention of venous thromboembolism in the general population and stroke in patients with acute coronary syndrome, but is not yet approved for use in patients with APS. In the new open-label trial, which was the largest of its kind to date, 190 patients with APS were randomly allocated to receive either 20 mg rivaroxaban per day or VKAs at a dose adjusted to achieve a target INR of 2.0-3.0. The primary efficacy end point was a new thrombotic event occurring during the 36 months of follow-up. Equal numbers of patients receiving each treatment discontinued therapy early for reasons other than thrombotic events, and eight patients died (five of those receiving rivaroxaban and three of those receiving VKAs), mostly as a result of cancer.

In the per protocol analysis, 11 patients receiving rivaroxaban (11.6%) had a thrombotic event compared with 6 patients receiving VKAs (6.3%), producing a risk ratio (RR) of 1.83 (CI 0.71-4.76) for rivaroxaban that did not reach statistical significance. The results of the intention-to-treat analysis were similar (RR 2.0; CI 0.78-5.11) and also did not reach statistical significance. The upper limit of the CI margin exceeded the predefined noninferiority margin of 1.40, leading the authors to conclude that rivaroxaban did not show noninferiority to dose-adjusted VKAs for preventing recurrent thrombosis in patients with APS.

These results contrast with those of the 2016 RAPS trial, which was the first randomized controlled trial to investigate the noninferiority of rivaroxaban compared with warfarin in patients with APS. The threshold for noninferiority was also not met in the RAPS trial, but peak thrombin generation was lower after 6 weeks in those treated with rivaroxaban than in those treated with warfarin, and no thrombotic events were recorded during that time, leading the authors to suggest that rivaroxaban might be considered as an alternative therapy to warfarin in these patients. Differences in the primary efficacy end points and trial durations might explain the discrepancies in results between the current study and the RAPS trial.

Notably, the rate of arterial thrombosis, especially stroke, was much higher in the new study in those receiving rivaroxaban (9 incidences) than in those receiving VKAs (0 incidences). These results echoed those of the 2018 TRAPS study, which examined the noninferiority of rivaroxaban to warfarin for patients with APS who had triple antiphospholipid antibody (aPL) positivity. Although the TRAPS study was terminated early, the results suggested an increased risk of arterial thrombotic events in patients receiving rivaroxaban compared with those receiving warfarin.

The results of the TRAPS study led EULAR to recommend against the use of rivaroxaban in patients with APS who are triple-aPL-positive in their 2019 APS management recommendations. Whether the results of the present trial will lead to this recommendation being extended to all patients with APS remains to be seen.

Joanna Collison

ORIGINAL ARTICLE Ordi-Ros, J. et al. Rivaroxaban versus vitamin K antagonist in antiphospholipid syndrome: a randomized noninferiority trial. *Ann. Intern. Med.* https://doi.org/10.7326/M19-0291 (2019)

IN BRIEF

SPONDYLOARTHRITIS

Ixekizumab superior to adalimumab for PsA

Targeting IL-17A with ixekizumab was superior to targeting TNF with adalimumab in a head-to-head trial involving 566 biologic DMARD-naive patients with psoriatic arthritis (PsA). All patients enrolled had active skin and joint disease and had previously responded poorly to conventional synthetic DMARDs. At 24 weeks, 36% of patients receiving ixekizumab had reached the primary end point compared with 28% of those receiving adalimumab. Patients receiving ixekizumab also had fewer serious adverse events than those receiving adalimumab.

ORIGINAL ARTICLE Mease, P. J. et al. A head-to-head comparison of the efficacy and safety of ixekizumab and adalimumab in biological-naïve patients with active psoriatic arthritis: 24-week results of a randomised, open-label, blinded-assessor trial. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-215386 (2019)

AUTOIMMUNITY

Increased risk of rheumatic disease in IPAF

Patients with interstitial pneumonia with autoimmune features (IPAF) have a 14-fold higher risk of progressing to a rheumatic disease than patients with interstitial lung disease (ILD) but not IPAF, according to the results of a retrospective cohort study. The term IPAF is used to describe individuals with ILD who have some features of autoimmunity, but who do not fulfil the classification criteria for an autoimmune rheumatic disease. Of the 174 patients included in the study, 8 out of 50 patients with IPAF (16%) developed rheumatic diseases after a median of 5.2 years, compared with 2 out of 124 patients with ILD (1.6%).

ORIGINAL ARTICLE Alevizos, M. K. et al. Risk of progression of interstitial pneumonia with autoimmune features to a systemic autoimmune rheumatic disease. *Rheumatology* https://doi.org/10.1093/rheumatology/kez404 (2019)

MYOSITIS

Combined immunosuppression for ILD in myositis

A combined immunosuppressive treatment regimen of high-dose glucocorticoids, tacrolimus and intravenous cyclophosphamide was effective in a Japanese cohort of 29 patients with anti-melanoma differentiation-associated gene 5 $(MDA5)^+$ dermatomyositis-associated interstitial lung disease (ILD) in a prospective study. The 6-month survival of patients receiving combined immunosuppression was higher than that of a historical cohort of 15 patients who received step-up therapy of high-dose glucocorticoids with additional immunosuppressants (89% versus 33%, respectively).

ORIGINAL ARTICLE Tsuji, H. et al. A multicenter prospective study of the efficacy and safety of combined immunosuppressive therapy with high-dose glucocorticoid, tacrolimus, and cyclophosphamide in interstitial lung diseases accompanied by anti-melanoma differentiation-associated gene 5-positive dermatomyositis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41105 (2019)

THERAPY

Checkpoint inhibitor-induced arthritis is persistent

Inflammatory arthritis that developed following immune checkpoint inhibitor (ICI) therapy for cancer persisted for several months following cessation of ICI therapy and necessitated management by a rheumatologist. At 6 months follow-up, 20 out of 41 patients for whom data were available had active inflammatory arthritis. Overall, three-quarters of the 60 patients included in the follow-up study required immunosuppressive treatment for their inflammatory arthritis, which did not seem to affect cancer progression.

ORIGINAL ARTICLE Braaten, T. J. et al. Immune checkpoint inhibitor-induced inflammatory arthritis persists after immunotherapy cessation. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2019-216109 (2019)

Credit: A-Digit/DigitalVision Vectors

SJÖGREN SYNDROME

Stratifying Sjögren syndrome into symptom-based subgroups

In a new study, symptom-based stratification of patients with primary Sjögren syndrome (pSS) revealed the existence of endotypes that differ with respect to their clinical and biological characteristics and response to therapy. "To our knowledge, this is the first report showing distinct subsets of an immune-mediated inflammatory disease and linking clinical and pathobiological heterogeneity, with direct clinical implications," reports corresponding author Wan-Fai Ng.

Heterogeneity in the clinical presentation of pSS presents challenges in the design of new treatments and in their evaluation in clinical trials. Understanding the differences between patient subgroups could influence pSS management.

In the study, a team of biostatisticians, bioinformaticians, data scientists and clinicians first undertook exploratory clustering analysis of symptom scores for pain, fatigue, dryness, anxiety and depression reported by 608 patients in the UK Primary Sjögren's Syndrome Registry (UKPSSR). The analysis identified four distinct subgroups: low symptom burden, high symptom burden (HSB), dryness dominant with fatigue (DDF) and pain dominant with fatigue. A multinomial logistic regression model was then used to develop a tool to stratify other patients with pSS into these four symptom-based subgroups.

Comparison of the subgroups in the UKPSSR cohort revealed substantial differences in salivary flow, ocular dryness, serum IgG concentrations, peripheral blood lymphocyte counts and prevalence of anti-SSA or anti-SSB antibodies; whole-blood transcriptomic profiles also varied across the subgroups. These differences were also observed in two independent validation cohorts of patients in Norway ($n = 62$) and France ($n = 334$).

"A vital lesson that we have learned is the importance of collecting patient reported outcomes, not only because they matter to the patients, but also because they help us to do better research," Ng says.

Application of the symptom-based stratification scheme in a reanalysis of data from two placebo-controlled phase III trials in pSS showed that treatment response varied across the subgroups. In the TRACTISS trial, patients in the DDF subgroup showed improved salivary flow in response to rituximab treatment, and in the JOQUER trial, patients in the HSB subgroup showed improvement in symptoms in response to hydroxychloroquine treatment, whereas no treatment effect was seen in the other subgroups.

"We believe that our findings have key implications for drug development, particularly in clinical trial design, as well as informing molecular targets," says Ng. "Knowledge of these subtypes will also help us to develop a more personalised management plan for individual patients."

Sarah Onuora

ORIGINAL ARTICLE Tarn, J. R. et al. Symptom-based stratification of patients with primary Sjögren's syndrome: multi-dimensional characterisation of international observational cohorts and reanalyses of randomised clinical trials. *Lancet Rheumatol.* **1**, e85–94 (2019)

RHEUMATOID ARTHRITIS

Targeting the CRP–HIF1α axis in RA improves response to leflunomide

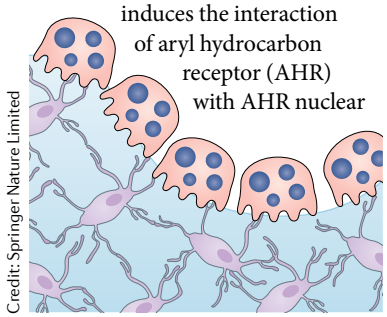
In a substantial proportion of patients with rheumatoid arthritis (RA), treatment with the DMARD leflunomide fails to halt the progression of bone erosion, despite inhibiting inflammation. A new study reveals that C-reactive protein (CRP)–hypoxia inducible factor 1α (HIF1α) signalling attenuates the response to leflunomide, and suggests that co-administration of a HIF1α inhibitor could improve the therapeutic response to leflunomide in selected patients.

"We performed binary logistic regression analysis to determine the relationships between the limited leflunomide response and commonly used clinical factors," says corresponding author Ge Zhang. "Surprisingly, we revealed that serum CRP concentrations showed predictive value for classifying the patients with RA with limited response to leflunomide."

> combined treatment with leflunomide and acriflavine, an FDA-approved HIF1α inhibitor, prevented bone loss

In patients with RA and high serum concentrations of CRP (CRP-high), leflunomide attenuated bone erosion to a lesser extent than in CRP-low patients; notably, leflunomide had similar immunomodulatory effects in both groups. Rats with collagen-induced arthritis (CIA) had similarly differential responsiveness to leflunomide treatment on the basis of CRP concentrations.

Further experiments revealed that in CRP-low rats, leflunomide induces the interaction of aryl hydrocarbon receptor (AHR) with AHR nuclear translocator (ARNT) to inhibit hepatic CRP expression and thus attenuate bone erosion. In CRP-high rats, however, CRP upregulates expression of HIF1α, which competes with AHR for ARNT binding and interferes with leflunomide–AHR–CRP signalling, ultimately limiting the response to leflunomide.



Credit: Springer Nature Limited

Knockdown of HIF1α in vitro and hepatocyte-specific deletion of HIF1α in mice with CIA improved leflunomide–AHR–CRP signalling and, in the mice, inhibited bone erosion. Moreover, combined treatment with leflunomide and acriflavine, an FDA-approved HIF1α inhibitor, prevented bone loss in CRP-high rats with CIA.

Together, the results suggest that CRP could have clinical value for prediction of response to leflunomide treatment, and that the combination of leflunomide and acriflavine could be used as precision medicine for CRP-high patients with RA.

Sarah Onuora

ORIGINAL ARTICLE Liang, C. et al. HIF1α inhibition facilitates leflunomide-AHR-CRP signaling to attenuate bone erosion in CRP-aberrant rheumatoid arthritis. *Nat. Commun.* **10**, 4579 (2019)

THERAPY

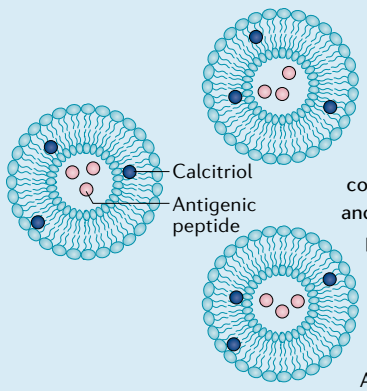
Liposomal targeting of DCs to induce tolerance

New findings highlight targeting of dendritic cells (DCs) with antigen-containing liposomes as a promising strategy for inducing autoantigen-specific immunological tolerance in autoimmune diseases. "Our data show that immune regulation could be restored in inflammatory autoimmune diseases where autoantigen is widely expressed — in models of rheumatoid arthritis (RA) and renal vasculitis," reports corresponding author Ranjeny Thomas.

"Liposomes have an established clinical track record for targeted drug delivery and provide a platform for co-delivery to DCs of the lipophilic NF-κB inhibitor calcitriol with hydrophilic antigenic peptide," says Thomas. "Importantly, co-delivery of antigen in the liposomes ensures that immune regulation is antigen-specific and not generalized," explains Thomas.

Indeed, calcitriol-containing liposomes suppressed the cytokine-secreting and antigen-presenting capacity of DCs in vitro. In mice immunized with an antigenic peptide, administration of liposomes containing both calcitriol and the peptide suppressed the expansion of antigen-specific effector T cells and promoted the expansion of antigen-specific regulatory T cells.

An important question was whether an inflammatory setting could inhibit the induction of tolerance by activating DCs. "We show that active inflammation actually supported tolerance induction with liposomes encapsulating peptide and calcitriol," reports Thomas. "Inflammation promoted PDL1 expression by DCs, PDL1$^+$ DCs were more likely to take up the liposomes and PDL1 was required for the induction of regulatory T cells."



Credit: Springer Nature Limited

In mouse models of RA and Goodpasture's vasculitis (also known as anti-glomerular basement membrane disease), liposomes containing calcitriol and disease-associated peptides inhibited the development of autoimmunity and also suppressed existing disease. Analysis using peptide–MHC class II tetramer staining to visualize the autoreactive CD4$^+$ T cell compartment suggested that liposomal treatment decreased disease severity in an antigen-specific manner.

> liposomal treatment decreased disease severity in an antigen-specific manner

"The research opens up new potential for drug development and trials in patients with autoimmune diseases and in individuals at high risk of disease development," concludes Thomas.

Jessica McHugh

ORIGINAL ARTICLE Galea, R. et al. PD-L1- and calcitriol-dependent liposomal antigen-specific regulation of systemic inflammatory autoimmune disease. *JCI Insight* **4**, e126025 (2019)

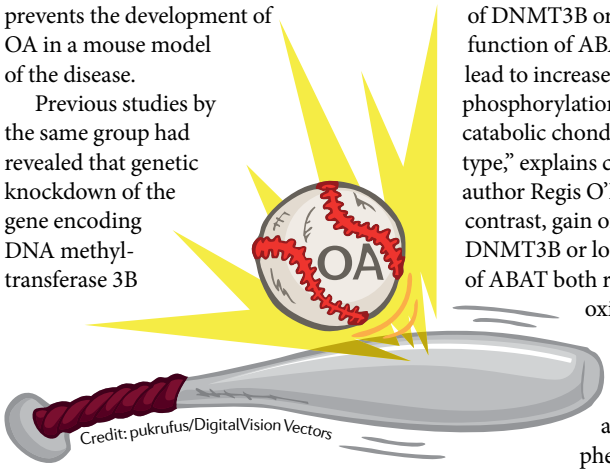
OSTEOARTHRITIS

Taking ABAT to OA

New research establishes the enzyme 4-aminobutyrate aminotransferase, mitochondrial (ABAT) as a key regulator of cellular metabolism and a potential therapeutic target in osteoarthritis (OA). The research also demonstrates that vigabatrin, a small-molecule inhibitor of ABAT that is already approved for clinical use as an anticonvulsant, prevents the development of OA in a mouse model of the disease.

Previous studies by the same group had revealed that genetic knockdown of the gene encoding DNA methyltransferase 3B (DNMT3B) in articular chondrocytes led to OA progression via changes in mitochondrial metabolism. In the current study, the group identified ABAT as an important downstream target of DNMT3B in chondrocytes and elucidated its role in regulating chondrocyte mitochondrial function and the development of OA.

"We found that loss of function of DNMT3B or gain of function of ABAT both lead to increased oxidative phosphorylation and a catabolic chondrocyte phenotype," explains corresponding author Regis O'Keefe. "By contrast, gain of function of DNMT3B or loss of function of ABAT both reduce oxidative phosphorylation and result in an anabolic phenotype."



Credit: pukrufus/DigitalVision Vectors

> "intraperitoneal administration of vigabatrin … completely prevented the development of injury-induced OA"

Consistent with these findings, lentivirus-mediated overexpression of ABAT in mouse knees accelerated OA development following medial ligament injury (MLI) surgery, whereas lentiviral suppression of ABAT partially attenuated cartilage destruction. Notably, intraperitoneal administration of vigabatrin at a dose of 200 mg/kg for 6 weeks following MLI surgery completely prevented the development of injury-induced OA. In primary articular chondrocyte cell cultures, vigabatrin treatment also reduced mitochondrial respiration and inhibited IL-1β-mediated expression of catabolic genes including *Runx2*, *Mmp13* and *Col10a1*.

The investigators envisage that ABAT inhibition could be developed as a targeted therapy for OA, and are exploring the possibility of delivering ABAT small interfering RNA into arthritic joints via nanoparticles.

Sarah Onuora

ORIGINAL ARTICLE Shen, J. et al. Inhibition of 4-aminobutyrate aminotransferase protects against injury-induced osteoarthritis in mice. *JCI Insight* **4**, e128568 (2019)

SYSTEMIC LUPUS ERYTHEMATOSUS

Targeting mitochondrial dysfunction in SLE

Emerging evidence suggests that mitochondrial abnormalities contribute to immune dysregulation and organ damage in systemic lupus erythematosus (SLE). In a new study, targeting mitochondrial dysfunction using the drug idebenone improved clinical and immunological features of lupus-like disease in mice, highlighting idebenone as a promising new drug for SLE.

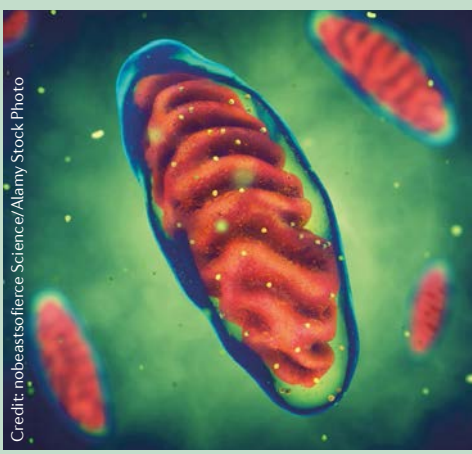
Idebenone is a coenzyme Q10 synthetic quinone analogue that has antioxidant properties. Notably, this drug is already approved in some countries for the treatment of other conditions associated with mitochondrial dysfunction (such as Duchenne muscular dystrophy).

"In two mouse models of SLE, we found that administration of idebenone reduced renal inflammation and renal function, attenuated systemic immune dysregulation in the innate and adaptive immune systems and improved mitochondrial metabolism," states Mariana Kaplan, corresponding author on the study.

> "treatment with idebenone inhibited spontaneous NET formation"

Treatment with idebenone improved the survival of the mice and was well tolerated. "Importantly, the use of idebenone was associated with improvements in endothelium-dependent vasorelaxation, suggesting that this drug could target lupus vasculopathy, a prominent feature in this disease," explains Kaplan.

One mechanism by which mitochondrial dysfunction and aberrant production of mitochondrial reactive oxygen species (mROS) might contribute to SLE is by promoting the formation of neutrophil extracellular traps (NETs) and activation of the type I interferon pathway. Indeed, in neutrophils from mice with lupus or from patients with SLE, ex vivo treatment with idebenone inhibited spontaneous NET formation. Furthermore, in the mouse neutrophils, this treatment was associated with decreased mROS synthesis.


Credit: nobeastsofierce Science/Alamy Stock Photo

"We are continuing to investigate potential therapeutic targets that modify mitochondrial function and immunometabolism in SLE," says Kaplan. "Our hope is to be able to identify the best compounds that could be tested in clinical trials in SLE in the future."

Jessica McHugh

ORIGINAL ARTICLE Blanco, L. P. et al. The coenzyme Q10 analog idebenone attenuates murine lupus by improving mitochondrial metabolism and reducing inflammation. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41128 (2019)

THERAPY

Allogeneic HSCT for autoimmune disease: a shared decision

Keith M. Sullivan and Stefanie Sarantopoulos

Autologous haematopoietic stem cell transplantation (HSCT) can be an effective treatment for refractory autoimmune diseases. With reports of the use of allogeneic HSCT emerging, how do these two very different types of stem cell transplantation compare and is allogeneic HSCT advisable?

Refers to Greco, R. et al. Allogeneic HSCT for autoimmune diseases: a retrospective study from the EBMT ADWP, IEWP, and PDWP working parties. *Front. Immunol.* **10**, 1570 (2019)

Autoimmune diseases are a leading cause of morbidity and mortality that disproportionally affect women. Control of disease with biologics and DMARDs is often imperfect and alternative approaches are required. A new report by Greco and colleagues highlights one such potential therapy — allogeneic haematopoietic stem cell transplantation (HSCT)[1] — but is this approach advisable?

The outcomes and complications of HSCT can differ considerably depending on the source of haematopoietic stem cells for transplantation[2]. For autologous HSCT, a person's own stem cells are collected before HSCT from the bone marrow or from the peripheral blood (after mobilization) and reinfused intravenously after cytoreductive conditioning. To reduce the risk of reinfusing autoreactive or abnormal cells, $CD34^+$ selection of the cell product might be employed. Within 10–14 days of transplant, neutrophil counts return to normal, although full recovery of the haematologic and immunologic systems might take several months. Allogeneic transplantation infuses normal haematopoietic stem cells from a suitably matched related or unrelated donor, lowering the risk of return of the underlying disease. However, allogeneic HSCT might have associated risks of morbidity and mortality from graft-versus-host disease (GVHD), a complication not seen with autologous HSCT.

In a retrospective study, Greco et al.[1] assessed the long-term outcomes and toxicity of allogeneic HSCT using registry data provided by the European Society for Blood and Marrow Transplantation (EBMT) and associated working parties. This registry contained data collected from 128 patients who received allogeneic HSCT for refractory autoimmune disease between 1997 and 2014. Disorders included haematologic ($n = 49$), gastrointestinal ($n = 20$), and neurologic ($n = 12$) autoimmune diseases and rheumatic autoimmune diseases ($n = 47$). Most of the recipients were children (median age 12.7; range 0.2–62 years). Peripheral blood ($n = 67$), bone marrow ($n = 52$) or cord blood ($n = 9$) was collected from unrelated ($n = 51$), HLA-matched related ($n = 46$), HLA-mismatched related ($n = 15$), cord-blood ($n = 9$) or identical twin ($n = 7$) donors. The conditioning regimens of 122 transplants consisted of myeloablative regimens ($n = 74$) or reduced intensity regimens ($n = 48$) and the median post-transplant follow-up period was 49 months (ranging from 21 to 87 months).

> “an important challenge for autologous HSCT is to reduce the recurrence of autoimmune disease”

At 100 days post-transplant, 20.8% of the patients had developed grade II–IV (moderate-to-severe) acute GVHD, and 27.8% had developed chronic GVHD at 5 years[1]. The transplant-related, non-relapse mortality was 20% at 5 years. The relapse rate (return of autoimmune disease) was also 20% at 5 years, with overall survival and progression-free survival at 5 years of 70% and 59%, respectively. Multivariable analyses found that male sex, age <18 years, reduced intensity conditioning and more recent year of transplant were all associated with improved outcomes

How do these results compare with autologous HSCT? Another study has previously analysed data from the EBMT registry for the outcomes and toxicity of autologous HSCT for autoimmune disease[3], using data collected from 1,951 patients transplanted between 1994 and 2015. Compared with the report by Greco et al., the median age of these patients was older (median age 37; range 3–76 years). The most common identified rheumatic diseases were systemic sclerosis ($n = 443$), inflammatory arthritis ($n = 162$) and SLE ($n = 107$). Many of the transplants involved non-myeloablative regimens and unmodified (not CD34-selected) mobilized autologous peripheral blood stem cells. Among the 1,951 autologous transplants, the 5-year non-relapse mortality was 5%, disease relapse

Box 1 | Points to consider for HSCT

A number of aspects are important to consider when deciding whether to use autologous or allogeneic haematopoietic stem cell transplantation (HSCT) for the treatment of autoimmune rheumatic diseases:

- If available, patients should be enrolled in clinical trials
- Transplants should be performed at experienced centres with established collaborations of rheumatology and transplant physicians supporting the patient during and after the transplant
- Transplants should be performed early in the course of autoimmune disease before organ failure has developed
- Allografts with HLA-matched bone marrow could be considered in children with refractory autoimmune diseases. However, parents must understand the substantial risks of transplant mortality and graft-versus-host disease
- For adults, evidence on balance favours autologous transplantation over allogeneic transplantation
- Patients should be monitored life-long for toxicity and relapse by a multidisciplinary team and results reported to transplant registries

> For allogeneic transplants to be widely adopted, associated complications must be lowered

was 46%, and progression-free survival was 49%. As with allogeneic HSCT, multivariate analyses of these autologous transplants showed younger age during transplant and more recent year of transplant as factors associated with improved outcomes. Additionally, centre experience (>23 transplants for autoimmune disease), learning time (>6 years from the first autoimmune disease transplant) and accreditation by the Joint Accreditation Committee of the International Society for Cellular Therapy and EBMT (JACIE) were independently associated with improved progression-free survival.

What do these two registry reports do to inform clinical practice? These reports[1,3] support pre-existing assumptions that autologous transplantation was safer than allogeneic transplantation but could be associated with higher rates of relapse of disease[2]. Hence, an important challenge for autologous HSCT is to reduce the recurrence of autoimmune disease. Moreover, the EBMT data[1,3] are consistent with registry reports from North America and South America[4]. On both sides of the Atlantic, mortality was lower in centres with higher levels of transplant experience than other centres. For example, in a 2018 study of fully myeloablative, CD34$^+$ selected autologous HSCT for severe SSc in eight specialized transplant centres in North America, the rates of relapse of SSc and non-relapse mortality were low (9% and 3% at 54 months posttransplant, respectively)[5].

For allogeneic transplants for autoimmune diseases to gain wider acceptance, non-relapse mortality and associated GVHD and infections must be reduced. To this end, three approaches could be considered. First, allogeneic HSCT should be limited to children as the rates of acute and chronic GVHD are substantially lower in younger than in older individuals. For example, allogeneic HSCT has been successfully used in children with refractory juvenile idiopathic arthritis[6]. Second, allogeneic bone marrow rather than peripheral blood stem cells should be used as the source of stem cells given that the rates of chronic GVHD are lower with this approach, especially among young children[7]. Finally, post-transplant cyclophosphamide could be administered to lessen the rates of GVHD[8]. For allogeneic transplants to be widely adopted, associated complications must be lowered, or else the ultimate trade-off for treating one chronic debilitating autoimmune disease could be the development of another chronic disease requiring years of post-transplant immunosuppression[9].

So, is allogeneic HSCT ever advisable over autologous HSCT for the treatment of autoimmune disease? Past experience can help inform this decision (BOX 1). In the end, the choice to use any developing therapy should be on the basis of a shared decision by the physician, patient and family, reflecting their values and situation[10]. As rheumatologists and transplant physicians, the availability of HSCT represents a special opportunity to listen closely to the patient and assess, teach and assist in the shared decision of stem cell transplantation for autoimmune diseases.

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Competing interests
The authors declare no competing interests.

RHEUMATOID ARTHRITIS

Can machine learning predict responses to TNF inhibitors?

Nisha Nair and Anthony G. Wilson

A machine learning model to predict whether patients with rheumatoid arthritis will respond to TNF inhibitors has been produced following an international crowd-sourced competition, but is the mixture of clinical and omics biomarkers used in this model optimal for clinical use?

Refers to Guan, Y. et al. Machine learning to predict anti-TNF drug responses of rheumatoid arthritis patients by integrating clinical and genetic markers. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41056 (2019)

Rheumatoid arthritis (RA) is a common inflammatory autoimmune disease that affects up to 1% of Western populations[1]. Although remarkable advances in treatment have occurred over the past two decades following the introduction of biologic therapies, a substantial proportion of patients with RA do not respond adequately to these medications. Administering effective treatment early in the disease course is imperative for improving patient outcomes. As such, identifying strong predictors of treatment response is an important clinical and research priority. In a new study, Guan et al.[2] propose a machine learning model that integrates both clinical and omics biomarkers to predict the response of patients with RA to TNF inhibitors.

Efforts to identify genetic, genomic and epigenetic biomarkers of treatment response and outcomes have already shown promise[3–5]. However, individually these omics biomarkers are not useful predictors of response owing to small individual effect sizes, and large study populations are required to identify robust predictive therapeutic response models[6]. Advances in machine learning

> For allogeneic transplants to be widely adopted, associated complications must be lowered

was 46%, and progression-free survival was 49%. As with allogeneic HSCT, multivariate analyses of these autologous transplants showed younger age during transplant and more recent year of transplant as factors associated with improved outcomes. Additionally, centre experience (>23 transplants for autoimmune disease), learning time (>6 years from the first autoimmune disease transplant) and accreditation by the Joint Accreditation Committee of the International Society for Cellular Therapy and EBMT (JACIE) were independently associated with improved progression-free survival.

What do these two registry reports do to inform clinical practice? These reports[1,3] support pre-existing assumptions that autologous transplantation was safer than allogeneic transplantation but could be associated with higher rates of relapse of disease[2]. Hence, an important challenge for autologous HSCT is to reduce the recurrence of autoimmune disease. Moreover, the EBMT data[1,3] are consistent with registry reports from North America and South America[4]. On both sides of the Atlantic, mortality was lower in centres with higher levels of transplant experience than other centres. For example, in a 2018 study of fully myeloablative, CD34$^+$ selected autologous HSCT for severe SSc in eight specialized transplant centres in North America, the rates of relapse of SSc and non-relapse mortality were low (9% and 3% at 54 months posttransplant, respectively)[5].

For allogeneic transplants for autoimmune diseases to gain wider acceptance, non-relapse mortality and associated GVHD and infections must be reduced. To this end, three approaches could be considered. First, allogeneic HSCT should be limited to children as the rates of acute and chronic GVHD are substantially lower in younger than in older individuals. For example, allogeneic HSCT has been successfully used in children with refractory juvenile idiopathic arthritis[6]. Second, allogeneic bone marrow rather than peripheral blood stem cells should be used as the source of stem cells given that the rates of chronic GVHD are lower with this approach, especially among young children[7]. Finally, post-transplant cyclophosphamide could be administered to lessen the rates of GVHD[8]. For allogeneic transplants to be widely adopted, associated complications must be lowered, or else the ultimate trade-off for treating one chronic debilitating autoimmune disease could be the development of another chronic disease requiring years of post-transplant immunosuppression[9].

So, is allogeneic HSCT ever advisable over autologous HSCT for the treatment of autoimmune disease? Past experience can help inform this decision (BOX 1). In the end, the choice to use any developing therapy should be on the basis of a shared decision by the physician, patient and family, reflecting their values and situation[10]. As rheumatologists and transplant physicians, the availability of HSCT represents a special opportunity to listen closely to the patient and assess, teach and assist in the shared decision of stem cell transplantation for autoimmune diseases.

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RHEUMATOID ARTHRITIS

Can machine learning predict responses to TNF inhibitors?

Nisha Nair and Anthony G. Wilson

A machine learning model to predict whether patients with rheumatoid arthritis will respond to TNF inhibitors has been produced following an international crowd-sourced competition, but is the mixture of clinical and omics biomarkers used in this model optimal for clinical use?

Refers to Guan, Y. et al. Machine learning to predict anti-TNF drug responses of rheumatoid arthritis patients by integrating clinical and genetic markers. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41056 (2019)

Rheumatoid arthritis (RA) is a common inflammatory autoimmune disease that affects up to 1% of Western populations[1]. Although remarkable advances in treatment have occurred over the past two decades following the introduction of biologic therapies, a substantial proportion of patients with RA do not respond adequately to these medications. Administering effective treatment early in the disease course is imperative for improving patient outcomes. As such, identifying strong predictors of treatment response is an important clinical and research priority. In a new study, Guan et al.[2] propose a machine learning model that integrates both clinical and omics biomarkers to predict the response of patients with RA to TNF inhibitors.

Efforts to identify genetic, genomic and epigenetic biomarkers of treatment response and outcomes have already shown promise[3–5]. However, individually these omics biomarkers are not useful predictors of response owing to small individual effect sizes, and large study populations are required to identify robust predictive therapeutic response models[6]. Advances in machine learning

have improved the possibility of constructing accurate models in large and complex health-care datasets by deviating from traditional statistical analyses; such models have the computational capacity to analyse many different variables to generate a powerful predictive model for treatment response[6]. The Dialogue on Reverse Engineering Assessment and Methods (DREAM): RA Responder Challenge was a crowd-sourced competition with the aim of developing methods to identify predictors of TNF inhibitor response in patients with RA[7]. In the model proposed by Guan et al.[2], which was the winning entry for this competition, the authors constructed machine learning models to predict changes in disease activity scores associated with treatment with TNF inhibitors and used these scores to assign patients to either responder or non-responder groups.

The DREAM: RA Responder Challenge organizers randomly selected 1,892 patients with RA from across 13 cohorts of patients with European ancestry; data from this group of patients was then utilized by Guan et al.[2] as the training dataset. The validation dataset consisted of data from 680 individuals from the CORRONA registry[8]. Guan et al.[2] used these data to develop a Gaussian process regression (GPR) model, which can predict an unknown dependent variable for any independent variables in a mixed heterogeneous dataset of samples that have both independent and dependent variables. Using a bespoke kernel function (a type of pattern analysis algorithm), an individual was weighted in proportion to how similar they are to another individual on the basis of clinical and genetic factors, and then any differences in factors between the individuals were accepted as input variables. This model addressed the difficulty posed by datasets in which there is heterogeneity between patients with the same disease, as patients were matched with those who had similar clinical and genetic factors; this approach proved useful in this study[2] to identify sub-cohorts of non-responders to TNF inhibitor therapy. Data used in this GPR model[2] included demographic data (such as age and sex), clinical data (such as baseline disease activity and treatment type) and genetic information in the form of microarray single-nucleotide polymorphism (SNP) data.

Notably, Guan et al.[2] used the change in the 28-joint disease activity score (DAS28) as the outcome measure for their predictive model. Although the DAS28 is one of the most commonly used outcome measures for RA, it is heavily influenced by subjective outcome measures such as the visual analogue score and tender joint count. The 2019 re-weighted DAS score[9], which includes the less subjective components of this metric such as swollen joint count and C-reactive protein concentrations, might have been a better outcome measure to use in this model[2], as it can work well in a variety of patient populations. Nonetheless, the model proposed by Guan et al.[2] shows promise, as 78% of individuals' response statuses were correctly classified in the training cohort, although the correlation coefficients seem relatively weak (0.406 and 0.393 in the training and validation cohorts, respectively) and, as such, more work is needed to refine the model to improve its potential clinical utility. However, the authors have considered the practical applications of their modelling strategies and the GPR analysis presented in their manuscript can easily be interpreted in a clinical environment. The answer to improving the model itself seems to lie in the choice of variables that are used, as well as the sample size of the training and validation datasets.

Credit: Andriy Popov/Alamy Stock Photo

> "A machine learning algorithm is only as good as the sample size that the model is trained with"

Guan et al.[2] reported that genetic predictors added only a small contribution to the overall model and that clinical biomarkers, in particular the baseline DAS28 score, are more important than genetic biomarkers for predicting response to TNF inhibitors. This claim is perhaps not strictly true since previous studies have shown that multiple genetic factors are associated with treatment response and that these associations are polygenic with small effect sizes[3]. Even if the genetic contribution to risk seems small, it still adds value and genetic risk scores are necessary for analyses to address the contributions of multiple genes. Polygenic risk scores (PRS) calculate the quantitative effect of multiple genetic loci associated with a particular trait. The success of these risk scores in predicting disease susceptibility have been notable in cardiovascular disease (CVD); PRS have been developed to include millions of variants associated with different CVD outcomes, and these scores have been successful in predicting risk of susceptibility when tested in different populations[10]. By contrast, the model proposed by Guan et al.[2] is only applicable to populations of individuals with European ancestry. For this model to be applicable across heterogeneous cohorts of individuals with mixed ancestry, large genetic association studies of diverse populations of patients with RA will first be required to inform such models, and/or PRS could perhaps be added to a modified predictive model for treatment response.

A machine learning algorithm is only as good as the sample size that the model is trained with. Although the training dataset used by Guan et al.[2] was not small, the outcomes of the DREAM: RA Responder Challenge[5] have shown that even larger datasets are required to utilize the full potential of the genetic variables in the model. Despite the study by Guan et al.[2] verifying that clinical factors are perhaps the strongest predictors of treatment response, genetic factors or, indeed, genomic or epigenetic factors, should still be considered. For example, differential methylation at the *LRPAP1* locus has been

associated with non-response to etanercept in patients with RA, and also correlated with the SNP rs3468 in this gene, showing that epigenetic factors are strong candidate biomarkers for treatment response and that correlation with genetic factors provides a strong model for predicting responses[4].

Overall, Guan et al.[2] report a promising application of machine learning in the development of a model to predict response to TNF inhibitor therapy that suggests that response to therapy is heavily influenced by clinical factors. Although we agree that clinical variables have a high predictive power, the potential value of additional omics biomarkers should not be underestimated, as such biomarkers might substantially increase the clinical utility of a machine learning-derived predictive model.

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Shared and distinct mechanisms of fibrosis

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Abstract | Fibrosis is defined as an excessive deposition of connective tissue components and can affect virtually every organ system, including the skin, lungs, liver and kidney. Fibrotic tissue remodelling often leads to organ malfunction and is commonly associated with high morbidity and mortality. The medical need for effective antifibrotic therapies is thus very high. However, the extraordinarily high costs of drug development and the rare incidence of many fibrotic disorders hinder the development of targeted therapies for individual fibrotic diseases. A potential strategy to overcome this challenge is to target common mechanisms and core pathways that are of central pathophysiological relevance across different fibrotic diseases. The factors influencing susceptibility to and initiation of these diseases are often distinct, with disease-specific and organ-specific risk factors, triggers and sites of first injury. Fibrotic remodelling programmes with shared fibrotic signalling responses such as transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), WNT and hedgehog signalling drive disease progression in later stages of fibrotic diseases. The convergence towards shared responses has consequences for drug development as it might enable the development of general antifibrotic compounds that are effective across different disease entities and organs. Technological advances, including new models, single-cell technologies and gene editing, could provide new insights into the pathogenesis of fibrotic diseases and the development of drugs for their treatment.

Fibrosis describes the excessive deposition of connective tissue components in an organ in response to a trigger or injury. The accumulation of extracellular matrix (ECM) proteins often disrupts the physiological architecture and can lead to organ malfunction. Fibrotic tissue remodelling can affect virtually every organ system. Although most individual fibrotic diseases have a low incidence, fibrotic tissue responses are highly prevalent in chronic diseases such as asthma, chronic obstructive pulmonary disease (COPD), atherosclerosis or chronic inflammatory bowel diseases[1]. Fibrotic tissue responses often contribute strongly to disease outcomes and overall morbidity, even if the condition is not commonly associated with fibrosis. Particularly common examples include myocardial remodelling in heart failure, vascular remodelling in atherosclerosis, epithelial–mesenchymal transition and desmoplastic reactions in tumours, and airway remodelling in COPD and asthma[2]. Indeed, up to 45% of all deaths in the developed world have been estimated to be attributed to fibrotic tissue responses[3]. In general, the global incidence of fibrosis as well as the associated health-care burden are increasing and, therefore, fibrosis is increasingly recognized as one of today's major health-care challenges[3,4].

Normal wound healing and fibrotic diseases have many commonalities. In both conditions, an initial injury initiates a cascade of reparative processes in damaged tissues to restore organ integrity. The reparative cascade involves an early inflammatory response to the initiating trigger, which leads to leukocyte infiltration, activation and accumulation in affected tissues[3]. Although the inflammatory responses vary across different fibrotic conditions, they share polarization towards a T helper 2 (T_H2) cell–M2 macrophage-mediated response with abundant release of profibrotic mediators as a common feature[3]. These mediators promote the activation and accumulation of myofibroblasts[3]. Myofibroblasts are a heterogeneous population of cells, which are defined by the expression of contractile proteins and their abundant release of ECM proteins. A variety of different cell types can acquire at least a partial myofibroblast phenotype, including resident fibroblasts and various cells of the vascular wall, such as pericytes, endothelial cells and smooth muscle cells; furthermore, epithelial cells, bone-marrow-derived fibrocytes and bone-marrow-derived progenitor cell populations (including adipogenic progenitors) can also contribute to ECM protein release[5] (FIG. 1). In normal wound healing, myofibroblasts

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Key points

- In fibrotic diseases, disease-specific triggers initiate site-specific injuries, which activate distinct cells that drive fibrosis in a genetically susceptible individual.
- The inflammatory responses vary across different fibrotic conditions but share polarization towards a T helper 2 cell–M2 macrophage-mediated response, with abundant release of profibrotic mediators as a common feature.
- Although myofibroblasts are a heterogeneous population of cells that are derived from various cellular precursors, they are activated by a shared set of core pathways, including transforming growth factor-β, platelet-derived growth factor, WNT and hedgehog signalling.
- Structural changes in fibrotic tissues, such as tissue stiffness and hypoxia, generate an important feed-forward loop that leads to chronicity of tissue-repair responses in fibrotic diseases.
- The chronic profibrotic milieu induces epigenetic imprinting in myofibroblasts, which serves as a self-amplifying loop to consolidate fibroblast activation in the later stages of fibrotic diseases.

Epigenetic modifications
Heritable differences in gene expression that are not encoded by changes of the nucleotide sequence.

undergo apoptosis and the reparative responses are terminated after the damage has been repaired[6]. In fibrotic diseases, however, tissue remodelling and fibroblast activation persist as a chronic, uncontrolled process[5]. Fibrotic tissue remodelling can therefore be considered an exaggerated and prolonged wound healing response[7]. Thus, fibrotic diseases might be driven not only by pathological activation of tissue repair responses, but in particular by impaired termination.

Although the mechanisms that lead to chronicity of tissue repair responses in fibrotic diseases are not well understood, structural changes of fibrotic tissues could have an important role. The progressive deposition of ECM proteins increases the stiffness of the affected tissues[8] and impairs diffusion of nutrients and oxygen[9,10], which further promote cell injury and myofibroblast activation[9]. Moreover, the chronic extracellular profibrotic milieu induces epigenetic modifications in myofibroblasts[10–12], which consolidates their activated phenotype and renders them partially independent of external stimulation, thus resulting in a vicious feed-forward amplification loop. These factors could therefore promote self-sustaining activation of myofibroblasts that then drives progressive tissue remodelling, in particular in the later stages of fibrotic diseases (FIG. 1).

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Nintedanib, a tyrosine kinase inhibitor[13,14], and pirfenidone, a drug that inhibits transforming growth factor-β (TGFβ) signalling by incompletely understood mechanisms[15], have been approved within the past decade by the FDA and European Medicines Agency for the treatment of idiopathic pulmonary fibrosis (IPF) and are therefore the first molecular targeted antifibrotic drugs in clinical use[16]. In September 2019 the FDA approved nintedanib as the first antifibrotic drug for the treatment of systemic sclerosis (SSc)-associated interstitial lung disease (ILD)[17]. Even though there remains an exceptionally high medical need for effective antifibrotic therapies in other fibrotic disorders, the development of targeted therapies for many individual fibrotic diseases is hindered by the extraordinarily high costs of drug development and the rare incidence of many of these fibrotic disorders. A potential strategy to overcome this problem could be to target shared pathways of fibrosis that are of central pathophysiological relevance in different fibrotic diseases and across multiple tissues.

In this Review, we discuss core pathways and mechanisms that are shared across different tissues and might therefore be candidates for general antifibrotic strategies. We also highlight organ-specific and disease-specific differences in fibrotic diseases and discuss the lessons that might be learned from those differences for future drug development efforts. We particularly focus on shared and distinct mechanisms during initiation and progression of fibrotic diseases, but also briefly discuss genetic risk factors for fibrotic disease. We use SSc, the prototypical systemic fibrotic disease, as a paradigm to highlight commonalities and differences in fibrotic diseases with a particular focus on skin and lung, but also validate our conclusions with results from other fibrotic diseases and in other organs. Finally, we provide a short outlook on how modern technological advances and new human tissue-based experimental models could provide new insights into the pathogenesis of fibrotic diseases, and help identify novel therapeutics.

Susceptibility to fibrotic diseases

Genetic associations

Genome-wide association studies (GWAS) have greatly improved our understanding of genetic susceptibility to fibrotic diseases. These studies have yielded numerous susceptibility loci for individual fibrotic diseases (see TABLE 1 for an overview). As this topic is covered by a number of excellent reviews[18–21], we will not discuss individual susceptibility loci here but rather compare the biological processes and pathways affected by these polymorphisms.

Most of the gene products of loci associated with susceptibility to SSc, such as *IRF4* (encoding interferon regulatory factor 4), *IRF5* (interferon regulatory factor 5), *STAT4* (signal transducer and activator of transcription 4), *IL12A* (IL-12 subunit-α), *IL12RB1* (IL-12 receptor β1 subunit), *BANK1* (B cell scaffold protein with ankyrin repeats 1) and *IRAK1* (IL-1 receptor-associated kinase 1), have been implicated in inflammation and autoimmunity, highlighting the autoimmune nature of SSc[18]. These alterations might exacerbate the inflammatory response

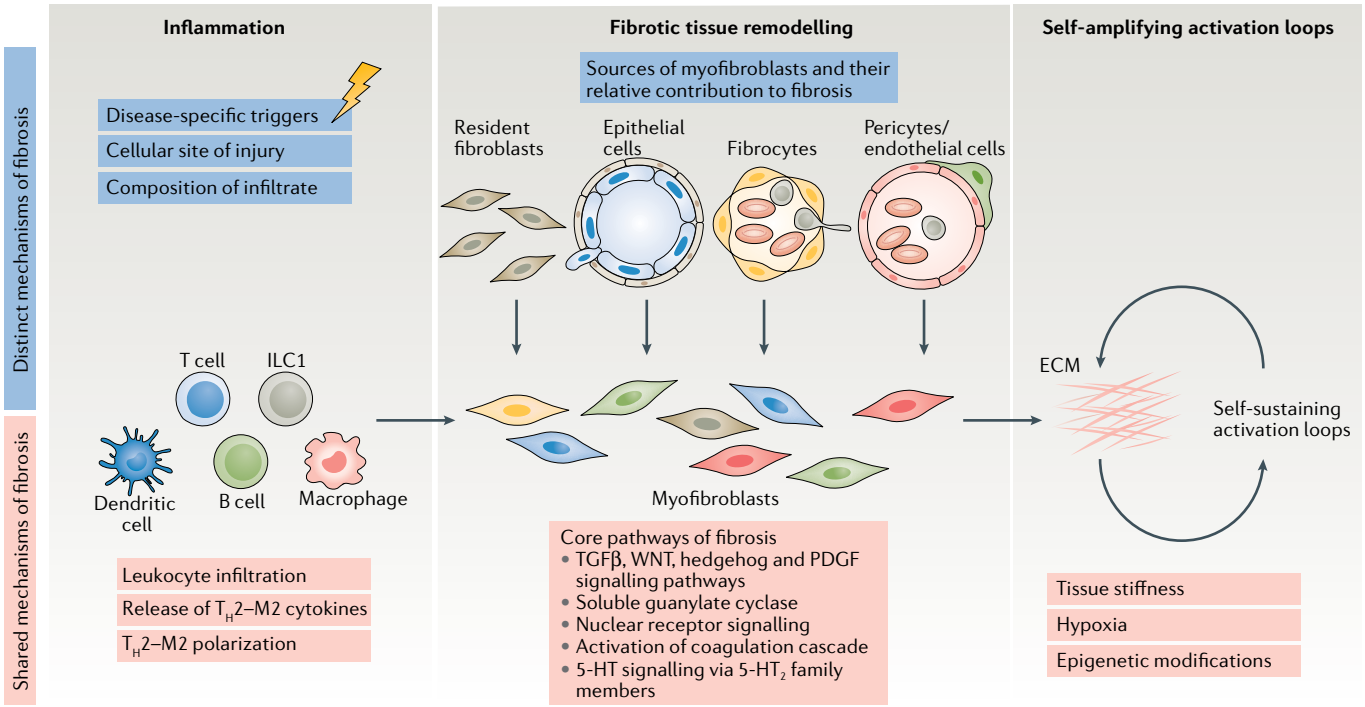

Fig. 1 | **Common and distinct mechanisms in different stages of fibrotic tissue remodelling.** This overview of the shared and distinct fibrotic mechanisms involved in the initiation and progression of fibrotic diseases highlights the existence of distinct fibrotic mechanisms driving the early phases of tissue fibrosis and the predominance of shared fibrotic mechanisms in the later phases of fibrotic tissue remodelling. Disease-specific and site-specific triggers drive leukocyte tissue infiltration and activation and T helper 2 (T_H2) cell–M2 macrophage polarization, with subsequent secretion of myriad profibrotic cytokines. This profibrotic milieu activates resident fibroblasts and induces transdifferentiation of various cell types including epithelial cells, pericytes and endothelial cells and bone-marrow-derived fibrocytes to myofibroblasts. Progressive tissue remodelling induces self-sustaining activation loops such as tissue stiffness or hypoxia, which foster a persistent activated phenotype of myofibroblasts in fibrotic diseases. 5-HT, 5-hydroxytryptamine (serotonin); 5-HT_2, 5-HT receptor 2; ECM, extracellular matrix; ILC1, group 1 innate lymphoid cell; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor-β.

to endothelial cell injury and promote the subsequent development of autoimmunity. In IPF, several susceptibility loci have been identified[22], with a single nucleotide polymorphism (SNP) within the mucin gene *MUC5B* being the most prominent risk factor for the disease identified to date. Importantly, additional risk loci have been found in genes that link IPF to epithelial cell dysfunction, such as *DSP* (encoding desmoplakin), *TLR3* (Toll-like receptor 3) or *AKAP13* (A-kinase anchoring protein 13)[23], as well as to impaired innate immunity, such as *TOLLIP* (Toll-interacting protein)[19]. Changes in these genes could affect epithelial integrity and the ability of the epithelium to cope with repetitive injuries[24,25]. Notably, changes in genes associated with regulation of telomere biology have also been linked to IPF, including *TERT* (encoding telomerase reverse transcriptase), *TERC* (telomerase RNA component), *DKC1* (dyskerin pseudouridine synthase 1), *TINF2* (TERF1-interacting nuclear factor 2), *RTEL1* (regulator of telomere elongation helicase), *PARN* (poly(A)-specific ribonuclease) and *NAF1* (nuclear assembly factor 1 ribonucleoprotein)[26]. In the liver, however, the loci most commonly linked to nonalcoholic fatty liver disease regulate lipid metabolism and promote hepatic lipid accumulation and toxicity[20]. Thus, most of the associations between genetic variants and susceptibility to fibrotic diseases are disease-specific and directly related to the primary site and mechanism of injury.

Telomere function

Telomere length is commonly used as a surrogate marker of telomere function, with short telomeres indicating reduced telomere function. Accumulating evidence highlights that telomere shortening increases susceptibility to fibrosis[26]. Telomere dysfunction in alveolar type 2 epithelial cells may interfere with the ability of the pulmonary epithelium to cope with injury and bias these cells towards initiating fibrotic remodelling[26]. Moreover, critically reduced telomere length triggers a chronic DNA damage response that activates the p53–p21 signalling pathway and induces cellular senescence[27]; as discussed in more detail in a later section, several reports have highlighted that cellular senescence, characterized by the senescence-associated secretory phenotype (SASP), can promote pathological repair responses and tissue fibrosis in several organs[28,29]. Mice with short telomeres do not develop de novo fibrosis per se, but are biased towards fibrotic remodelling in response to chronic injury such as that induced by cigarette smoke, low-dose bleomycin or carbon tetrachloride[30].

Telomere length is influenced by age, genetic variants and mutations in telomere-related genes, inherited

Table 1 | **Gene variants associated with SSc, IPF and NAFLD**

Clinical association of genetic variant	Allele or gene (variant)	Gene function
Anti-topoisomerase I antibodies	*HLA-DRB1*11:04/11:01*	MHC class II
	*HLA-DPB1*13:01*	MHC class II
	*HLA-DPB1*13:01/*01:01/*10:01*	MHC class II
Anti-centromere antibodies	*HLA-DPB1*04:02*	MHC class II
	*HLA-DQB1*05:01*	MHC class II
Anti-telomere antibodies	*HLA-DRB1*15:02*	MHC class II
	*HLA-DQB1*06:01*	MHC class II
	*HLA-DPB1*03:01/*09:01*	MHC class II
Juvenile SSc	*HLA-DRB1*10*	MHC class II
	*HLA-DQA1*05*	MHC class II
SSc	*IRF4* (*rs9328192*)	Interferon signalling
	IL12A (*rs77583790*)	IL-12 signalling
	IL12RB1 (*rs436857*)	IL-12 signalling
	ATG5 (*rs9373839*)	Autophagy
SSc-associated ILD	*HLA-DPB1*03:01*	MHC class II
	IRAK1 (*rs1059702*)	IL-1 signalling
SSc and SSc-associated ILD	*IRF5* (*rs2004640*)	Interferon signalling
	STAT4 (*rs7574865*)	Interferon signalling
dcSSc	*BANK1* (*rs10516487*)	B cell receptor-induced calcium mobilization from intracellular storages
	COL4A3 (*rs55816283*)	ECM protein
	COL5A2 (*rs116298748*)	ECM protein
	COL22A1 (*rs72727814*)	ECM protein
	COL13A1 (*rs41277962*)	ECM protein
	CTGF (*G-945C*)	ECM protein
dcSSc and SSc-associated ILD	*COL4A4* (*rs200450557*)	ECM protein
	TERT (*rs34094720*)	Telomere biology
IPF	*AKAP13* (*rs62025270*)	PKA-mediated signalling
	TERT (*rs2736100*)	Telomere biology
	OBFC1 (*rs11191865*)	Telomere biology
	MUC5B (*rs35705950*)	Mucin composition
	MUC2 (*rs7934606*)	Mucin composition
	ATP11A (*rs1278769*)	Phospholipid translocation, host defence
	DSP (*rs2076295*)	Desmosome composition
	DPP9 (*rs12610496*)	Cellular barrier function
	HLA-DRB1 (*rs2395655*)	MHC class II
	TLR3 (*rs3775291*)	Innate immunity
	TOLLIP (*rs111521887*, *rs5743894*, *rs2743890*)	Innate immunity
	IL1RN (*rs408392*, *rs419598*, *rs2637988*)	IL-1 signalling
	IL8 (*rs4073*, *rs2227307*)	IL-8 signalling
	FAM13A (*rs2609255*)	Signal transduction
	TGFB1 (*rs1800470*)	TGFβ signalling
NAFLD	*PNPLA3* (*rs738409*)	Hydrolysis of triglycerides and retinyl esters
	TM6SF2 (*rs12137855*)	VLDL secretion
	MBOAT7 (*rs641738*)	Phospholipid remodelling pathway (Lands cycle)
	GCKR (*rs1260326*)	De novo lipogenesis

dcSSc, diffuse cutaneous systemic sclerosis; ECM, extracellular matrix; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NAFLD, nonalcoholic fatty liver disease; PKA, protein kinase A; SSc, systemic sclerosis; TGFβ, transforming growth factor-β. Adapted with permission from REF.[18], Springer Nature Limited; REF.[19], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/); and REF.[20], republished with permission of AME Publishing Company, from New insights into genetic predisposition and novel therapeutic targets for nonalcoholic fatty liver disease. Barbara, M., Scott, A. & Alkhouri, N. **7**, 2018, permission conveyed through Copyright Clearance Center, Inc.

telomere length and environmental factors such as cigarette smoke. Telomere-related gene mutations and SNPs are associated with organ fibrosis and especially with pulmonary fibrosis[26]. Mutations in genes linked to telomere maintenance account for the disease in up to 25% of patients with familial IPF, 10% of those with sporadic IPF and 10% of those with connective tissue disease-associated ILD[26]. In addition to pulmonary fibrosis, increased prevalence of missense mutations in *TERT* and *TERC* genes has also been observed in patients with hepatic cirrhosis[31–33]. Age-related telomere dysfunction in cardiomyocytes has been linked to myocardial fibrosis[34]. Short telomeres have also been found in patients with SSc and ILD; however, in contrast to IPF, telomere shortening was observed only in lymphocytes, suggesting that telomere shortening might be a consequence rather than a driver of pulmonary fibrosis in SSc-associated ILD[35]. Multiple studies have demonstrated an association between short telomeres and chronic kidney disease, but to date there is no clear evidence for a direct pathogenic role of telomere shortening in kidney cells[36,37]. No association between telomere shortening and dermal fibrosis has yet been reported.

Disease initiation

In SSc, the primary site of injury is thought to be the microvascular endothelium[38]. Aberrant activation and subsequent apoptosis of endothelial cells in a genetically susceptible individual is thought to be the first manifestation of SSc[38]. Although endothelial cell injury has not yet been shown to be sufficient to induce an SSc-like disease, the consensus is that the initial microvascular injury promotes perivascular injury and autoimmunity, which triggers fibroblast activation and tissue fibrosis[39]. The initial vascular injury might be caused by a virus, for example cytomegalovirus, but results are conflicting and do not allow definite conclusions to be drawn on the contribution of viruses or other agents to the initial vascular injury in SSc[40]. Autoantibodies against endothelial cells might maintain chronic vascular injury even after clearance of the initial trigger[41].

In IPF, several lines of evidence suggest that the disease is initiated by microinjuries to the epithelium[42]. Repetitive exposure to cigarette smoke, inhaled toxins, gastroesophageal reflux or infections damages alveolar and airway epithelial cells, including their progenitor pool. These cells might further carry intrinsic genetic alterations (as described above) that render them susceptible to extensive phenotypic changes during pulmonary fibrogenesis, including injury-induced cellular senescence[27]. Thus, the regenerative ability of these damaged epithelial cells is impaired in susceptible individuals, resulting in maladaptive repair responses with ongoing epithelial cell injury and phenotypic reprogramming, ECM alterations and myofibroblast differentiation[43]. Although the epithelium is not the first site of injury in SSc, epithelial cell damage might precipitate SSc-associated ILD[44], which often occurs in later stages of the disease. Cells of epithelial origin are also considered the site of primary injury in most forms of fibrotic hepatic and renal diseases[45,46], although suspected triggers and mechanisms of injury are disease-specific.

Inflammatory responses

The early stages of fibrotic disease are characterized by complex inflammatory events involving both the innate and adaptive immune systems. The inflammatory responses differ between tissues and organs, but some key features are shared (FIG. 1). A better understanding of these inflammatory events could have implications for the development of therapies for fibrotic diseases.

Innate immunity

Macrophages and monocytes show extraordinary plasticity in response to a wide variety of stimuli. These stimuli include pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which are sensed through TLRs, RIG-I-like receptors and NOD-like receptors[47]; apoptotic cell debris, sensed through scavenger and phosphatidylserine receptors[48,49]; and a variety of cytokines, sensed through cytokine receptors[50]. The T_H2 cell-derived cytokines IL-4 and IL-13 lead to M2-polarized macrophages (also referred to as 'alternatively activated' macrophages); by contrast, stimulation by IFNγ, TNF or TLRs typically leads to an inflammatory, M1 phenotype[50]. It is worth noting that, given the complexity of the signals that macrophages integrate, the M1–M2 paradigm oversimplifies the range of activities of these cells, and that other, more complex paradigms have been proposed[51]. Macrophages are important in the physiological response to wound injury. The early inflammatory phase of the wound response depends on circulating monocyte-derived M1 macrophages[52]. In the later phases, these macrophages are replaced by a specific, reparative population of M2 macrophages that express CD206 (also known as MRC1) and CD301b (also known as MGL2)[53]. Similar waves of macrophages with distinct phenotypes are seen in inflammatory and fibrotic diseases[54]. Following carbon tetrachloride-induced liver injury in mice, for example, early depletion of macrophages reduces tissue injury, whereas later depletion leads to increased injury, indicating distinct roles for macrophages at different stages after injury[55]. Macrophages also show similar patterns of phenotypic differences in early inflammation and later repair in pulmonary, dermal and renal fibrosis[25,56].

IL-13 and M2 macrophages have key roles in dermal, pulmonary and hepatic fibrosis. In the lung, IL-13 induces fibrosis through activation of TGFβ1 (REFS[57,58]), whereas in the liver IL-13 acts through a TGFβ-independent pathway[59]. Macrophages mediate IL-13-induced inflammation and fibrosis in the lung[60] and liver[61]. Unexpectedly, however, selective deletion of the IL-4–IL-13 receptor (IL-4 receptor α-chain) on macrophages actually delays fibrosis resolution in the liver[61], eventually suggesting context-dependent differences in the contribution of macrophages to fibrosis progression and reversal.

Another major insight into the roles of macrophages in fibrosis comes from the understanding of their origins[62]. Macrophages derived from yolk sac and fetal progenitors (which have capacity for local self-renewal) and monocyte-derived migrating macrophages seem to

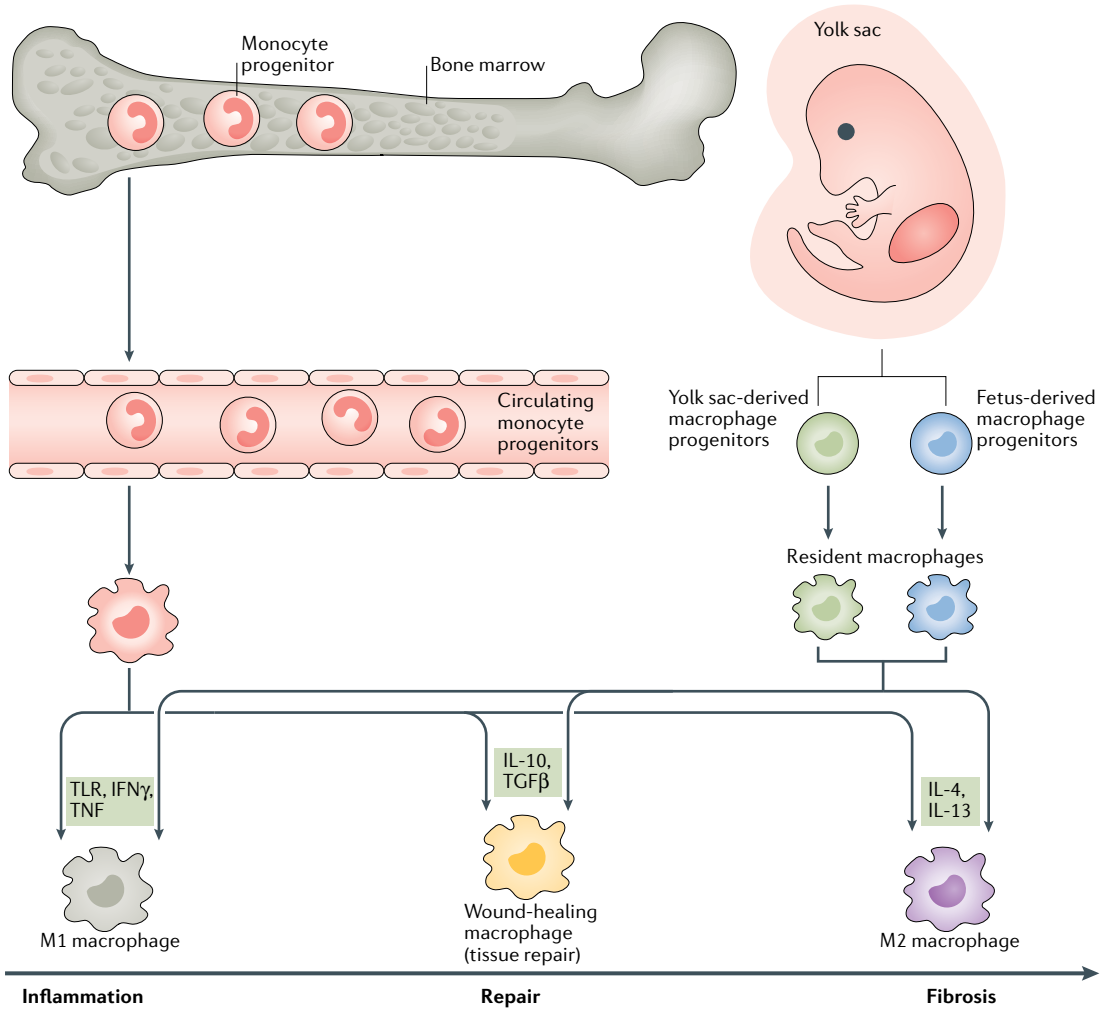

Fig. 2 | **Macrophage activation and differentiation in tissue repair and fibrosis.** Tissue-resident macrophages and circulating bone marrow-derived macrophage progenitors can differentiate into M1, M2 and wound-healing macrophages with specific cytokine production profiles and distinct functions in different stages of fibrotic diseases. TGFβ, transforming growth factor-β; TLR, Toll-like receptors.

have different roles in inflammation, repair and fibrosis[50] (FIG. 2). In several models of fibrosis, early deletion of macrophages reduces inflammation, whereas deletion of macrophages at later time points limits tissue repair and fibrosis[63]. Other observations also suggest that IL-4 and IL-13 activate monocyte-derived and resident macrophages differently[64].

Together, these and other studies indicate that macrophages are key regulators of inflammation and fibrosis. Single-cell RNA studies have identified a novel population of profibrotic alveolar macrophages present in the fibrotic lung of patients with IPF[65,66], thus supporting the notion that specific effects of macrophages depend on the underlying macrophage/monocyte phenotype, the origin of the macrophages, the tissue being affected, and the inflammatory profibrotic stimulus.

Apart from macrophages, innate lymphoid cells (ILCs), specifically group 2 ILCs (ILC2s), are also emerging as innate immune effector cells with central roles in the pathogenesis of fibrosis. Not only are ILC2s another major source of IL-13, but they also produce other profibrotic mediators such as IL-5, IL-6 and IL-9 (REF.[67]). ILCs are activated by epithelial cell-derived cytokines such as IL-33, IL-25 and thymic stromal lymphopoietin (TSLP), concentrations of which have been reported to be increased in various fibrotic diseases[68,69]. ILCs activated by IL-33 or IL-25 drive fibrosis in models of hepatic and pulmonary fibrosis in an IL-13-dependent manner, whereas the roles of other ILC-derived mediators such as IL-5 and IL-9 are currently less well studied[70].

Adaptive immunity

The crosstalk between the innate and adaptive immune systems and parenchymal cells in fibrotic diseases has been well appreciated over the years. Activated epithelial and endothelial cells secrete many inflammatory mediators, such as TGFβ1 (REF.[71]), IL-1β[71] and CXC and CC chemokines[72], which chemoattract immune cells within the fibrotic tissue. The infiltrating cells consist of T cells[73], monocytes[74], B cells[75], mast cells[76], ILCs[77], plasmacytoid dendritic cells (pDCs)[78] and γδ T cells[78]. These infiltrating immune cells, secrete TGFβ[79], IL-1β[79], IL-6 (REF.[79]), IL-13 (REF.[79]) and other mediators[79], further amplifying the inflammatory and profibrotic responses.

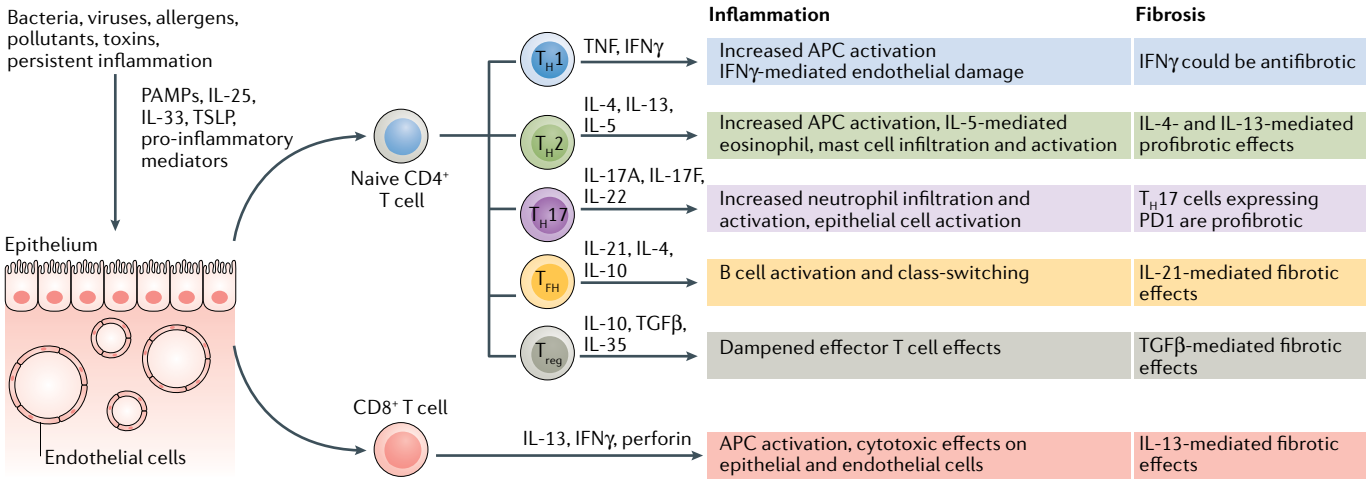

Fig. 3 | **T cell subpopulations in fibrotic tissue remodelling.** Injury to epithelial cells or endothelial cells promotes the release of pro-inflammatory mediators that trigger the activation and differentiation of T cells. Naive CD4$^+$ T cells can differentiate in mature T helper 1 (T_H1), T_H2, T_H17, regulatory T (T_{reg}) and T follicular helper (T_{FH}) cells, each of which has specific profiles of cytokine production and distinct effects on inflammation and fibrosis. CD8$^+$ T cells are also recruited at inflammatory sites where they exert cytotoxic effects on epithelial and endothelial cells as well as IL-13-mediated profibrotic effects. APC, antigen-presenting cell; PAMP, pathogen-associated molecular pattern; PD1, programmed cell death 1; TGFβ, transforming growth factor-β; TSLP, thymic stromal lymphopoietin.

T cells in fibrosis. The different subtypes of T cells have varying modulatory effects on inflammation and fibrosis (FIG. 3). Moreover, the contribution of the different T cell populations to the fibrotic process varies between different organs and stage of fibrosis, as summarized in TABLE 2. The phenotype of infiltrating T cells in fibrotic tissues is heterogeneous and can vary depending on the stage and activity of individual fibrotic diseases. However, a common feature of T cell responses in fibrotic diseases is polarization towards T_H2 cell-mediated responses. In particular, T_H2 cell-derived IL-13 has been shown in multiple studies to have a crucial role in the pathogenesis of numerous fibrotic diseases including dermal, pulmonary and renal fibrosis as well as SSc[58,80]. IL-13 can directly activate fibroblasts and increase TGFβ secretion[58]. Furthermore, IL-13 in combination with IL-4 drives human fibroblast-to-myofibroblast transition in a JUN N-terminal kinase-dependent manner[81]. T_H2 cells also promote fibrosis in the unilateral ureteral obstruction (UUO) mouse model of renal fibrosis[82], and IL-13 is thought to have a central role in T_H2 cell-mediated tissue fibrosis.

T_H2 cells are not the only T cell population that can secrete IL-13; CD8$^+$ T cells can also release IL-13, as shown by increased secretion of IL-13 by peripheral blood effector/memory CD226^{high}CD8$^+$ T cells from patients with SSc in comparison with those from healthy individuals[83]. CD226^{high}CD8$^+$ T cells, which are expanded in the blood of SSc patients with diffuse cutaneous involvement (dcSSc) and in those with SSc-associated ILD, might promote fibrosis by secretion of IL-13 and cytotoxicity-mediated endothelial cell damage[83]. CD8$^+$CD28$^-$ T cells from the skin and peripheral blood of patients with SSc have also been reported to secrete increased levels of IL-13 in comparison with those from healthy individuals[84]. Similarly, a 2019 study showed that CD3$^+$ T cells in lung tissue from patients with IPF have downregulated CD28 expression and increased pro-inflammatory cytokine expression[85], although the level of CD226 expression in these cells is not known.

The role of T_H1 cells is considered controversial, and IFNγ released from T_H1 cells has both pro-inflammatory and antifibrotic roles in IPF[86], and in SSc it might also promote vascular damage[83]. In the UUO model, IFNγ$^+$CD8$^+$ T cells can limit T_H2 cell differentiation and ameliorate experimental renal fibrosis[87]. By contrast, mouse studies have demonstrated that T cell specific deletion of angiotensin (AT1) receptor leads to upregulation of pro-inflammatory T_H1 cell-derived cytokines including IFNγ and IL-1β, which cause increased infiltration of pro-inflammatory and profibrotic macrophages in the kidney, resulting in renal fibrosis[88].

Traditionally, the role of T follicular helper (T_{FH}) cells is to drive B cell antibody production, although a population of T_{FH}-like cells expressing inducible T cell costimulator (ICOS) and programmed cell death 1 (PD1) is reportedly increased in the skin of patients with SSc and their numbers seem to correlate with dermal fibrosis[89]. Treatment with an anti-ICOS monoclonal antibody blocked the expansion of T_{FH}-like cells and ameliorated dermal fibrosis in mice[89]. T_{FH} cells might drive fibroblast activation via production of IL-21, as neutralization of IL-21 reduces dermal inflammation and fibrosis in mouse graft-versus-host disease[89]. IL-21 acts as a key driver of T_H17 and T_H2 cell responses and can thereby promote fibrosis and lung injury through IL-13 (REF.[90]).

Evidence published in 2018 points to an important role of PD1$^+$CD4$^+$ T cells in fibrotic diseases[91]. The number of PD1$^+$CD4$^+$ T cells is increased in patients with IPF and in mice with bleomycin-induced pulmonary fibrosis[91]. PD1$^+$CD4$^+$ T cells from patients with IPF have increased expression of TGFβ and IL-17A in comparison with cells from healthy individuals[91]. Indeed, PD1$^+$ T_H17

Table 2 | **T cell subsets in patients with SSc and IPF**

T cell subset	SSc skin	SSc lung	IPF
CD4⁺ T cells			
T_H2 cells	In early SSc, IL-4-expressing T_H2 cells block collagen production through secretion of TNF[383]	Increased numbers of T_H2 cells in the peripheral blood of patients with SSc-associated ILD, based on the ratio of $CCR5^+$ to $CRTH2^+$ T cell frequencies (indicative of type 1 and type 2 immune polarization, respectively)[384]	Increased numbers, profibrotic[86]
T_H17 cells	Increased IL-17A secretion inhibits collagen deposition[385,386]	IL-22-producing cells more than IL-17A producing cells correlated with SSc-associated ILD[387]	Increased numbers in IPF compared with healthy lung tissue[388]
T_{reg} cells	Higher numbers of $FOXP3^+$ cells in early disease in the skin, but not in the peripheral blood, compared with numbers in healthy individuals[389]; decreased numbers of $FOXP3^+$ T_{reg} cells and $TGFβ^+IL\text{-}10^+$ cells in the skin of patients with early SSc[390]; T_{reg} cells from skin produce IL-4 and IL-13 (REF.[391])	Increased functionally impaired T_{reg} cells[392]	Decreased $CD4^+CD25^+FOXP3^+$ T_{reg} cells in blood and bronchoalveolar lavage[393]
T_H1 cells	No change in the peripheral blood[387]	Decreased in the peripheral blood[384]	Antifibrotic[86]
T_{FH} cells	$ICOS^+PD1^+$ T_{FH}-like cells increased[89]	*Icos*$^{-/-}$ mice protected from lung fibrosis[394]; role in human lung fibrosis to be determined	Unknown
CD8⁺ T cells			
$CD8^+$ T cells	Increased numbers[83,395]	Increased peripheral $CD226^{high}CD8^+$ memory T cells are associated with lung involvement[83]	Increased $CD8^+$ T cells in lung tissue correlated with poor prognosis[396]

ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; SSc, systemic sclerosis; T_{FH} cell, T follicular helper cell; T_H cell, T helper cell; T_{reg} cell, regulatory T cell.

cells are the predominant $CD4^+$ T cell subset expressing TGFβ in IPF[91]. Co-culture of $PD1^+CD4^+$ T cells with fibroblasts stimulates the release of type I collagen, whereas inactivation of the PD1 pathway reduces TGFβ and IL-17A secretion from $CD4^+$ T cells and inhibits collagen release from the fibroblasts in a STAT3-dependent manner and also ameliorates bleomycin-induced pulmonary fibrosis in mice[91]. Increased numbers of T_H17 cells are also found in COPA syndrome, an autosomal dominantly inherited autoimmune disorder with prominent ILD[92]. IL-17A and IL-17A-mediated induction of CC-chemokine ligand 5 (CCL5; also known as RANTES) have been linked to the pathogenesis of renal fibrosis[93]. Moreover, IL-17 and IL-17 receptor A are important for bile duct ligation-induced and carbon tetrachloride-induced hepatic fibrosis in mice[94], and inhibition of IL-17 secretion by RORγt inhibitor protects against carbon tetrachloride-induced hepatic fibrosis[95].

B cells in fibrosis. Many fibrotic diseases are associated with infiltration of B cells[96,97], increased levels of B cell activating factor (BAFF; also known as TNFSF13B)[98,99] and autoantibodies of various specificities[100,101]. Activated memory B cells that express CD80, CD86 and CD95 are increased in the blood of patients with SSc, and B cells from these patients secrete TGFβ1 and IL-6 and activate fibroblasts[102].

Autoantibodies were long thought not to be direct drivers of fibrotic disease[103], including SSc, but were seen as markers for disease classification[104]. However, pathogenic and functional autoantibodies against tyrosine kinase receptors, such as platelet-derived growth factor (PDGF) receptors, and G protein-coupled receptors, such as endothelin and angiotensin receptors, have now been identified in SSc[105–108]. These autoantibodies are capable of inducing signal transmission and might thus contribute to the aberrant activation of these receptors. Linking autoantibodies and secretion of type I interferons, Kim et al. showed that interfering with Fcγ receptor IIa (FcγRIIa) or treatment with RNAse suppresses IFNα production by peripheral blood mononuclear cells (PBMCs) upon stimulation with SSc serum containing anti-topoisomerase I antibodies, suggesting that topoisomerase I antibodies are taken up by pDCs via FcγRIIa and subsequently activate TLR7, inducing the production of IFNα[109]. Although the presence of autoantibodies (antinuclear antibodies and anti-smooth muscle antibodies) did not correlate with the severity of fibrosis on liver histology in a large cohort of patients with nonalcoholic steatohepatitis (NASH)[110], elevated levels of BAFF in early-stage NASH could indicate a role for B cells in disease initiation in particular. Indeed, blocking BAFF prevented fibrosis progression in two mouse models of NASH, namely the methionine–choline-deficient (MCD) diet and the choline-deficient amino acid (CDAA) diet[111]. B cells have also been found to have an important role in renal fibrosis as they secrete chemokines, such as CCL2, that result in monocyte infiltration into the kidneys, and depletion of B cells by administration of anti-CD20 antibodies reduced monocytes influx and ameliorated fibrosis in the UUO model of renal fibrosis[112]. Autoantibodies against other targets, such as matrix metalloproteinases (MMPs), have also been described, but their functional role in fibrosis is less well understood[108]. Autoantibodies might also activate fibroblasts to secrete pro-inflammatory cytokines, such as IL-6, IL-8, TGFβ1 and procollagen Iα1, in an Fc receptor-independent manner[113].

Although IPF has not generally been considered an autoimmune disease, some findings suggest that autoantibodies occur in IPF and might also correlate with prognosis[114]. The presence of autoantibodies against

heat shock protein 70 has been associated with increased deterioration of lung function and mortality in patients with IPF[114]. Moreover, circulating levels of autoantibodies against vimentin are increased in patients with IPF and are inversely correlated with lung function[115]. In addition, circulating B cells from patients with IPF are more extensively antigen-differentiated and have greater proportions of plasmablasts than B cells from healthy individuals, and plasma BAFF levels are strongly associated with IPF manifestations and patient outcomes[103]. Interestingly, a 2017 study[116] using proteome-wide profiling of human tissue fibrosis, including skin and lung fibrosis, identified the marginal zone B cell and B1 cell-specific protein (MZB1) as a common marker of pulmonary and dermal fibrosis. MZB1 expression marked a $CD20^-$ plasma B cell subset in fibrotic lung and skin tissue, underlining the need for future investigations to elucidate the causative role of antibody-mediated autoimmunity in organ fibrosis[116]. Results from prospective, multicentre, randomized controlled studies of B cell depletion in SSc or IPF are currently not available. However, retrospective studies and small case series have shown mixed results[117–120]. A controlled study with the CD20 antibody rituximab in IPF is currently ongoing[121].

Implications for treatment. Although inflammation has been thought to be more important for disease progression in SSc than in IPF, the data presented here highlight the complexity and also the heterogeneity of the general inflammatory response in fibrotic diseases. A deeper understanding of the cell populations and inflammatory mediators that are involved in the different fibrotic responses is required to shed light on organ-specific responses, which will inform the development of drugs targeting inflammation. The concept of differences in the inflammatory responses between different fibrotic diseases is supported by the results of clinical trials. In IPF, for example, drugs with broad anti-inflammatory modes of action, such as corticosteroids[122] and anti-TNF antibodies[123], fail to demonstrate clinical benefit, and the combination of azathioprine, prednisone and N-acetylcysteine worsened outcomes in the PANTHER-IPF trial[124]. By contrast, several anti-inflammatory, immunomodulatory drugs demonstrated efficacy in large randomized controlled trials in SSc, including mycophenolate mofetil[125] and cyclophosphamide[126] as well as high-dose chemotherapy with subsequent stem cell transplantation[127]. Other treatments, such as methotrexate[128] and the anti-IL-6 receptor antibody tocilizumab[129], showed favourable trends for the primary end points and significant improvement in key secondary end points of dermal and pulmonary fibrosis in SSc.

Inflammation is also considered to have a prominent role in many forms of hepatic[130] and renal fibrosis[131], although this conclusion is mainly based on preclinical models with less evidence from clinical trials.

Fibrotic tissue remodelling

Following initial inflammatory events, the progression of fibrosis is facilitated by progressive deposition of ECM by myofibroblasts, leading to fibrotic tissue remodelling. Myofibroblast differentiation and activation is driven by a core set of profibrotic pathways that are shared across different diseases and organs. In the following section, after discussing the cellular sources for the pool of myofibroblasts in different organs, we discuss the molecular mechanism of myofibroblast differentiation and persistence in fibrotic diseases. Common and distinct mechanisms implicated in myofibroblast differentiation in fibrotic diseases are illustrated in FIG. 1.

Cellular sources of myofibroblasts

As highlighted in the Introduction to this article, various cell types are capable of acquiring myofibroblast features, including secretion of ECM proteins under profibrotic conditions; however, the functional contribution of the different cellular sources to tissue contraction and collagen deposition, in particular in human fibrotic diseases, is less well understood. The cellular sources of myofibroblasts and their relative contributions to the pool of myofibroblasts vary across different tissues. Although systemic comparisons using comparable lineage tracing methods across different organs are limited, the available data[132] suggest that tissue-specific differences in the sources of myofibroblasts might be partially driven by differences in the primary cellular site of injury. In many epithelium-derived organs, such as lung and kidney, numerous studies have demonstrated that epithelial cells are a source of myofibroblasts, although the relative contributions of these cells to the myofibroblast pool vary widely depending on the lineage tracing approach and the models used[133–135]. In the skin, however, an intact basal membrane is a tight barrier that hinders transdifferentiation of keratinocytes (the epithelial cells of the skin) into myofibroblasts, thus preventing epithelial–mesenchymal transition as a relevant source of myofibroblasts. Although the multiple epidermal stem cell populations located in the hair follicle are known to be of central importance for tissue repair in normal wound healing[136], the role of such epidermal stem cells in SSc and other fibrotic skin diseases remains under investigation. Epidermal keratinocytes could promote myofibroblast differentiation from other cell types by secretion of soluble mediators that maintain a pro-inflammatory and profibrotic milieu. Keratinocyte-derived growth factors and cytokines include TGFβ isoforms, activins, connective tissue growth factor, vascular endothelial growth factor (VEGF) isoforms, insulin-like growth factor 1 (IGF1) and IGF-binding proteins, epidermal growth factor, and oncostatin M, IL-33 and nuclear factor-κB (NF-κB)-regulated cytokines[137].

Evidence suggests that adiponectin-positive adipogenic precursor cells can differentiate into myofibroblasts in experimental dermal fibrosis[138]. The loss of adipocyte markers and acquisition of myofibroblast features can be driven by TGFβ-dependent downregulation of the master adipogenic transcription factor peroxisome proliferator-activated receptor-γ (PPARγ)[138]. This transdifferentiation might promote the progressive subcutaneous tissue atrophy that is seen in the majority of patients with SSc who have advanced disease. Other key profibrotic pathways, such as the WNT and PDGF signalling pathways, have also been shown in mice to be able to induce a corresponding phenotype shift from

adipocyte to fibroblast phenotypes with atrophy of the subcutaneous tissue and dermal fibrosis[139,140]. Similar signalling pathways regulate the phenotypic and functional behaviour of a subset of fibroblasts in the lung. Lipofibroblasts, a fibroblast population rich in neutral lipids, have emerged as a subpopulation of resident fibroblasts with important roles during embryonic differentiation and in the response to injury[141,142]. Lipofibroblasts are required for epithelial type II homeostasis and production of surfactant proteins[143]. They differentiate from mesodermal precursor cells in a manner dependent on fibroblast growth factor 10 (FGF10) and PPARγ[141,144,145]. TGFβ inhibits PPARγ signalling and promotes downregulation of adipogenic markers and upregulation of myofibroblast markers[146]. Consistent with this mechanism, the number of lipofibroblasts decreases during fibrotic tissue responses, and rebounds during resolution of fibrosis[142]. Thus, similar to the findings with adipocyte precursors in the skin, downregulation of PPARγ drives transdifferentiation of adipocyte precursors from a lipid-storing to a myofibroblastic phenotype in other organs, such as the lung.

The contribution of resident fibroblasts might also vary between different tissues. Resident fibroblasts are more abundant in the skin as an organ rich in collagen and fibre, as compared with lung or kidney, and could thus represent a relatively larger recruitment pool in the skin than in many other organs. As discussed in the previous section, inflammatory responses differ depending on the initiating trigger and the primary cell type affected. It is tempting to hypothesize that those differences in injury site and response to injury mobilize individual cellular sources of myofibroblasts to different extents. However, experimental confirmation of this hypothesis is currently lacking.

Core signalling pathways

To understand shared and distinct molecular mechanisms of fibrosis in different tissues and different fibrotic diseases, we adopt the elegant concept of 'core' and 'regulatory' pathways of fibrosis introduced by Mehal et al. a few years ago[147]. They defined core pathways as pathways that are "essential to convert an initial stimulus to the development of fibrosis", whereas they defined regulatory pathways as those that "influence the core pathway but do not directly convert the initial stimulus into the basic component of fibrosis". Although regulatory pathways can have substantial effects on fibrosis, they demonstrate greater variability between different organs and disease entities. Conversely, core pathways will be conserved across different organs, diseases and also individuals. When the article was published in 2011, the authors highlighted that only a few pathways would fulfil the strict criteria for a core pathway, as cross-validation across different tissues and diseases was lacking for many targets[147]. However, several pathways have since been cross-validated to be functionally essential in different organs and diseases, thereby fulfilling the criteria for core pathways of fibrosis. A selection of shared pathways with potential translational relevance is discussed in the following paragraphs.

TGFβ signalling. TGFβ is a master regulator of physiological and pathological tissue repair responses[148,149]. Various cell types can release TGFβ, including platelets, monocytes/macrophages, T cells, epithelial cells and fibroblasts[150]. In particular, deregulation of TGFβ1 and TGFβ2 activity has been linked to the pathogenesis of fibrotic diseases such as SSc. Activation of TGFβ signalling, for example by fibroblast-specific overexpression of constitutively active TGFβ receptor type 1 (TGFβRI), is sufficient to induce a systemic fibrotic disease with progressive fibrosis in multiple tissues[151]. Moreover, numerous preclinical studies have demonstrated that inhibition of TGFβ signalling exerts potent antifibrotic effects in various animal models across different organs[152]. In mice with bleomycin-induced pulmonary fibrosis, epithelial-specific deletion of TGFβRII resulted in an attenuated fibrotic response in the lung[153]. In patients with SSc, the expression of a set of TGFβ-regulated genes correlated with modified Rodnan skin score (mRSS) and with myofibroblast counts[154]. Nevertheless, the first attempt to inhibit TGFβ signalling in patients with SSc using metelimumab (CAT-192), an antibody specifically targeting TGFβ1, failed to show efficacy in a placebo-controlled trial[155]. The results of this trial, however, do not provide strong evidence against inhibition of TGFβ signalling in SSc, because CAT-192 has only weak affinity for TGFβ1 in vivo. In a more recent open-label trial of fresolimumab, a high-affinity neutralizing antibody that targets all three TGFβ isoforms (TGFβ1, TGFβ2 and TGFβ3), in patients with SSc[154], mRSS score (as a surrogate marker of skin fibrosis) decreased upon treatment with fresolimumab. This decrease was accompanied by reduced mRNA levels of TGFβ-regulated genes and decreased myofibroblast counts in fibrotic skin[154].

Upon activation from its latent form (discussed in more detail below), TGFβ binds to TGFβRII, which subsequently dimerizes with and phosphorylates TGFβRI. This ligand binding can activate a plethora of different downstream pathways (FIG. 4). The best-studied downstream pathway is SMAD signalling, also known as canonical TGFβ–SMAD signalling[156]. Apart from SMAD signalling, TGFβ can activate multiple alternative pathways relevant to the pathogenesis of fibrosis, such as mitogen-activated protein kinase pathways mediated by extracellular-signal-regulated kinase (ERK), p38 and JUN N-terminal kinase (JNK) as well as RHO-associated kinase (ROCK) and RAC-α serine/threonine protein kinase (AKT) pathways[157]. The activation of multiple intracellular signalling cascades enables cross-regulation of a number of growth factor pathways and modulation of tissue repair at multiple levels. Selected intracellular pathways activated by TGFβ, and interactions between TGFβ and other growth factors that have been shown to regulate tissue remodelling across different organs, are discussed in the following paragraphs.

Aberrant activation of latent TGFβ. An important distinctive feature of TGFβ is its secretion into the ECM in a biologically inactive form (FIG. 4). TGFβ is released in complexes of TGFβ with latency-associated peptide (LAP). The third component of these complexes, latent TGFβ-binding protein, promotes storage in the ECM,

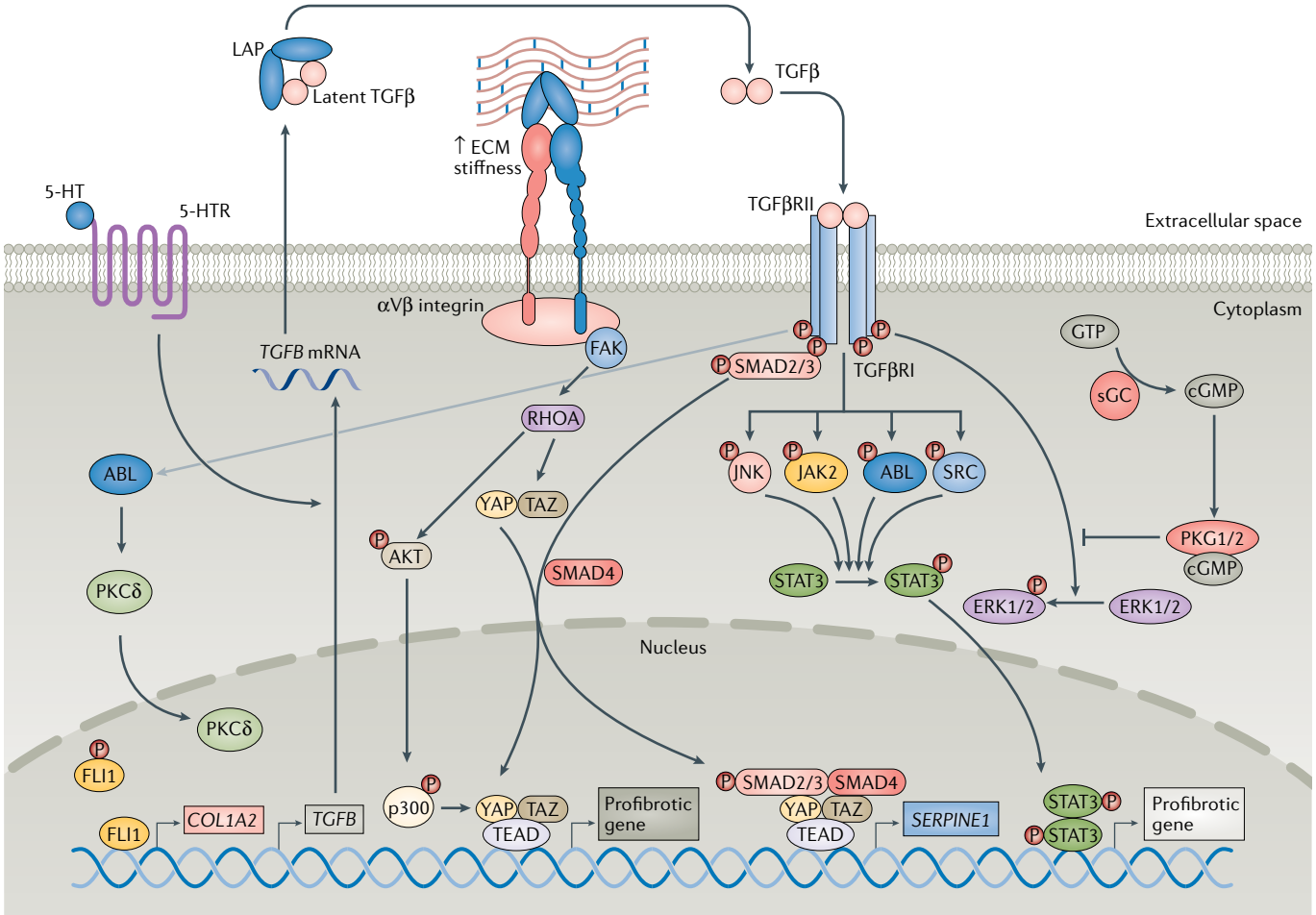

Fig. 4 | **Schematic representation of interactions of TGFβ signalling with other profibrotic signalling pathways.** Fibrotic tissue remodelling leads to increased extracellular matrix (ECM) stiffness that is sensed by αVβ integrins, activating an intracellular signal transduction pathway involving the RAS homologue gene family, member A (RHOA) and the transcription factors yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) that leads to transcription of profibrotic genes, including the transforming growth factor-β (TGFβ) target gene *SERPINE1*. Increased ECM stiffness also enhances activation of latent TGFβ, leading to increased TGFβ signalling. Serotonin (5-hydroxytryptamine (5-HT)) signalling mediated by 5-HT receptors (5-HTRs) is enhanced in fibrotic diseases and leads to increased transcription of TGFβ, thereby contributing to exaggerated TGFβ signalling in fibroblasts. TGFβ signalling leads to phosphorylation of the *COL1A2*-repressor Friend leukaemia integration 1 (FLI1) and its dislocation from the promoter of *COL1A2*, permitting gene transcription and enhancing fibrosis. TGFβ signalling also activates JUN N-terminal kinase (JNK), Janus kinase 2 (JAK2), ABL and SRC kinases, which all lead to phosphorylation of signal transducer and activator of transcription 3 (STAT3) and activation of STAT3-dependent profibrotic transcriptional programmes. Upregulation of profibrotic signalling in fibrosis is accompanied by activation of antifibrotic signalling pathways such as soluble guanylate cyclase (sGC)–protein kinase G (PKG), which attempts to block TGFβ-induced activation of extracellular-signal-regulated kinase (ERK) signalling. AKT, RAC-α serine/threonine protein kinase; cGMP, cyclic guanosine monophosphate; FAK, focal adhesion kinase; GTP, guanosine triphosphate; LAP, latency-associated peptide; PKCδ, protein kinase Cδ; TEAD, transcriptional enhancer factor TEF; TGFβR, transforming growth factor-β receptor.

which serves as a large reservoir of TGFβ[158,159]. Activation of latent TGFβ1 from this reservoir is enhanced in fibrotic diseases[160], and is thought to be a key mechanism of increased TGFβ signalling. The activation of latent TGFβ can be mediated by different mechanisms[161]. Multiple lines of evidence demonstrate that αv integrin is important for the activation of latent TGFβ in vitro and in vivo[162]. Inhibition of αv integrins has potent antifibrotic effects in mouse models of carbon tetrachloride-induced hepatic fibrosis and bleomycin-induced pulmonary fibrosis[163–168]. A humanized monoclonal antibody against αvβ6 (STX-100) was evaluated in a phase II clinical trial in patients with IPF[169], the results of which have not yet been published. In addition to αv integrin, thrombospondin 1, reactive oxygen species (ROS) and several proteases (including plasmin, cathepsin D, MMP2, MMP9 and MMP14) have all been shown to be capable of activating TGFβ in vitro[161,162].

Soluble guanylate cyclase. Soluble guanylate cyclase (sGC) catalyses the formation of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate (GTP) upon binding of nitric oxide (NO). Activation of sGC with increasing levels of cGMP

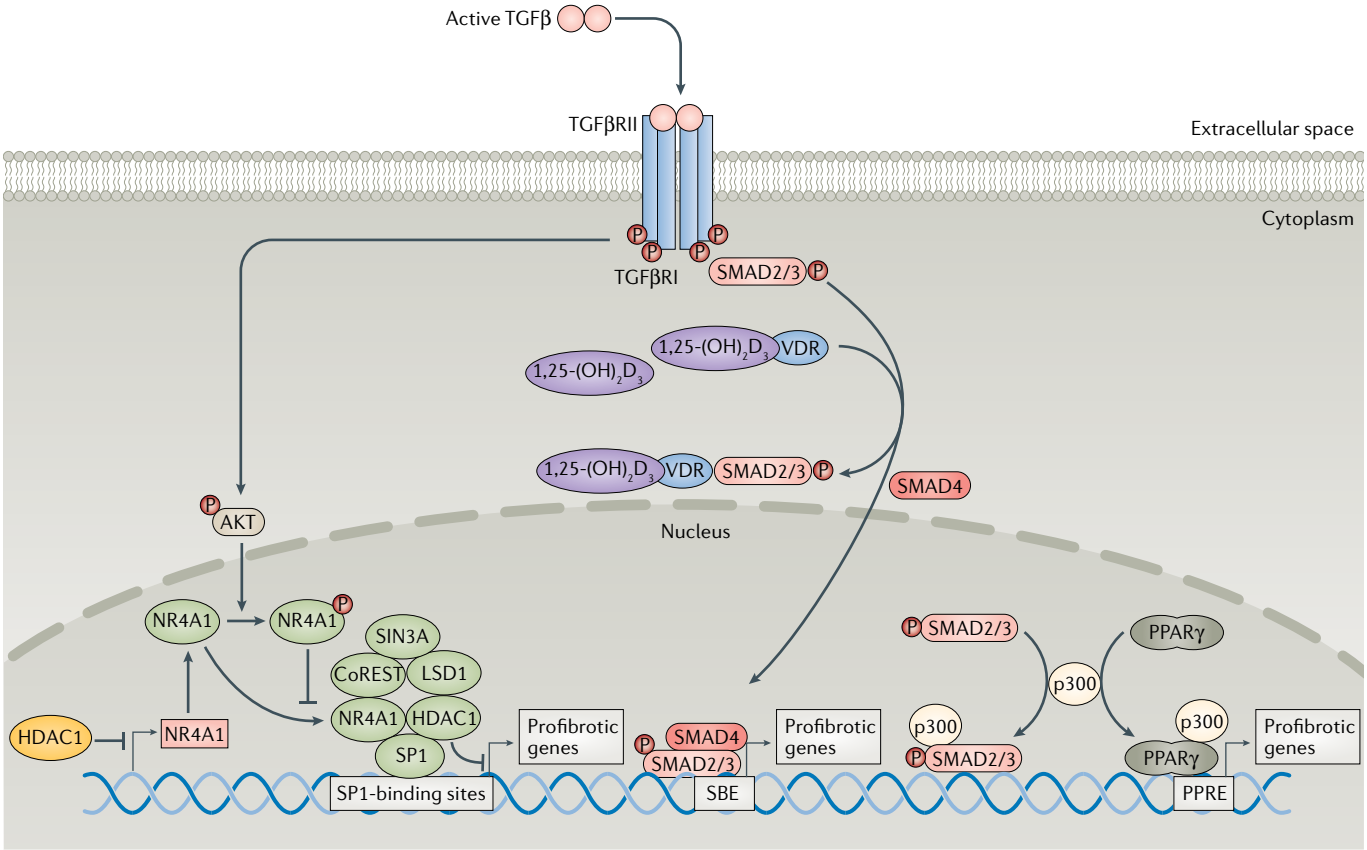

Fig. 5 | **Schematic representation of the role of nuclear receptors as downstream mediators of TGFβ signalling in fibrosis.** Nuclear receptor subfamily 4 group A member 1 (NR4A1), vitamin D receptor (VDR) and peroxisome proliferator-activated receptor-γ (PPARγ) nuclear receptors have inhibitory effects on transforming growth factor-β (TGFβ) signalling. NR4A1 forms a repressor complex together with transcription factor SP1, REST co-repressor 1 (CoREST), lysine-specific demethylase 1 (LSD1), transcriptional co-repressor SIN3A and histone deacetylase 1 (HDAC1); this complex represses the transcription of SP1-dependent profibrotic genes. In fibrotic tissue remodelling, however, activation of TGFβ signalling counter-regulates NR4A1-dependent repression of profibrotic genes. Vitamin D-bound VDRs compete with SMAD4 for the binding of SMAD2/3 dimers, limiting TGFβ-induced SMAD3-dependent transcription of profibrotic genes. Similarly, PPARγ competes with SMAD2/3 for the binding of p300 co-activator and in turn activates transcription of antifibrotic genes with PPAR response element (PPRE). AKT, RAC-α serine/threonine protein kinase; SBE, SMAD-binding element; TGFβR, transforming growth factor-β receptor.

inhibits TGFβ-dependent fibroblast activation in an ERK-dependent manner[170,171] (FIG. 4). Chronic stimulation of fibroblasts with TGFβ decommissions this inhibitory mechanism by downregulation of sGC–cGMP–protein kinase G (PKG) signalling[172]. Stimulators of sGC have antifibrotic effects in experimental models of dermal, pulmonary, hepatic and renal fibrosis[173,174], providing evidence that downregulation of sGC signalling is a common mechanism in fibrotic diseases. In a randomized, controlled phase II study of the sGC stimulator riociguat in patients with dcSSc[175], although the primary end point (change in mRSS) was not met, treatment with riociguat was associated with beneficial trend for mRSS as well as favourable results for several secondary readouts, including forced vital capacity as a standard parameter for pulmonary fibrosis.

Nuclear receptors. Nuclear receptors are a superfamily of transcriptional regulators. Several nuclear receptors, such as PPARγ, vitamin D receptor (VDR) and nuclear receptor subfamily 4 group A member 1 (NR4A1), have emerged as crucial players in fibrotic tissue remodelling across different organs. These receptors might exert their effects, at least in part, by modulating TGFβ signalling (FIG. 5).

PPARγ (also known as NR1C3) is a nuclear receptor with well-characterized antifibrotic effects. Its expression is decreased in fibrotic tissues of patients with SSc and also in cultured fibroblasts from patients with SSc[146]. This downregulation is caused by TGFβ–SMAD signalling[146]. The downregulation of PPARγ, in turn, positively regulates canonical TGFβ signalling[176]. Mechanistically, PPARγ competes with SMAD3 for the transcriptional coactivator histone acetyltransferase p300 and thus blocks SMAD-mediated transcription of profibrotic target genes[176,177] (FIG. 5). Stimulation of PPARγ signalling inhibits TGFβ-induced fibroblast activation and blocks collagen release[176,178–180]. Selective PPARγ agonists have consistently demonstrated antifibrotic effects in murine models of dermal, hepatic, myocardial and renal fibrosis[178,181–183]. However, translation of these promising results to clinical applications has long been prevented by safety concerns, as several selective PPARγ

agonists have been withdrawn from the market because of increased risk of cardiovascular events and bone fracture[184–186]. Nevertheless, pan-PPAR agonists, which activate PPARα, PPARγ and PPARδ, have not shown these safety issues, but maintain the antifibrotic potential of selective PPARγ agonists. The pan-PPAR agonist IVA337, also known as lanifibranor, was well-tolerated and ameliorated experimental dermal and pulmonary fibrosis in bleomycin-challenged mice and mice with transgenic overexpression of the transcription factor FOS-related antigen-2 (REFS[187,188]). Lanifibranor has also been evaluated in a randomized, controlled, phase II trial in patients with dcSSc, but failed to demonstrate antifibrotic efficacy[189].

NR4A1 (also known as Nur77 or TR3) is another example of an antifibrotic nuclear receptor that is downregulated in fibrotic diseases[190]. In normal wound healing, with only temporary activation of TGFβ signalling, NR4A1 inhibits the expression of profibrotic genes by transrepression of the transcription factor SP1 (REF.[190]). However, in fibrotic diseases, the chronic activation of TGFβ signalling decommissions the inhibitory effects of NR4A1 by two mechanisms: on the epigenetic level by histone deacetylase-induced silencing of the gene encoding NR4A1, and on the posttranslational level by phosphorylation of NR4A1 (REFS[190,191]) (FIG. 5). NR4A1 agonists can prevent inactivation of NR4A1 and exert antifibrotic effects in experimental models of dermal, pulmonary, renal and hepatic fibrosis[190]. However, agonists with pharmacokinetics suitable for clinical application are not yet available.

VDR (also known as NR1I1) has also been implicated in the pathogenesis of fibrotic tissue remodelling in multiple organs. Activation of VDR by its natural ligand calcitriol (1,25-$(OH)_2D_3$; the active metabolite of vitamin D_3) or by synthetic agonists can inhibit canonical TGFβ–SMAD signalling[192]. VDR binds to phosphorylated SMAD3 and blocks SMAD3-dependent transcription[192] (FIG. 5). This mechanisms could have direct relevance for the pathogenesis of SSc, as the expression of VDR is decreased in the skin of patients with SSc[192] and vitamin D deficiency is common in patients with SSc or other chronic diseases[193–198]. Moreover, vitamin D deficiency is sufficient to induce hepatic fibrosis in mice in the absence of additional profibrotic stimuli[199]. Treatment with VDR agonists ameliorates fibrosis in murine models of pulmonary, intestinal, renal and hepatic fibrosis[199–203].

Hedgehog and WNT signalling. Abundant evidence demonstrates that hedgehog signalling and WNT signalling are central components of fibrotic tissue remodelling[204–212]. These pathways are often referred to as developmental (morphogen) pathways, as they are essential to organ development and generation. Under homeostatic conditions, these pathways are known to regulate stem cell behaviour and function; moreover, both hedgehog and WNT signalling have been shown to be pathologically activated in many fibrotic diseases, and targeted inhibition of these pathways has antifibrotic effects in different organs[212–214]. These individual morphogen pathways are highly regulated and interlinked via multiple mechanisms with each other in general, and with TGFβ signalling in particular.

Expression of sonic hedgehog (SHH) and of the hedgehog transcription factor GLI2 is upregulated in the skin of patients with SSc[206,215]. Moreover, SHH concentrations are elevated in the serum of patients with SSc and correlate with the fibrotic burden[215]. The activation of hedgehog signalling in SSc is caused, at least in part, by TGFβ, which not only induces the expression of SHH, but also directly binds to and activates the promoter of *GLI2* in fibroblasts[206] (FIG. 6). Hedgehog signalling stimulates fibroblast-to-myofibroblast transition and promotes dermal fibrosis[206]. Pharmacological or genetic inactivation of hedgehog signalling ameliorated experimental fibrosis in murine models of dermal, pulmonary, renal and hepatic fibrosis[216–219], indicating that hedgehog signalling is a core pathway of fibrosis. These findings could have translational implications, as inhibitors of hedgehog signalling, such as Smoothened inhibitors and GLI2 inhibitors, are in clinical use (for basal cell carcinoma) and in clinical trials, respectively[220].

β-Catenin-dependent WNT signalling, also commonly referred to as 'canonical' WNT signalling, is active in multiple fibrotic conditions across different organs and species, with overexpression of WNT proteins, downregulation of endogenous WNT inhibitors and accumulation of nuclear β-catenin[208,209,221–225]. In the lung, β-catenin overexpression has primarily been observed in epithelial cells and fibroblasts[226] in human and murine fibrotic conditions. TGFβ can activate canonical WNT signalling in lung and skin fibroblasts, with nuclear translocation of β-catenin and increased transcription of WNT target genes[227] (FIG. 6). Further activation of WNT signalling by TGFβ occurs via downregulation of endogenous WNT antagonists[228]. For example, TGFβ inhibits the transcription of Dickkopf-1 (DKK1) in a p38-dependent manner and also induces epigenetic silencing of DKK1 as well as of another WNT inhibitor, secreted frizzled-related protein-1 (SFRP1), by DNA methylation[227,229,230]. Several studies have demonstrated that canonical WNT signalling is a core pathway of fibrosis that is sufficient and required for fibrotic tissue remodelling in various organs[208,212,221,223,227,228,231–236]. β-Catenin-independent signalling, known as 'non-canonical' WNT signalling, has been less extensively investigated in tissue fibrosis; however, studies have demonstrated that expression of WNT5A, which is largely known as a non-canonical WNT ligand, is increased in fibroblasts in patients with IPF[237,238] and is strongly regulated by TGFβ[239]. Conversely, TGFβ has been shown to drive secretion of WNT proteins[240], including non-canonical WNT proteins[239].

Given that both WNT and hedgehog signalling pathways are known to be crucial for development as well as for tissue repair under non-fibrotic conditions[241–244], one intriguing concept suggests that repair in fibrosis is misdirected by TGFβ signalling, among other pathways. Current data support the notion that the parallel, non-regulated activity of several core pathways at the same time leads to altered signalling crosstalk, which consequently drives aberrant tissue repair and fibrosis across organs[212,245–247].

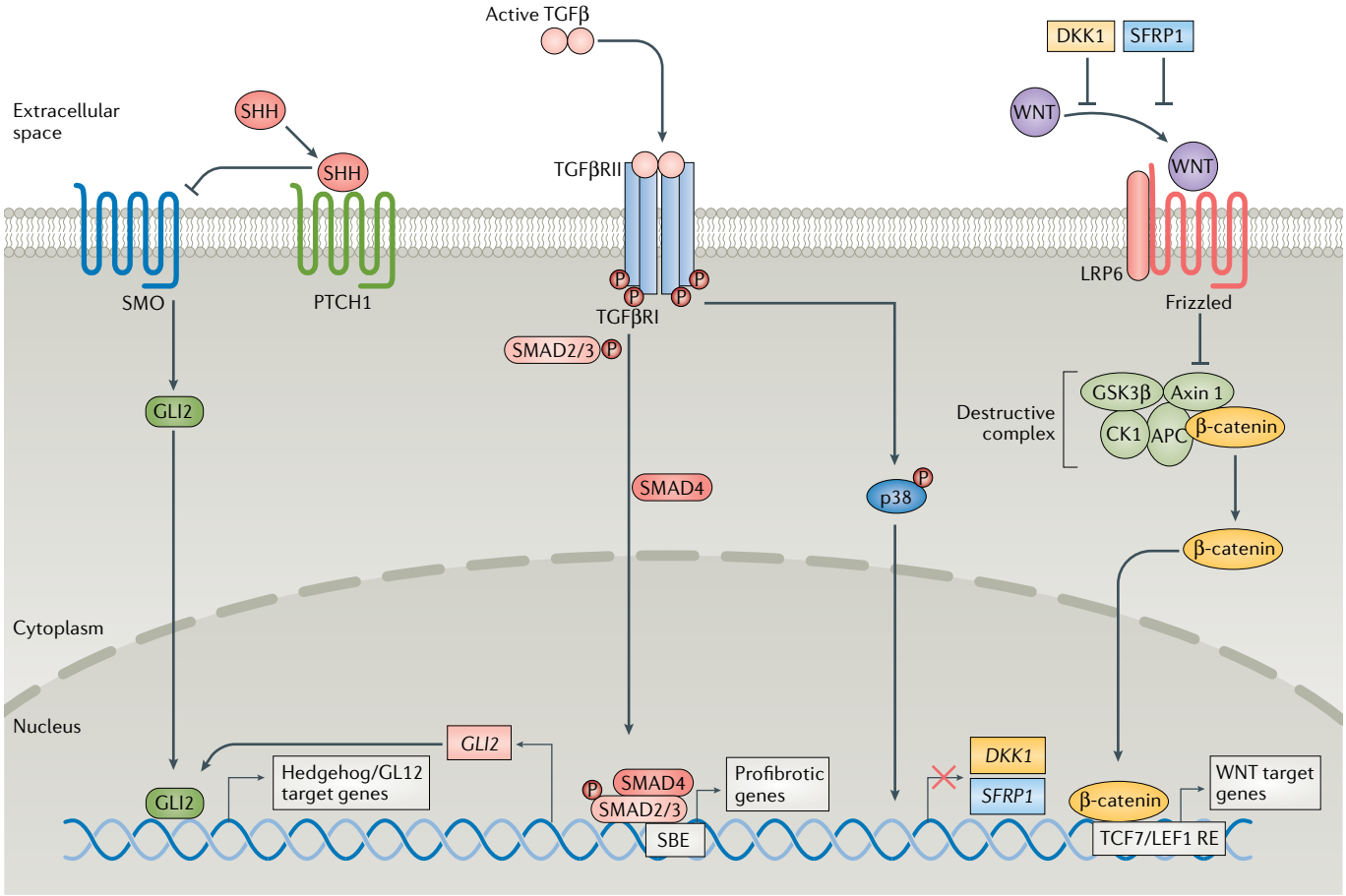

Fig. 6 | **Schematic representation of developmental signalling pathways and their interactions with TGFβ signalling.** Hedgehog and WNT developmental signalling pathways are activated in fibrotic diseases, with extensive crosstalk with transforming growth factor-β (TGFβ) signalling. TGFβ signalling induces transcription of *GLI2*, with subsequent transcription of profibrotic zinc-finger protein GLI2 and hedgehog target genes. TGFβ signalling also inhibits the transcription of the WNT signalling inhibitors secreted frizzled-related protein 1 (SFRP1) and Dickkopf-related protein 1 (DKK1), leading to destabilization of the β-catenin degradation complex and enhanced β-catenin-dependent transcription of profibrotic WNT-target genes. APC, adenomatous polyposis coli protein; CK1, casein kinase 1; GSK3β, glycogen synthase kinase 3β; LEF1, lymphoid enhancer-binding factor 1; LRP6, low-density lipoprotein receptor-related protein 6; PTCH1, protein patched homologue 1; RE, responsive element; SBE, SMAD-binding element; SHH, sonic hedgehog; SMO, smoothened; TCF7E, transcription factor 7; TGFβR, transforming growth factor-β receptor.

Platelet-derived growth factor. PDGF has four isoforms (A, B, C and D), which form several dimeric proteins (AA, BB, AB, CC and DD) that are important mesenchymal cell mitogens and also stimulate collagen gel contraction[248]. The two PDGF receptor tyrosine kinases, PDGFRα and PDGFRβ, are typically expressed by mesenchymal cells in all organs[249]; PDGFRα is a marker for fibroblasts[250], and PDGFRβ is expressed more broadly by mesenchymal cells, including pericytes[249]. PDGF receptor engagement activates mitogen-activated protein kinase and phosphoinositide 3 kinase, as well as small RHO family GTPases involved in cell motility, SRC and other non-receptor tyrosine kinases, and phospholipase Cγ[251]. Multiple studies examining the effects of PDGF inhibition or overexpression have implicated PDGF in fibrosis in mouse bone marrow, lung, kidney, liver and heart[249]. PDGF promotes fibrosis via its mitogenic and perhaps also chemoattractant properties[249]. PDGF also synergizes with TGFβ to augment fibrosis[252], through crosstalk mechanisms that include PDGF regulation of TGFβ levels[253]. Nintedanib, one of the few clinically approved antifibrotic agents, is a tyrosine kinase inhibitor that blocks the activity of PDGF receptors as well as that of FGF and VEGF receptors. In patients with IPF, nintedanib inhibits fibroblast-to-myofibroblast differentiation and myofibroblast proliferation[13]. However, imatinib, which also inhibits PDGF receptor tyrosine kinases, did not show efficacy in clinical trials in patients with IPF[254]. Hence, whether nintedanib acts primarily through PDGF receptor tyrosine kinase inhibition, and thus whether PDGF receptors are a key target for fibrosis, remain uncertain.

Platelets and coagulation. The endothelium is thought to be the site of first injury in SSc. Apoptosis of endothelial cells with subsequent microvascular alterations precede fibrotic manifestations in patients with SSc[255]. Exposure of sub-endothelial ECM and reduced blood

Mitogens
Chemical substances that induce cell division.

Mesangial cells
Specialized cells that form the renal mesangium.

Senolytic therapies
Drugs that selectively induce the death of senescent cells.

Mitophagy
The selective removal of mitochondria by autophagy.

flow rates in the damaged vessels result in activation and degranulation of platelets[256]. Aberrant platelet activation has also been described in other fibrotic diseases[257], although the underlying mechanisms are less clear. Although serotonin (5-hydroxytryptamine (5-HT)) is mainly known as a neurotransmitter, most 5-HT in the human body is stored in platelets[258]. Consistent with platelet activation, concentrations of circulating 5-HT are elevated in patients with SSc in comparison with those in healthy individuals[259]. 5-HT has been implicated in the pathogenesis of fibrotic tissue remodelling of the skin, lungs, liver, arterial wall and heart valves[260–264]. Moreover, inhibition of platelet activation ameliorates experimental fibrosis of the skin, heart, aortic valves, kidneys and liver[260,265,266]. However, 5-HT is not the only profibrotic mediator stored in platelets: platelet granules also contain TGFβ, PDGF, FGFs and VEGF as well as bioactive lipids such as lysophosphatidic acid and sphingosine-1-phosphate[267]. More specific evidence for a role of platelet-derived 5-HT in fibrotic tissue remodelling is provided by the observation that knockout of tryptophan hydroxylase 1 (encoded by *Tph1*), the rate-limiting enzyme for 5-HT synthesis in non-neuronal tissues, ameliorates experimental fibrosis[260,268]. 5-HT stimulates the release of collagen in cultured dermal or cardiac fibroblasts and mesangial cells[260,269]. These stimulatory effects of 5-HT on fibroblasts are mediated by 5-HT_2 receptors, in particular 5-HT_{2B}. Stimulation of 5-HT_{2B} activates TGFβ–SMAD3 signalling in fibroblasts, and neutralizing antibodies against TGFβ reduce the profibrotic effects of 5-HT, providing another example of excessive crosstalk between TGFβ and other profibrotic mediators[260]. Knockout or pharmacological inhibition of 5-HT_{2B} ameliorates fibrosis in mouse models of dermal and pulmonary fibrosis and in an in vitro model of aortic valve remodelling[260,264]. Moreover, in a small, unblinded proof-of-concept trial in patients with SSc, treatment with a non-selective 5-HT_2 inhibitor decreased mRSS on clinical examination, reduced dermal fibrosis on histology and decreased transcription of TGFβ target genes[270]. 5-HT has also been shown to be a central mediator of hepatic and pulmonary fibrosis[271,272]. However, in these two organs, the profibrotic signals of 5-HT might be transmitted by 5-HT_{2A} receptors[272,273]. 5-HT_{2A} receptors are upregulated in activated hepatic stellate cells[273], and treatment with the 5-HT_{2A} inhibitor mirtazapine attenuated experimental hepatic fibrosis by inhibition of TGFβ–SMAD3 and TGFβ–ERK signalling[262]. In pulmonary fibrosis, profibrotic signalling could be transmitted by both 5-HT_{2A} and 5-HT_{2B} (REF.[272]).

Activation of the coagulation cascade upon tissue injury has also been implicated in the pathogenesis of fibrotic diseases in different organs, including lung, skin, kidney, heart and liver[274]. Thrombin in particular might promote tissue fibrosis, but other components of the coagulation cascade, such as factor Xa, might also promote fibroblast activation[275,276]. Thrombin induces the expression of profibrotic and pro-inflammatory mediators such as connective tissue growth factor[277] and CCL2 (REF.[278]), stimulates proliferation of fibroblasts, and promotes transdifferentiation of resting fibroblasts into myofibroblasts and the release of ECM proteins[279]. Dabigatran, a direct thrombin inhibitor, ameliorated experimental bleomycin-induced pulmonary fibrosis in preventive and therapeutic regimens[279]. Direct thrombin inhibitors have also shown efficacy in rodent models of cardiac[280] and hepatic[281] fibrosis, suggesting that activation of the coagulation cascade is a shared mechanism in the pathogenesis of fibrotic disorders.

Cell death, senescence and metabolism

Cell death, senescence and altered metabolism can initiate fibrotic tissue remodelling responses[282]. Oxidative stress caused by environmental toxins, such as bleomycin, asbestos, radiation and tobacco exposure in the lungs or alcohol exposure in the liver, increases ROS, which trigger DNA damage, activate tumour protein p53, injure mitochondria and promote cell death or senescence; epithelial cell death is associated with fibrosis in the liver, lung and kidney[43,283,284].

Mitochondrial dysfunction, dysregulated mitophagy and/or altered mitochondrial biogenesis affect cellular metabolism and can in turn increase production of ROS[282]. Deficient antioxidant activity also affects the redox status of the cell[285] and may thereby promote fibroblast activation. The transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) upregulates antioxidant genes that neutralize ROS, and some studies have indicated that NRF2 deficiency can lead to or aggravate fibrotic disease[285].

Levels of cell stress that do not lead to death induce a state of cellular senescence, mediated by p53 through cyclin-dependent kinase inhibitor 1 (CDKN1; also known as p21) and CDKN2 (also known as p16), and this senescence is linked to altered cellular metabolism[286]. Cell senescence is also induced by shortening of telomeres[287]. Telomere mutations in patients with IPF[288], a disease strongly associated with ageing, emphasize the importance of senescence in fibrosis. Indeed, senolytic therapies in experimental pulmonary fibrosis models have been shown to attenuate and reverse fibrosis by targeting epithelial cells as well as fibroblasts[28,289]. Moreover, a first-in-human open-label study suggested that senolytics could be beneficial in patients with IPF[290]; however, these findings need to be confirmed in larger randomized controlled trials. Senescent cells secrete a distinct series of proteins[28,289], known as the SASP[291], and show decreased mitophagy[292]. SASP and TGFβ (a component of SASP)[293], in combination with TGFβ-induced telomere dysfunction, leads fibroblasts to differentiate into myofibroblasts[294]. However, senescence can also limit fibrosis, as in the liver, where senescent hepatic stellate cells inhibit fibrosis[295].

Self-amplifying activation loops

Progressive deposition of ECM by injured and activated cells alters central physical properties of the tissue. Particularly in the later stages of fibrotic diseases, excessive deposition of ECM leads to stiffening of fibrotic tissues. Fibrotic tissue remodelling also impairs perfusion and the deposited ECM hinders diffusion[10]. These mechanisms lead to profound decreases in the oxygen levels in fibrotic tissues. Increased tissue

CpG islands
Regions of DNA with a high frequency of cytosine–guanine dinucleotides.

stiffness and hypoxia both further promote epithelial cell injury and fibroblast-to-myofibroblast differentiation[9,296,297], and fibrotic ECM further drives a myofibroblast phenotype by regulation of miRNAs in these cells[12]. These physical stimuli can thus coordinate vicious cycles of self-amplifying fibroblast activation and tissue fibrosis during disease progression (FIG. 1). These mechanisms are highly conserved across different fibrotic diseases.

Tissue stiffness

Stiff substrates have long been known to promote fibroblast activation, accompanied by increased expression of contractile proteins and enhanced release of collagen[298]. Elegant studies have shown that transcriptional coactivator YAP1 and transcriptional coactivator with PDZ-binding motif (TAZ, also known as WW domain-containing transcription regulator protein 1) function as central molecular mechanosensors[299]. Inhibition of YAP–TAZ signalling inhibits stiffness-induced as well as TGFβ-induced fibroblast-to-myofibroblast transformation, whereas constitutive activation of YAP promotes fibroblast activation and ECM release[300]. Moreover, pharmacological inhibition of YAP or knockout of *Yap/Taz* in Gli1-positive precursor cells ameliorated UUO-induced fibrosis[300]. Stiffness-induced myofibroblast differentiation further requires p300 and α6 integrin[175]. Stiff substrates activate p300 in hepatic stellate cells by AKT-induced phosphorylation (at serine 1834), consequently promoting the transcription of profibrotic genes[175]. Conversely, targeted inactivation of p300 ameliorates carbon tetrachloride-induced fibrosis[175]. α6 integrin is induced by stiff matrices in pulmonary fibroblasts and mediates MMP-2-dependent myofibroblast invasion[301]. Conditional ablation of α6 integrin or blockade of stiffness-induced α6 integrin expression protects against bleomycin-induced pulmonary fibrosis[301].

Hypoxia

Hypoxia-inducible factor 1α (HIF1α) is an oxygen-sensitive transcription factor that mediates most of the cellular effects of hypoxia. When cellular oxygen levels are in the normal range, HIF1α is hydroxylated and acetylated, which induces binding of von-Hippel-Lindau (pVHL) protein to HIF1α and subsequent proteasomal degradation of HIF1α[302]. With progressive hypoxia, the lack of molecular oxygen prevents hydroxylation of HIF1α by HIF-prolyl hydroxylases and induces accumulation of HIF1α[302]. HIF1α translocates into the nucleus, dimerizes with HIF1β (also known as aryl hydrocarbon receptor nuclear translocator) and regulates oxygen-sensitive genes via hypoxia-responsive elements in their regulatory regions[302,303]. Hypoxia promotes fibroblast-to-myofibroblast transition and stimulates the transcription of several ECM proteins via HIF1α-dependent and HIF1α-independent mechanisms. Chronic hypoxia has also been shown to stimulate collagen release in renal tubular interstitial cells and hepatic stellate cells[304,305], underlining the general relevance of hypoxia in the pathogenesis of fibrotic diseases.

Epigenetic modifications

Fibroblasts explanted from fibrotic tissues share an activated profibrotic phenotype with increased expression of contractile proteins and enhanced release of ECM. A characteristic feature of these fibroblasts is the persistence of the activated phenotype for several passages in vitro. The stable expression of this phenotype even after withdrawal of the initiating stimulus provides a typical example of epigenetic modification. Prolonged exposure of fibroblasts to a profibrotic environment induces epigenetic alterations that stabilize the activated phenotype and render myofibroblasts partially independent of external stimulation. Epigenetic alterations could contribute to stabilization of the activated phenotype of fibroblasts in fibrotic diseases[306,307]. DNA methylation, histone modifications (such as acetylation, deacetylation or methylation) and non-coding RNAs (such as microRNAs (miRNAs) or long non-coding RNAs (lncRNAs)) have been implicated in the pathogenesis of SSc and other fibrotic diseases, and could be targets for antifibrotic therapies[307–312]. Although the principle mechanisms are shared across different diseases and tissues, in some cases the individual modifications might induce a profibrotic phenotype by regulating different target genes in different cell types. Examples are discussed in more detail below, with a focus on DNA methylation and miRNAs as the two most intensely studied epigenetic changes in fibrotic diseases.

DNA methylation. DNA can be methylated at position C5 of the pyrimidine ring of cytosine residues by a family of three DNA methyltransferases (DNMTs): DNMT1, DNMT3A and DNMT3B[313]. The interaction of methyl-CpG-binding domain (MBD) proteins with these methylated cytosine residues in CpG islands of regulatory DNA promotes the recruitment of repressor complexes, thereby silencing gene transcription[314]. Specific inhibitors of DNMTs, such as decitabine, are approved for the treatment of acute myeloid leukaemia[315]. Several studies have implicated DNA methylation-induced silencing of antifibrotic genes in the pathogenesis of fibrotic tissue remodelling[228,307,316–318]. The first evidence for this concept was provided in experimental models of renal fibrosis[309], in which setting long-term stimulation with TGFβ induced hypermethylation of the *RASAL1* promoter, resulting in aberrant RAS signalling. The best studied target of DNA methylation in SSc is Friend leukaemia integration factor 1 (FLI1), a transcription factor of the ETS family[307,319,320]. FLI1 can inhibit TGFβ signalling and limit fibroblast activation[321]. Chronic activation of TGFβ signalling, however, inhibits FLI1 expression and activity via epigenetic and posttranslational mechanisms. TGFβ induces methylation of the FLI1 promoter[309,322] and promotes PKCδ-dependent phosphorylation of FLI1 that stimulates its degradation[323]. DNA methylation also promotes activation of profibrotic pathways other than TGFβ; for example, activation of canonical WNT signalling by silencing of the endogenous WNT antagonists DKK1 and SFRP1 (REF.[228]). Epigenetic silencing of additional antifibrotic genes has been reported in patients with other fibrotic diseases[309,316,317,322]. Treatment with the DNMT inhibitor

Antagomirs
Chemically modified oligonucleotides that are used to silence microRNAs by binding specifically to particular microRNAs.

5-aza-2′-deoxycytidine has consistently been shown to exert antifibrotic effects in rodent models of dermal, pulmonary and renal fibrosis[228,309,324].

MicroRNAs. miRNAs are non-coding RNAs with a length of 20–25 nucleotides. Binding of miRNAs to their respective target mRNAs promotes degradation of the mRNAs[325]. To date, around 50 miRNAs have been implicated in the pathogenesis of fibrotic diseases[325]. Most of these miRNAs are expressed in a highly cell-specific and/or context-specific manner, and are thus selectively involved in fibrosis of specific organs. Two examples of broadly relevant miRNAs with regulatory effects across different organs are miR-21 and miR-29. miR-21 is upregulated in the lungs of mice challenged with bleomycin and of patients with IPF, with prominent expression in myofibroblasts in both mice and humans[326]. miR-21 amplifies canonical TGFβ signalling: TGFβ induces the expression of miR-21, which in turn downregulates SMAD7 to promote canonical TGFβ signalling[326]. In addition to pulmonary fibrosis, miR-21 has been linked to the pathogenesis of renal[327] and cardiac[328] fibrosis. Antagomirs against miR-21 attenuate bleomycin-induced pulmonary fibrosis[326] as well as interstitial myocardial fibrosis induced by pressure-overload[328]. Moreover, treatment with miR-21 small-hairpin RNA ameliorates UUO-induced renal fibrosis[327]. In contrast to miR-21, miR-29 is an antifibrotic miRNA. miR-29 inhibits the translation of multiple collagen genes and of several enzymes involved in ECM turnover[329], and the levels of miR-29 are downregulated in fibrotic tissues across different diseases including SSc and cardiac, renal, pulmonary and hepatic fibrosis[312,329–332]. The deregulation of miR-29 expression is in part mediated by aberrant TGFβ signalling, providing another example of how profibrotic mediators such as TGFβ hijack the epigenetic machinery in fibrotic diseases[329].

Future prospects

Insights from gene expression studies

Altered expression of mRNAs and lncRNAs[333] have been characterized in fibroblasts from patients with SSc and an assortment of different end-target tissue biopsies. Correlation of clinical phenotypes with gene expression signatures objectively defined using various 'omic' technologies, such as RNA sequencing (RNA-seq) and DNA microarray, has enabled disease stratification into pathway-centric groups, with potential implications for personalized medicine. Various studies have demonstrated that distinct gene signatures can be identified in the skin of patients with SSc[334–339]. Profiling of gene expression in the skin also reflects the extent of skin and end-organ damage in patients with SSc[334,340–342], including gene signatures that are associated with ILD[340].

Gene expression studies in biopsy-obtained skin tissue have also identified 'molecular' gene expression subsets that have been termed 'intrinsic' subsets of SSc, namely the inflammatory, fibroproliferative, limited and normal-like subsets. These subsets are referred to as intrinsic because they are typically intrinsic to an individual patient, rather than to a specific organ, and are analogous to those subsets found in more prevalent diseases such as breast cancer[343,344]. The intrinsic subsets of SSc have been reproduced in skin samples from patients with SSc in multiple independent cohorts[336–338]. Notably, a 2015 study[339] reproduced the inflammatory and normal-like SSc subsets but could not identify a fibroproliferative subset. However, re-analysis of the data from this study[339] using a machine-learning-based classifier trained on three independent cohorts was able to reproduce all three subsets associated with dcSSc[345]. The inflammatory subset of SSc is characterized by infiltrating immune cells that include T and B cells and macrophages[336–338,346]. The major pathway driving fibrosis in these patients is the profibrotic IL-13–IL-4 pathway, in which both cytokines signal through the shared IL-4 receptor α-chain[346], with concomitant activation of NF-κB[341]. By contrast, the major pathways driving fibrosis in the fibroproliferative subset are TGFβ and PDGF[340,341,347], although TGFβ can span and link the two subsets[341,348].

Similarly, unbiased gene expression studies in patients with IPF have suggested the existence of at least two molecular subtypes of the disease, on the basis of molecular signatures: one subtype is associated with 'classic' fibrosis markers, in which phenotype fibroblastic foci are dominant, and another subtype with a cilium gene signature, the phenotype of which was associated with distorted epithelial regions and honeycombing[349].

Studies of SSc have typically been underpowered, owing to the rarity of the disease. The integration of smaller datasets for detailed meta-analyses helps addresses this issue of statistical power. These meta-analyses enable a better understanding of SSc pathophysiology and provide an opportunity to integrate multiple independent cohorts analysed on different platforms. In the first network analysis of gene expression in skin from patients with SSc, Mahoney et al.[348] demonstrated that the genetic polymorphisms associated with SSc are found almost exclusively in inflammation-related genes. Mahoney et al.[348] found multiple hubs of gene expression that are related to adaptive immunity, interferon activation, innate immune processes associated with alternatively activated macrophages and ECM deposition. Patients with fibroproliferative disease were enriched in genes associated with proliferation and ECM deposition. Genes associated with ECM deposition were found in both inflammatory and fibroproliferative subsets. Furthermore, the different nodes of gene expression were interconnected with an alternatively activated macrophage and interferon node, which showed connections between one another and to the ECM deposition node, which is in turn connected to the proliferation node[348]. These findings show that the intrinsic subsets could be mechanistically interconnected. Specifically, the combined results of Mahoney et al.[348] and those of a follow-up study by Johnson et al.[341] indicate a mechanism for SSc whereby the interferon response promotes M2 macrophage–dendritic cell–innate immune system activation, which stimulates ECM production and proliferative responses via TGFβ signalling[341]. A separate study[350] took a different approach and integrated seven datasets, primarily from skin samples, and identified a common 415-gene signature that was differentially expressed in skin from patients with SSc as compared with skin from healthy controls across all cohorts. The

study also defined a SSc skin severity score, termed 4S, changes in which correlated with changes in mRSS in individual patients[350].

A key question is whether or not the changes in gene expression seen in skin are a common feature of all tissues in a patient with SSc. The available data suggest that these changes are a common feature, as many of the changes identified in skin have also been identified in other tissues analysed to date, namely blood, and oesophagus and lung tissues. For example, consistently differentially expressed genes can be identified in the PBMCs from patients with SSc[351,352]. Analysis of PBMC samples from patients with limited cutaneous SSc (lcSSc) with and without pulmonary arterial hypertension demonstrated consistent differences[351]. Therefore, high-throughput gene expression in these easily accessible cells can be used to identify the subset of patients with this devastating disease complication. Interestingly, a study of skin samples from patients with lcSSc identified subgroups with gene expression signatures associated with cardiovascular system development, and signatures for cell adhesion, ECM and immune and inflammatory responses[352], and the same subsets found in skin have subsequently also been identified in oesophageal biopsies[353].

Finally, a novel integrative multi-tissue network analysis by Taroni et al.[354] focused on identifying conserved biological processes found across different SSc-affected tissues. This approach builds on the methods developed by Mahoney et al.[348], who performed meta-genomic network analysis on three independent skin datasets and identified conserved gene expression changes[348]. Taroni et al.[354] identified a common pathogenic gene expression signature in ten independent datasets from multiple end-target tissues (skin, lung and oesophagus) and PBMCs from patients with SSc, and demonstrated that a common immune-fibrotic axis is associated with the most severe disease phenotypes of SSc. Moreover, as the analysis by Taroni et al.[354] included PBMCs from patients with and without SSc and lung samples from patients with pulmonary fibrosis or pulmonary arterial hypertension, the results suggested that similar pathways might be differentially expressed between patients with pulmonary fibrosis and patients with pulmonary arterial hypertension. These results suggest the intriguing possibility that not only do common mechanisms drive fibrosis across organ systems in patients with SSc, but also that the mechanisms might be common to other fibrotic conditions.

An important aspect of the gene expression subsets found in end-target tissues is that they might be able to predict response to therapy. For example, in a 2013 study, patients who showed clinical improvement in mRSS during treatment with mycophenolate mofetil mapped to the inflammatory intrinsic subset, whereas patients in the fibroproliferative subset failed to show any clinical improvement[338]. Similarly, a pilot study and a phase II double-blind placebo-controlled clinical trial showed that patients whose disease improved under abatacept therapy mapped to the inflammatory subset[355,356]. Prior results have suggested that tyrosine kinase inhibitors, such as imatinib, nilotinib and dasatinib[357], might target either the fibroproliferative subset or, more precisely, patients with activated TGFβ signalling[347,358,359]. The finding that SSc subsets respond differently to therapy raises the intriguing possibility that they could be used for precision medicine in systemic autoimmune disease[360,361]. The SSc intrinsic subsets have now been measured in several placebo-controlled trials[356,362] and the results of these studies are expected to be reported in the coming year, providing a control test of the predictive power of gene expression changes for clinical trials in fibrosis.

Emerging technologies and models

Over the past decade, we have witnessed the development of several new model systems that aim to improve the predictive value of preclinical testing in rodent models for the outcome of human clinical trials, thus closing the translational gap (TABLE 3). These new

Table 3 | **New techniques and models relevant to fibrosis research**

Technical advance	Key feature	Applications
Human cell-based organoids	Generation of organ-like structures derived from pluripotent cells	Improved prediction of therapeutic responses?
In vitro human organ equivalents	Assembly of organ structures by sequential seeding of organ-relevant cell types after in vitro amplification onto decellularized organ matrices	Improved prediction of therapeutic responses?
Precision-cut human tissue slices	Ex vivo culture of thick tissue sections (200–500 μm) to maintain cell–cell and cell–matrix interactions in 3D in the tissue of interest	Studies of ex vivo responses of patient-derived diseased tissue to defined stimuli and treatments
Humanized mouse models	Reconstitution of immunodeficient mice with human bone marrow cells, often in combination with human tissue samples	Studies of interactions of the human immune system with tissues of interest
Single-cell RNA sequencing	Sequencing of the transcriptome of individual cells	Identification of novel subpopulations of cells
Mass cytometry	Simultaneous evaluation of up to 40 markers in cells or tissues by metal isotype tagged antibodies with minimal spill over between the channels	Characterization of individual subpopulations or parallel characterization of multiple cell populations
Metabolomics	Comprehensive analysis of cell metabolites with characterization of metabolic phenotypes	Characterization of metabolic disturbances underlying different pathological conditions? Discovery of biomarkers for disease diagnosis/progression? Discovery of potential therapeutic targets?

models include human cell-based organoids and human organ equivalents, in which organ-like structures are generated in vitro from pluripotent stem cells or from the sequential addition of differentiated cells[363], respectively. Humanized mice, which have been reconstituted with human bone marrow cells and carry human tissue transplants, could also enable studies of human cells in a complex, disease-relevant environment[364]. Application of precision-cut human tissue slices from healthy and diseased human tissue represents another promising tool for closely recapitulating the complexity of the native organ environment and architecture in a 3D model. These ex vivo techniques not only allow advanced imaging at high spatiotemporal resolution, but also enable, through the use of patient-derived diseased tissue, the analysis of late-stage disease mechanisms for drug discovery and validation in an individualized fashion[365–367].

Whole-exome and whole-genome sequencing are now affordable and becoming routine, and are revealing increasingly rare genetic associations in diseases including fibrotic diseases[368]. Moreover, genome editing is now an established, although still evolving, technology. The CRISPR–Cas9 system has been widely applied to generate knockout mice[369]. Advances in CRISPR–Cas9 are providing opportunities to edit specific nucleotides in genes[370]. CRIPSPR–Cas9 has already entered the clinical arena for the treatment of cancer[371] as well as of genetic haematological diseases[121], as a tool for repairing mutations in human DNA[370].

Single-cell technologies are one of the most important advances in many years toward dissecting human disease cellular and molecular biology. Studies using these technologies have revealed striking heterogeneity of cell types in various tissues, and have permitted a cell-by-cell understanding of gene expression[372]. Comparing transcriptomes of normal and diseased cells provides unprecedented insight into the molecular alterations in the latter, and this approach has been applied in a compelling manner toward understanding fibrotic diseases, notably IPF[65]. Single-cell RNA sequencing (scRNA-seq) can also examine single-cell B cell and T cell receptor usage, so that clonality can be easily established and variable region sequences obtained[373]. Other single-cell technologies are rapidly emerging. In particular, large numbers of antibodies to surface proteins can be identified in Cite-seq (cellular indexing of transcriptomes and epitopes by sequencing) or REAP-seq (RNA expression and protein sequencing assay) studies, by complexing antibodies to oligonucleotide 'barcodes' and sequencing these barcodes, enabling the simultaneous assessment of epitopes and transcriptomes in single cells[374,375]. Mass cytometry, or cytometry by time of flight (CyTOF), substantially extends the protein phenotyping traditionally achieved by flow cytometry, by enabling the simultaneous detection of up to 40 different proteins[376]. Along with other omics approaches, such as metabolomics, lipidomics and proteomics, and integrated cutting-edge big data approaches, CyTOF technology could provide the basis for selective targeting of disease-relevant, profibrotic cell subsets without affecting the populations required to maintain tissue homeostasis. A single-cell assay for transposase-accessible chromatin using sequencing (ATAC-seq), another rapidly emerging single-cell technology, extends our understanding of open chromatin[377]. ATAC-seq is a particularly robust technology for analysing complex tissues, in which the utility of this technology depends on examining cells of the same phenotype, as open chromatin is highly dependent on the cell phenotype. One of the benefits of applying all of these single-cell technologies toward understanding fibrotic diseases is that they can be used with fresh disease tissues, directly from human or animal models, without the need for the in vitro manipulation that generally alters disease-associated cell phenotypes.

These emerging sequencing technologies are generating unprecedented quantities of data. A human genome contains approximately three billion nucleotides, and tens to hundreds of genomes can be sequenced in a week in a typical high-throughput centre. A typical scRNA-seq experiment examines the expression of ~30,000 genes in up to 10,000 cells, or ~300 million data points, representing a 10,000-fold increase in data compared with gene expression from microarray technology from just 10 years ago. Clinical databases are also increasingly detailed and encompass ever-larger numbers of patients, providing the potential to understand increasingly subtle associations within clinical data. Systems biologists are rapidly developing analytical algorithms to detect and understand associations within these large datasets. One widely utilized method for analysing large single-cell datasets from CyTOF and scRNA-seq is t-distributed stochastic neighbour embedding (TSNE) plot, an algorithm for dimensional reduction[378]. TSNE and other techniques, such as uniform manifold approximation and projection (UMAP), are used to compress very large datasets into visually comprehensible plots[378,379]. Other systems biology approaches are rapidly providing alternative methods for discovering associations between dataset elements and for comparing datasets from healthy and diseased tissues. The inclusion of single-cell technologies in clinical trials will permit markedly more detailed dissection of therapeutic effects on the single-cell level. The application of these technologies represents a truly exciting new chapter in understanding the biology of disease, including fibrotic diseases.

Considerations for targeted therapies

Targeting the shared fibrotic signalling responses that drive disease progression in the later stages of fibrotic diseases, for example by inhibition of the core pathways of fibrosis or of self-amplifying activation loops, might be effective in a broad range of fibrotic diseases. However, many of these pathways are also required for tissue homeostasis. Broad spectrum inhibition upstream of those core pathways might thus be limited by adverse events. For example, neutralization of all TGFβ isoforms or inhibition of the TGFβ receptors TGFβRI or TGFβRII could be complicated by autoimmune reactions and the formation of keratoacanthomas[380]. To overcome these safety concerns, alternative strategies, which involve selective inhibition of either cell-specific

Keratoacanthomas
Benign tumours of the skin, originating from the hair follicle.

or disease-specific membrane-bound receptors or intracellular downstream mediators, have been developed to more selectively interfere with profibrotic signals and to better maintain the homeostatic functions of core pathways[381].

Targeting of early events in fibrotic diseases would theoretically enable more selective interference, but might require specific approaches for individual fibrotic diseases. Targeting of early stages of the disease is also complicated by our limited understanding of the pathophysiological processes in the very early stages of fibrotic diseases. In this context, new technologies, such as disease modelling in ex vivo precision-cut tissue slices using human tissue, can be utilized[366].

Despite these challenges, the list of potential molecular targets for the treatment of fibrosis is continuously growing (FIGS 4–6; TABLE 4). Several approaches are currently being evaluated in clinical trials (TABLE 4) and nintedanib and pirfenidone have already been approved for the treatment of IPF[382].

Table 4 | **Clinical trials in patients with SSc and IPF**

Drug	Target	Target population	Phase	Clinical trial identifier	Status
Systemic sclerosis					
GLPG1690	Autotaxin	SSc	II	NCT03798366	Recruiting
IVIG	Fc receptors?	dcSSc	II	NCT01785056	Active, not recruiting
Nintedanib	Multiple tyrosine kinases	SSc-associated ILD	III	NCT03313180	Active, recruiting
Pirfenidone	Not well defined, but including TGFβ signalling	SSc-associated ILD	II	NCT03221257	Recruiting
Tofacitinib	JAK1/3	Early dcSSc	I/II	NCT03274076	Active, not recruiting
GSK2330811	Oncostatin	dcSSc	I/II	NCT03041025	Recruiting
AVID200	TGFβ1/TGFβ3	dcSSc	I	NCT03831438	Recruiting
Abatacept	CTLA4	dcSSc	II	NCT02161406	Completed
Tocilizumab	IL-6	dcSSc	III	NCT02453256	Completed
Riociguat	Soluble guanylate cyclase agonist	dcSSc	II	NCT02283762	Completed
Brentuximab vedotin	CD30	dcSSc	I/II	NCT03222492	Recruiting
Romilkimab (SAR 156597)	IL-4 and IL-13	dcSSc	II	NCT02921971	Completed
Lenabasum (JBT-101)	CB2 agonist	dcSSc	III	NCT03398837	Active, recruiting
Lanifibranor (IVA337)	PPARs	Early dcSSc	II	NCT02503644	completed
Idiopathic pulmonary fibrosis					
Pirfenidone	Not well defined, but including TGFβ signalling	Pulmonary fibrosis with anti-myeloperoxydase antibodies	II	NCT03385668	Recruiting
Bevasizumab	VEGF	Radiation-/chemotherapy-induced pulmonary fibrosis	II	NCT01917877	Recruiting
TRK-250	RNA-based inhibition of TGFβ1 expression	IPF	I	NCT03727802	Recruiting
FG-3019	CTGF	IPF	II	NCT01262001	Completed
VAY736	BAFFR	IPF	II	NCT03287414	Recruiting
GLPG1205	GPR84	IPF	II	NCT03725852	Recruiting
ND-L02-s0201	HSP47 (collagen-specific chaperone)	IPF	II	NCT03538301	Recruiting
BG00011	αVβ6 integrin	IPF	II	NCT03573505	Active, not recruiting
CC-90001	JNK1	IPF	II	NCT03142191	Recruiting
GLPG1690	Autotaxin	IPF	III	NCT03711162	Recruiting
Elafibranor	PPARα/δ	Nonalcoholic steatohepatitis with fibrosis	III	NCT02704403	Recruiting

Clinical trial data were accessed from ClinicalTrials.gov on 16 September 2019. BAFFR, B cell activating factor receptor; CB2, cannabinoid receptor 2; CTGF, connective tissue growth factor; CTLA4, cytotoxic T lymphocyte antigen 4; dcSSc, diffuse cutaneous systemic sclerosis; GPR84, G protein-coupled receptor 84; HSP47, heat shock protein 47 kDa; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; IVIG, intravenous immunoglobulin; JAK, Janus kinase; JNK1, JUN N-terminal protein kinase 1; PPAR, peroxisome proliferator-activated receptor; SSc, systemic sclerosis; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

Conclusions

Although the risk factors, initiating triggers and the resulting inflammatory responses in the early stages of fibrotic disease differ between tissues and organs, they all activate a highly consistent set of core pathways of fibrosis that promote tissue and cell injury, including accumulation of myofibroblasts and fibrotic tissue remodelling. Epigenetic imprinting in response to the chronic profibrotic milieu renders fibroblasts partially independent of external stimulation and could promote disease progression in the absence of the initiating inflammatory response. The progressive deposition of ECM results in increasing tissue stiffness and hypoxia, which serve as general self-amplifying loops of fibroblast activation and tissue remodelling in more advanced disease stages. Thus, disease-specific responses to injury at early stages culminate in accumulation of myofibroblasts and activation of shared fibrotic signalling responses that drive disease progression in later stages of fibrotic diseases. This progress in the understanding of fibrotic mechanisms raises hope that we are about to enter a new era with effective targeted therapies for the treatment of fibrotic diseases.

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Chemokines in rheumatic diseases: pathogenic role and therapeutic implications

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Abstract | Chemokines, a family of small secreted chemotactic cytokines, and their G protein-coupled seven transmembrane spanning receptors control the migratory patterns, positioning and cellular interactions of immune cells. The levels of chemokines and their receptors are increased in the blood and within inflamed tissue of patients with rheumatic diseases, such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, vasculitis or idiopathic inflammatory myopathies. Chemokine ligand–receptor interactions control the recruitment of leukocytes into tissue, which are central to the pathogenesis of these rheumatic diseases. Although the blockade of various chemokines and chemokine receptors has yielded promising results in preclinical animal models of rheumatic diseases, human clinical trials have, in general, been disappointing. However, there have been glimmers of hope from several early-phase clinical trials that suggest that sufficiently blocking the relevant chemokine pathway might in fact have clinical benefits in rheumatic diseases. Hence, the chemokine system remains a promising therapeutic target for rheumatic diseases and requires further study.

Chemokines are a family of small (8–10 kDa) chemotactic cytokines that control the migratory patterns and positioning of immune cells[1], and over 50 chemokines and 19 chemokine receptors have so far been identified[1]. These secreted signalling proteins are classified into four main subfamilies according to the location of cysteine residues near the amino terminus of their primary amino acid sequence: XC chemokines (which contain a single N-terminal cysteine), CC chemokines (which have two adjacent cysteines near their amino acid terminus), CXC chemokines (which have two cysteines separated by one other amino acid), and CX_3C chemokines (which have two cysteines separated by three amino acids)[1]. 'Classical' chemokine receptors are proteins with seven transmembrane spanning domains that are coupled to G proteins and regulate immune cell migration, and include the CC, CXC, XC, and CX_3C-chemokine receptors (CCR, CXCR, XCR, CX_3CR). By contrast, 'atypical' chemokine receptors (ACKRs) do not couple to G proteins and do not induce cell migration even though they also have seven transmembrane spanning domains[1].

High levels of chemokines have been observed in numerous human rheumatic diseases, including rheumatoid arthritis (RA)[2], systemic lupus erythematosus (SLE)[3], systemic sclerosis (SSc)[4], vasculitis[5] and idiopathic inflammatory myopathies (IIM)[6]. Chemokines and their receptors are thought to induce the recruitment of immune cells into the affected organs in these diseases, including neutrophils, macrophages, dendritic cells, T cells and B cells, and are also considered to be involved in activation of leukocytes once in the tissue, promoting integrin activation and the production of proteases and inflammatory mediators[1].

In this Review, we summarize the pathogenic functions of chemokines and their receptors in various rheumatic diseases, including RA, SLE, SSc, vasculitis and IIM. Within these sections, we also provide an update on the clinical trials of drugs targeting chemokines and chemokine receptors in rheumatic diseases and discuss their potential as therapeutic targets.

The chemokine system in RA

RA is a chronic inflammatory autoimmune disease in which the immune system attacks multiple joints[7]. Without effective treatment, RA can result in irreversible joint destruction and considerable disability. Biologic therapies, such as inhibitors of TNF and IL-6, have revolutionized the treatment of RA. However, the use of these biologic drugs is associated with an increased risk of infection; furthermore, ~50% of patients are unresponsive to treatment, and patients who do respond to treatment often have residual disease[8,9]. Therefore, new therapeutic targets and treatment strategies are still needed.

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Key points

- Chemokines are a large family of secreted chemotactic cytokines that control the recruitment of immune cells into tissue and their cellular interactions once in tissue.
- Chemokine receptors are G protein-coupled seven transmembrane spanning proteins that are expressed on immune cells and regulate their migration and cell–cell interactions.
- Concentrations of chemokines are increased in the blood and tissues of patients with rheumatic diseases, suggesting their involvement in the pathogenesis of these diseases and highlighting them as therapeutic targets.
- Preclinical animal models of rheumatic diseases show the important functional roles of the chemokine system in the pathogenesis of these diseases.
- Unfortunately, the majority of clinical trials testing the efficacy of chemokine and chemokine receptor inhibitors have failed to show meaningful clinical benefit.
- However, several clinical studies have shown promise and suggest that targeting the relevant chemokine system and ensuring complete inhibition at all times might be needed for therapeutic benefit.

Animal models of inflammatory arthritis are valuable research tools for understanding the pathogenesis of arthritis as well as for studying new drugs[10], although careful interpretation of prophylactic versus therapeutic approaches in these models is required before translation to human applications. Several mouse models of arthritis, such as K/BxN arthritogenic serum-induced arthritis[11], type II collagen-induced arthritis (CIA)[12] and collagen antibody-induced arthritis (CAIA)[13], have been used to study the role of chemokines and their receptors in inflammatory arthritis[10]. K/BxN mice express the T cell receptor (TCR) transgene KRN and MHC class II molecule A(g7) and spontaneously develop inflammatory arthritis alongside high titres of autoantibodies to glucose-6-phosphate isomerase[14]. Transfer of serum or anti-glucose-6-phosphate isomerase antibodies from K/BxN mice into wild-type mice induces arthritis in a T cell-independent and B cell-independent manner. Neutrophils are the main initiators of inflammation in this model, making it well suited for studying the contribution of innate immunity in inflammatory arthritis[15]. By contrast, adaptive immunity contributes to the development of arthritis in the CIA model, which is initiated through intradermal immunization with type II collagen emulsified in complete Freund's adjuvant[12]. Like the K/BxN serum transfer model, the CAIA model is induced by the passive transfer of antibodies, in this case a cocktail of monoclonal antibodies that are directed against conserved autoantigenic epitopes in type II collagen, followed by endotoxin. In all of these models, chemokines are required for the development of inflammatory arthritis (TABLE 1).

Chemokines in RA

Multiple CXC chemokines (CXCL1, CXCL2, CXCL5, CXCL8, CXCL9, CXCL10, CXCL12, CXCL13 and CXCL16), CC chemokines (CCL2, CCL3, CCL4, CCL5, CCL18, CCL19, CCL20, CCL21 and CCL25), XC chemokines (XCL1 and XCL2) and the CX_3C chemokine CX_3CL1 are present at elevated levels in the serum, synovial fluid and synovial tissue of patients with RA compared with healthy individuals[16–24] (Supplementary Table 1). Many of these same chemokines are also found at higher levels in the serum, synovial fluid and synovial tissue in animal models of inflammatory arthritis compared with control animals[25–35]. Synovial macrophages and fibroblast-like synoviocytes (FLSs) are producers of many of the inflammatory chemokines in the inflamed joints of patients with RA[16,31]. In addition, follicular dendritic cells in the synovium produce CXCL13 (REF.[36]), and synovial endothelial cells express CC chemokines in patients with RA[37]. Synovial macrophages are the main producers of CXCL8 in patients with RA whereas neutrophils are an important source of CXCL2 in the K/BxN serum transfer mouse model of arthritis[25,38,39]. Unlike other chemokines, the main source of CX_3CL1 in the synovium of patients with RA is endothelial cells and not synovial macrophages[21].

The function of chemokines in the pathogenesis of RA still remains unclear; however, data from animal models have revealed important functions for chemokines in inflammatory arthritis. In the early phase of human RA and in animal models of inflammatory arthritis, tissue-resident synovial macrophages are likely to be important producers of chemokines that control neutrophil and monocyte migration into the joint (FIG. 1). After entering the inflamed joint, activated neutrophils produce CXCL2, which promotes the recruitment of additional neutrophils in a positive-feedback loop as shown in the K/BxN serum transfer mouse model of arthritis[25,40]. In this model, neutrophils also amplify the local response by secreting cytokines, such as IL-1, which induce chemokine production by FLSs in the joint[25]. In later phases of disease, FLSs and other cells might have important roles in recruiting T and B cells (FIG. 1).

CXC chemokines regulate various aspects of leukocyte recruitment into the inflamed joint (Supplementary Table 1). CXCL1, CXCL2, CXCL5 and CXCL8 promote neutrophil recruitment[38], CXCL10 participates in effector T cell trafficking[41] and CXCL13 regulates B cell and T follicular helper (T_{FH}) cell migration in mouse models of inflammatory arthritis[42,43]. Furthermore, CXCL8, CXCL12 and CXCL16 promote angiogenesis in the synovium of patients with RA in in vitro studies[26], whereas CXCL8, CXCL10 and CXCL13 are promising biomarkers of RA disease severity or activity[17,23,44]. The data from animal models of inflammatory arthritis suggest that the CC chemokines promote monocyte (CCL2, CCL3, CCL4, CCL5 and CCL7)[30,32], T cell (CCL18, CCL19, CCL20, CCL21 and CCL25)[29] and B cell (CCL20)[45] entry into the inflamed joint. In addition, CX_3CL1 induces monocyte recruitment into the inflamed joint in the CIA mouse model of arthritis[35]. Finally, production of the XC chemokine XCL1 is increased in FLSs in the CAIA model, suggesting that this chemokine might also contribute to the pathogenesis of inflammatory arthritis[46]. However, the functions of XCL1 and XCL2 (the latter is not expressed in mice) in patients with RA and in animal models of arthritis are still unknown.

Chemokine receptors in RA

Various CXC-chemokine receptors (CXCRs), including CXCR1, CXCR2, CXCR3, CXCR4, CXCR5 and CXCR6, are highly expressed in the joints of patients with RA and in animal models of arthritis[16,25,26,32,47,48]. CXCR1 and CXCR2 are expressed on neutrophils in

the synovium of patients with RA, suggesting that these receptors mediate neutrophil migration into inflamed joints[47], whereas CXCR3 is expressed on infiltrating T helper 1 (T_H1) cells[41,48] (FIG. 1). CXCR4 also regulates lymphocyte recruitment into the synovium of patients with RA[32]. CXCR5 mediates B cell and circulating T_{FH} cell recruitment into the synovium of patients with RA and in mouse models, such as CIA[42,43,45,49]. CXCR6 regulates T cell accumulation and angiogenesis in the joints of patients with RA and in animal models[16,26]. CXCR4 and CXCR5 also contribute to the establishment of lymphoid follicles in the arthritic joint[50–52].

The CC-chemokine receptors (CCRs) CCR1, CCR2, CCR5, CCR6, CCR7, CCR9 and CCR10 are also abundantly expressed in the joints of patients with RA and in animal models of inflammatory arthritis[24,25,29,32,48,53]. These receptors are thought to regulate the recruitment of monocytes (CCR1, CCR2, CCR5, CCR9 and CCR10), T cells (CCR5, CCR6) and dendritic cells (CCR9) into the synovium in patients with RA and in mouse models. The XC-chemokine receptor XCR1 is highly expressed on mononuclear cells[54] whereas the CX_3C-chemokine receptor CX_3CR1 is expressed on T cells in the synovium of patients with RA[21], suggesting that these receptors also contribute to the recruitment of immune cells into the joints of patients with RA (FIG. 1). Thus, different combinations of chemokine receptors are required for the entry of different cell types into the synovium of patients with RA. How these receptors collaborate to control leukocyte recruitment into the joints still remains unknown. Thus, further study is needed to dissect each receptor's function in the leukocyte migration cascade in inflammatory arthritis.

Stromal cells also express chemokine receptors in the synovium of patients with RA, although the function of chemokines in this context is unclear. Chemokine receptor signalling in synovial endothelial cells could be an important mediator of angiogenesis in RA. Synovial endothelial cells in patients with RA express CCR7, and signalling via the CCL21–CCR7 axis in endothelial cells promotes angiogenesis[55]. CCR7 is also associated with the formation of lymphoid follicles in the synovium[49]. In addition, synovial endothelial cells express CCR10, and activation by its ligand CCL28 could also promote RA angiogenesis via extracellular signal-regulated kinase (ERK) activation[56]. CCR9 is expressed on FLSs in the synovium of patients with RA and these cells produce pro-inflammatory cytokines in vitro upon stimulation with its ligand CCL25, although the consequences of this interaction in vivo is not yet clear[29]. These studies suggest that, in addition to haematopoietic-derived immune cells, chemokines also have an important function in stromal cells, such as in endothelial cells and FLSs, during RA pathogenesis.

ACKR1 (formally known as Duffy antigen receptor for chemokines (DARC)), and ACKR3 (formally known as CXCR7) are expressed on joint endothelial cells in the synovial tissue of patients with RA[19,57,58]. Furthermore, the expression of ACKR2 (formally known as D6) is increased in peripheral blood leukocytes as well as in leukocytes and stromal cells in the synovial tissue of patients with RA compared with healthy individuals[59]. ACKR1 can transcytose inflammatory chemokines, suggesting that ACKR1 has a functional role in leukocyte recruitment into the joint by transporting chemokines produced within the joint into the lumen of overlying blood vessels to initiate leukocyte arrest and transendothelial migration[57,60]. Meanwhile, ACKR3 has been linked to angiogenesis in the synovium of mice with CIA[19]. However, the functional role of ACKRs in the pathogenesis of human RA remains unknown.

Targeting the chemokine system in RA

Insights from animal models. In animal models of arthritis, blockade of a single chemokine (CXCL10, CXCL13, CCL2, CX_3CL1 or XCL1) has preventive and/or therapeutic effects, although these effects vary depending on the model studied[26,30,35,41,46,61,62] (TABLE 1). For instance, the CIA model is more heavily dependent on monocytes, and blockade of CCL2 can ameliorate arthritis in this model. However, CCL2 blockade is not sufficient to ameliorate arthritis in the K/BxN serum transfer model owing to the important involvement of neutrophils in this model[28]. A limitation in targeting a single chemokine is the potential redundancy from other chemokines that bind the same receptor, which enables immune cell recruitment despite effective inhibition of the target chemokine. Indeed, many chemokine receptors, including CXCR2, CXCR3 and CCR1, have multiple ligands. Notably, a broadly cross-reactive CXCR2 ligand-blocking antibody SA138 that targets CXCL1, CXCL2, CXCL3 and CXCL5 considerably attenuates arthritis in the K/BxN serum transfer model compared with blocking antibodies against only CXCL1 (REF.[63]). Thus, strategies that inhibit multiple inflammatory chemokines, rather than a single chemokine alone, might be a promising direction for new therapies for RA.

Inhibition of chemokine receptors (CXCR2, CXCR3, CXCR5, CXCR7, CCR1, CCR2, CCR5, CCR7 and CCR9) can also ameliorate inflammatory arthritis in animal models[19,25,29,49,64–72] (TABLE 1). CXCR3, CXCR5, CCR5, CCR7 and CCR9 contribute to the pathogenesis of CIA, but not the pathogenesis of the K/BxN serum transfer model. These receptors control the recruitment of T cells, B cells and monocytes, which are involved in the pathogenesis of CIA but not in the pathogenesis of the K/BxN serum transfer model. However, the pathogenesis of RA is undoubtedly more complex than animal models of arthritis, and therefore the inhibition of multiple chemokine receptors might prove more effective than inhibition of any single receptor.

Insights from clinical trials. Eight drugs that target chemokines, including CCL2 (ABN912)[73] and CXCL10 (MDX-1100)[20], and chemokine receptors, including CCR1 (CP-481,715 (REF.[74])), CCX354-C[75] and MLN3897 (REF.[76])), CCR2 (MLN1202)[77] and CCR5 (SCH351125 (REF.[78]), AZD5672 (REF.[79]) and UK-427,857 (REF.[80])), have been tested in patients with RA. For most of these inhibitors, with some notable exceptions, treatment resulted in no beneficial effects (TABLE 2). However, one small clinical trial of a small-molecule CCR1 antagonist (CP-481,715) showed that blockade of CCR1 reduced tender and swollen joint count and the number of macrophages

Table 1 | **Targeting chemokine pathways in animal models of rheumatic diseases**

Target	Model	Animal	Chemokine-targeting drug or chemokine deficiency	Outcome	Notes	Refs
Inflammatory arthritis (chemokine receptors)						
CCR1	K/BxN serum transfer	Mouse	CCR1 deficiency	Partially effective	Delayed onset of arthritis; inhibited early neutrophil recruitment into the joint	25,38
	CIA	Mouse	CCR1 antagonist (J-113893)	Very effective	Ameliorated arthritis; reduced bone destruction and inflammatory cell infiltration into the joint	66
CCR2	K/BxN serum transfer	Mouse	CCR2 deficiency	Not effective	No change	28
	AIA	Rat	CCR2 antagonist (INCB3344)	Very effective	Improved arthritis; reduced bone destruction	67
CCR3	K/BxN serum transfer	Mouse	CCR3 deficiency	Not effective	No change	28
CCR4	K/BxN serum transfer	Mouse	CCR4 deficiency	Not effective	No change	28
CCR5	K/BxN serum transfer	Mouse	CCR5 deficiency	Not effective	No change	28
	CIA	Monkey	CCR5 antagonist (SCH-X)	Very effective	Improved arthritis; reduced bone destruction and reduced serum concentrations of C-reactive protein	68
CCR6	K/BxN serum transfer	Mouse	CCR6 deficiency	Not effective	No change	28,68
	CIA	Mouse	CCR6 deficiency	Very effective	Improved arthritis	69
CCR7	K/BxN serum transfer	Mouse	CCR7 deficiency	Not effective	No change	28
	CIA	Mouse	CCR7 deficiency	Very effective	Ameliorated arthritis	70
	CIA	Mouse	CCR7 mAb (8H3–16A12)	Very effective	Ameliorated arthritis	70
CCR9	K/BxN serum transfer	Mouse	CCR9 deficiency	Not effective	No change	28
	CIA	Mouse	CCR9 deficiency	Very effective	Suppressed arthritis; reduced bone destruction	29
	CIA	Mouse	CCR9 inhibitor (CCX8037)	Very effective	Suppressed arthritis; reduced bone destruction	29
CXCR1	CAIA	Mouse	CXCR1/CXCR2 antagonist (SCH563705)	Very effective	Improved arthritis; reduced bone erosion	71
CXCR2	CAIA	Mouse	CXCR1/CXCR2 antagonist (SCH563705)	Very effective	Improved arthritis; reduced bone erosion	71
CXCR2	K/BxN serum transfer	Mouse	CXCR2 deficiency	Partially effective	Improved arthritis; inhibited neutrophil recruitment into the joints	25,28,38
CXCR3	K/BxN serum transfer	Mouse	CXCR3 deficiency	Not effective	No change	28
	CIA	Mouse	CXCR3 antagonist (JN-2)	Very effective	Improved arthritis; inhibited T cell recruitment into joints	64
	CIA	Mouse	CXCR3 deficiency	Very effective	Ameliorated arthritis; reduced bone destruction	41
CXCR5	CIA	Mouse	CXCR5 deficiency	Very effective	Suppressed arthritis; reduced T follicular helper cell and B cell migration	43
	K/BxN serum transfer	Mouse	CXCR5 deficiency	Not effective	No change	28
CXCR6	CIA	Mouse	CXCR6 deficiency	Mildly effective	Reduced arthritis and T cell infiltration	72
CXCR7	CIA	Mouse	CXCR7 inhibitor (CCX733)	Very effective	Improved arthritis	19
CX_3CR1	K/BxN serum transfer	Mouse	CX_3CR1 deficiency	Mildly effective	Mildly improved arthritis	28
Arthritis (chemokines)						
CCL2	K/BxN serum transfer	Mouse	CCL2 deficiency	Not effective	No change	28
	CIA	Rat	CCL2 mAb	Mildly effective	Ameliorated arthritis; reduced inflammatory cell infiltration into the joint	62
CCL3	K/BxN serum transfer	Mouse	CCL3 deficiency	Not effective	No change	28
CXCL1	K/BxN serum transfer	Mouse	CXCL1 mAb (SA129)[a]	Not effective	No change	63
	K/BxN serum transfer	Mouse	CXCL1 mAb (SA138)[a]	Very effective	Attenuated arthritis; reduced neutrophil recruitment	63
CXCL2	K/BxN serum transfer	Mouse	CXCL1 mAb (SA138)[a]	Very effective	Attenuated arthritis; reduced neutrophil recruitment	63
CXCL3	K/BxN serum transfer	Mouse	CXCL1 mAb (SA138)[a]	Very effective	Attenuated arthritis; reduced neutrophil recruitment	63

Table 1 (cont.) | **Targeting chemokine pathways in animal models of rheumatic diseases**

Target	Model	Animal	Chemokine-targeting drug or chemokine deficiency	Outcome	Notes	Refs
Arthritis (chemokines) (cont.)						
CXCL5	K/BxN serum transfer	Mouse	CXCL1 mAb (SA138)[a]	Very effective	Attenuated arthritis; reduced neutrophil recruitment	63
CXCL10	CAIA	Mouse	CXCL10 deficiency	Partially effective	Ameliorated arthritis; reduced bone erosion	41
CXCL13	CIA	Mouse	CXCL13 mAb (5261)	Very effective	Improved arthritis; reduced bone erosion	27
XCL1	CAIA	Mouse	XCL1 mAb (1A3A)	Very effective	Improved arthritis; reduced bone destruction	46
Systemic lupus erythematosus						
CCR1	NZB/W lupus-prone strain	Mouse	CCR1 antagonist (BL5923)	Mildly effective	Reduced tubulointerstitial and glomerular damage; suppressed $CD4^+$ T cell, monocyte and macrophage recruitment	106
CXCR4	NZB/W lupus-prone strain	Mouse	CXCR4 inhibitor (AMD3100)	Very effective	Delayed proteinuria and improved survival	107
CXCL12	NZB/W lupus-prone strain	Mouse	CXCL12 mAb (1B13A)	Very effective	Improved nephritis and survival	107
	MRL/lpr lupus-prone strain	Mouse	CXCL12 antagonist	Mildly effective	Ameliorated kidney damage	98
	MRL/lpr lupus-prone strain	Mouse	CCL2 antagonist together with CXCL12 antagonist	Very effective	Improved kidney damage; dual treatment was more effective than treatment with either antagonist alone	98
CXCL13	MRL/lpr lupus-prone strain	Mouse	CXCL13 mAb (MAB4701, R&D Systems)	Very effective	Attenuated kidney damage; reduced serum anti-dsDNA titres, renal immune complex deposition and renal cytokine production	104
CCL2	MRL/lpr lupus-prone strain	Mouse	CCL2 antagonist	Mildly effective	Ameliorated kidney damage	98
	MRL/lpr lupus-prone strain	Mouse	CCL2 antagonist together with CXCL12 antagonist	Very effective	Protected from kidney damage; dual therapy was more effective than treatment with either antagonist	98
CX_3CL1	MRL/lpr lupus-prone strain	Mouse	CX_3CL1 polyclonal antibody	Mildly effective	Suppressed kidney damage; reduced T cell, macrophage and monocyte migration	105
Systemic sclerosis						
CCL2	Bleomycin-induced scleroderma	Mouse	CCL2 antibody (Genzyme Techne)	Effective	Reduced collagen content and monocyte recruitment	125
CCL5	Bleomycin-induced scleroderma	Mouse	CCL5 antibody (Genzyme Techne)	Not effective	No change	125
CCR2	Cytokine-induced fibrosis	Mouse	CCR2 deficiency	Partially effective	Reduced collagen content, monocyte recruitment and macrophage recruitment	126
CX_3CR1	Cytokine-induced fibrosis	Mouse	CX_3CR1 deficiency	Very effective	Reduced collagen content, monocyte recruitment and macrophage recruitment	126
Vasculitis						
CCR2	CAWS-induced vasculitis	Mouse	CCR2 deficiency	Very effective	Inhibited monocyte recruitment	143
Idiopathic inflammatory myopathies						
CX_3CL1	EAM	Mouse	CX_3CL1 mAb (5H8–4)	Very effective	Reduced monocyte recruitment	158
CXCL10	CIM	Mouse	CXCL10 mAb	Very effective	Reduced myositis and $CD8^+$ T cell migration	159

AIA, antibody-induced arthritis; CAIA, type II collagen antibody-induced arthritis; CAWS, *Candida albicans* water-soluble fraction; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CIA, type II collagen-induced arthritis; CIM, C protein-induced myositis; CX_3CL1, CX_3C-chemokine ligand 1; CX_3CR1, CX_3C-chemokine receptor 1; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; dsDNA, double-stranded DNA; EAM, experimental autoimmune myositis; mAb, monoclonal antibody; XCL1; XC-chemokine ligand 1. [a]SA138 blocks multiple CXCR2 ligands, whereas SA129 only blocks CXCL1.

in the synovial tissue compared with treatment with a placebo[74]. Also, in a randomized controlled trial of a small-molecule CCR1 antagonist (CCX354-C), a greater proportion of patients reached an ACR20 response at week 12 in the treatment groups (43% or 52% in the groups of patients treated with either 100 mg twice daily or 200 mg once daily, respectively) than in the placebo group (39%)[75]. Similarly, in the clinical trial of a fully humanized anti-CXCL10 antibody (MDX-1100), 54% of the patients treated with both MDX-1100 and methotrexate fulfilled the ACR20 response criteria after 85 days of treatment compared with 17% of patients

treated with placebo and methotrexate[20] (TABLE 2). Finally, the effect of a humanized anti-CX_3CL1 monoclonal antibody (E6011) was also tested in a phase I–II, open-label, multiple ascending dose study in 37 patients with RA who had an inadequate response or intolerance to methotrexate and/or biological agents.

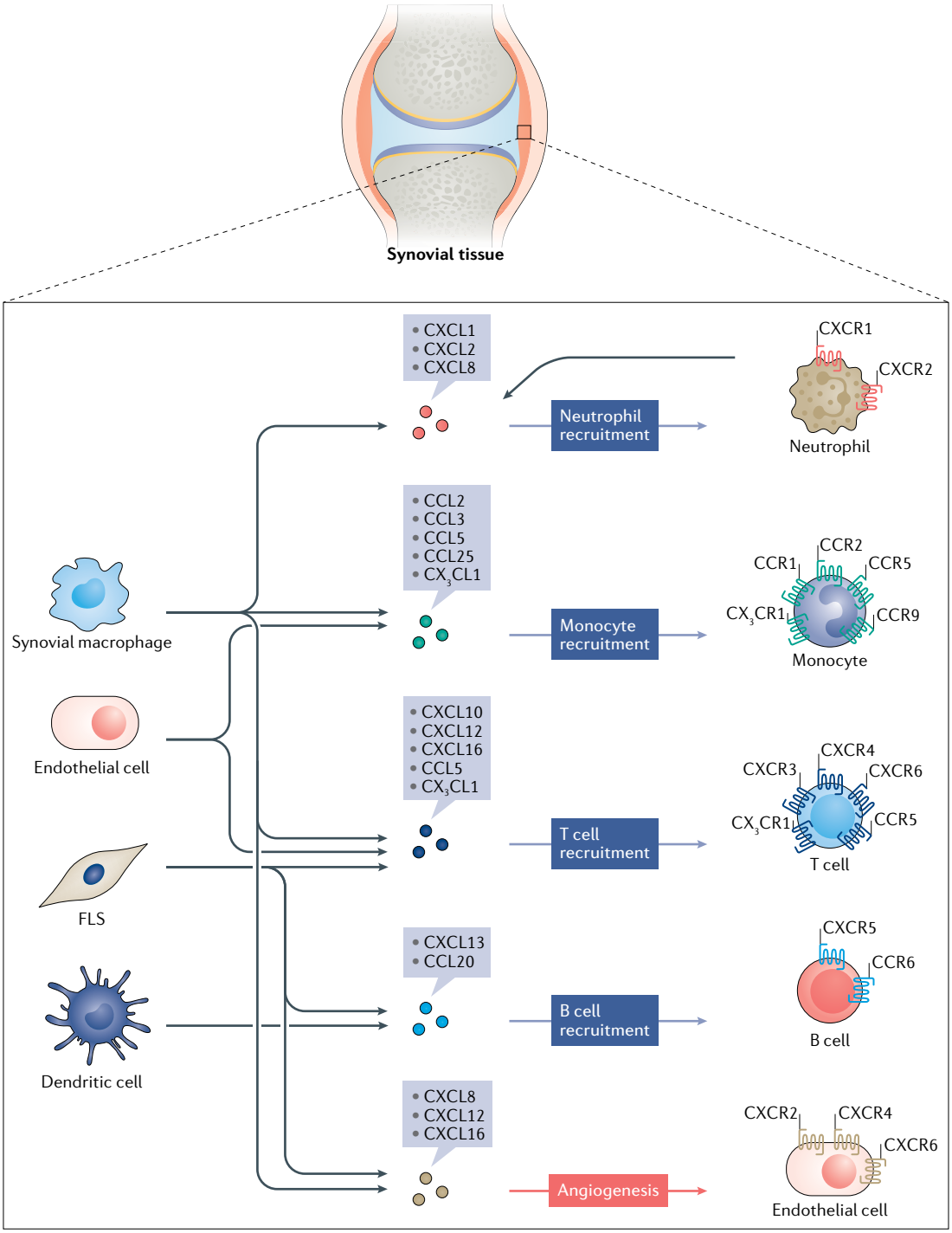

Fig. 1 | **Chemokines and chemokine receptors in RA.** In the synovial tissue in patients with rheumatoid arthritis (RA), synovial macrophages generate CXC-chemokine ligand 1 (CXCL1) and CXCL8, which promotes neutrophil recruitment into the joint. Once neutrophils enter the joint, they become activated and produce CXCL2, which amplifies neutrophil recruitment and stimulates additional chemokine production by macrophages. In RA, synovial macrophages also generate chemokines that promote monocyte recruitment via CC-chemokine receptor 2 (CCR2), CCR5, CCR9 and CX_3C-chemokine receptor 1 (CX_3CR1). Synovial macrophages, endothelial cells and fibroblast-like synoviocytes (FLSs) generate chemokines, which induce T cell trafficking via CXC-chemokine receptor 3 (CXCR3), CXCR4, CXCR6, CCR5 and CX_3C-chemokine receptor 1 (CX_3CR1). FLSs and dendritic cells can also produce CXCL13 and CC-chemokine ligand 20 (CCL20), which promote B cell recruitment via CXCR5. Finally, CXCL8, CXCL12 and CXCL16 produced by synovial macrophage and FLSs induces angiogenesis through CXCR2, CXCR4 and CXCR6, respectively, expressed on endothelial cells.

Table 2 | **Therapeutic effects of blocking chemokines and chemokine receptors in human rheumatic diseases**

Target	Drug (type of drug)	Type of study	Efficacy	Study outcome	Ref.
Rheumatoid arthritis					
CXCL10	MDX-1100 (antibody)	Phase II	Very effective	ACR20 response at week 12 was 54% (MDX1100 and methotrexate) and 17% (placebo and methotrexate)	20
CCL2	ABN912 (antibody)	Phase Ib	Not effective	No clinical improvement	73
CCR1	CP-481,715 (small-molecule inhibitor)	Phase Ib	Mildly effective	Reduced tender and swollen joint count, and macrophage infiltration into the synovial tissue compared with placebo	74
	CCX354-C (small-molecule inhibitor)	Phase II	Mildly effective	ACR20 response at week 12 was 39% (placebo), 43% (CCX354-C; 100 mg twice daily) and 52% (CCX354-C; 200 mg once daily)	75
	MLN3897 (small-molecule inhibitor)	Phase IIa	Not effective	ACR20 response at week 12 was 35% (MLN3897) and 33% (placebo)	76
CCR2	MLN1202 (antibody)	Phase IIa	Not effective	No clinical improvement after 6 weeks of treatment compared with placebo	77
CCR5	SCH351125 (small-molecule inhibitor)	Phase Ib	Not effective	ACR20 response at week 4 was 20% (SCH351125) and 33% (placebo)	78
	AZD5672 (small-molecule inhibitor)	Phase IIb	Not effective	ACR20 response at week 12 was 35% (AZD5672) and 38% (placebo)	79
	UK-427,857 (small-molecule inhibitor)	Phase IIa	Not effective	ACR20 response at week 12 was 23.7% (UK-427,857) and 23.8% (placebo)	80
CX_3CL1	E6011 (antibody)	Phase I/II	Effective? (No placebo)	~60% of treated patients had an ACR20 response at week 12	81
Systemic lupus erythematosus					
CCL2	Bindarit (small-molecule inhibitor)	Phase Ib	Unknown	Reduced proteinuria in patients with acute lupus nephritis	108

CCL2, CC-chemokine ligand 2; CCR, CC-chemokine receptor; CX_3CL1, CX_3C-chemokine ligand 1; CXCL10, CXC-chemokine ligand 10.

After 12 weeks, 75.0%, 33.3% and 8.3% of patients in the 100-mg group, 66.7%, 20.0%, and 13.3% in the 200-mg group, and 60.0%, 30.0%, and 20.0% in the 400-mg group had reached an ACR20 response, ACR50 response and ACR70 response, respectively[81]. Together, these clinical trials suggest that chemokines and their receptors, in particular CCR1, CXCL10 and CX_3CL1, are potentially important targets for new therapies for RA.

The lack of beneficial effects for some chemokine and chemokine receptor inhibitors in clinical trials to date requires closer examination to fully appreciate whether the therapeutic targets were truly inappropriate or whether other factors led to disappointing results and might be worth revisiting. Evidence suggests that the failure of individual inhibitors of CCL2, CCR2 or CCR5 might be because of functional overlap in their contributions to monocyte recruitment into the synovial compartment in patients with RA, whereas blockade of CCR1 alone might be effective but requires very high levels to achieve sustained binding of the inhibitor to the receptor[82]. Before the promising results with the CCR1 inhibitor CCX354-C, an earlier attempt to inhibit CCR1 using a different small-molecule antagonist (MLN3897) was ineffective in the treatment of patients with RA[76]. MLN3897 was less potent and had a shorter half-life than CCX354-C[83], suggesting that the lack of success of MLN3897 was not necessarily because CCR1 is a poor target. Thus, for chemokine inhibitors that have yet to be successful in clinical trials, it is important to determine the pharmacokinetic properties and extent of receptor inhibition before concluding whether inhibiting that particular target in a given disease process is beneficial.

The chemokine system in SLE

SLE is an autoimmune disease that has a predominance in females and affects multiple organs, including the kidney, skin, joint and central nervous system (CNS)[84]. During the course of the disease, immune complexes accumulate in these tissues and induce the infiltration of various types of leukocytes that promote inflammation[84]. Traditional therapies consist of broadly immunosuppressive agents, such as glucocorticoids, antimalarial drugs, cyclophosphamide, azathioprine, methotrexate and mycophenolate mofetil, that can have a range of targets and can result in serious adverse effects[84]. Biologic drugs and small molecules are being investigated with the aim of being more specific in reducing toxicity and improving efficacy compared with currently used treatments.

Two lupus-prone mouse models are commonly used to study the pathogenesis of SLE: NZB/W F1 and MRL/lpr mice. In these models, the mice spontaneously develop manifestations similar to lupus nephritis, arthritis, neuropsychiatric SLE and/or cutaneous SLE[85]. In the NZB/W F1 model, the oldest classical model of SLE, the mice develop an SLE-like phenotype, including lymphadenopathy, splenomegaly,

elevated serum anti-nuclear autoantibodies and anti-double-stranded DNA (dsDNA) antibodies[86,87]. Disease in MRL/lpr mice is caused by a mutation termed lymphoproliferation (*lpr*) that alters transcription of the FAS receptor[88]. Mice in these models have high concentrations of anti-nuclear autoantibodies, anti-single-stranded DNA, anti-dsDNA and anti-Sm antibodies, resulting in large amounts of immune complexes[89]. Chemokines and their receptors have important pathogenic roles in both human SLE and mouse models of SLE (Supplementary Table 1).

Chemokines in SLE

Concentrations of CXCL13 are increased in the serum and kidneys of patients with SLE compared with healthy individuals, and in NZB/W F1 mice compared with control wild-type mice[3,90,91]. In patients with SLE, levels of CXCL13 correlate with disease activity, especially in patients with lupus nephritis, implicating this chemokine as a biomarker of lupus nephritis activity[3,90]. In patients with SLE, increased serum concentrations of CXCL13 are associated with decreased serum concentrations of complement proteins, such as C3 and C4, increased titres of anti-dsDNA antibodies and an increased prevalence of inflammatory arthritis[3]. In NZB/W F1 mice, CXCL13 is mainly produced by renal dendritic cells and its production promotes CXCR5$^+$ B cell recruitment into the kidneys[91].

The cerebrospinal fluid of patients with neuropsychiatric SLE and the kidneys of patients with lupus nephritis contain CXCL12, the concentration of which is associated with disease activity[92]. The serum concentrations of CXCL12 and CXCL10 also positively correlate with disease severity in patients with SLE[93]. Furthermore, the concentrations of CXCL9 and/or CXCL10 are increased in the blood and kidneys of patients with active lupus nephritis compared with healthy individuals[3,94,95]. In NZB/W F1 mice, autoantibodies stimulate the production of CXCL12 by podocytes[96], whereas in MRL/lpr mice tubular epithelial cells are the main source of intrarenal CXCL9 and CXCL10 in lupus nephritis[97]. Although CXCL10 deficiency in MRL/lpr mice does not ameliorate the development of lupus nephritis, CXCL9 deficiency prevents the development of nephrotoxic serum nephritis and diminishes CXCR3$^+$ leukocyte recruitment into the kidney, suggesting that CXCL9 rather than CXCL10 might be more critical for CXCR3-dependent leukocyte trafficking in lupus nephritis[97].

In addition to the CXC chemokines, CC chemokines are also elevated in patients with SLE. In particular, serum levels of CCL2 tend to be higher in patients with SLE than in healthy individuals, but do not correlate with disease activity[3,95,98]. In patients with lupus nephritis, as well as in lupus-prone mice, CCL2 is mainly expressed in the tubulointerstitial region of the kidney[99]. Renal endothelial cells, epithelial cells and infiltrated leukocytes are potential sources of CCL2 and promote CCR2-dependent monocyte recruitment[99]. Urinary concentrations of CCL3 and CCL5 are also increased in patients with lupus nephritis compared with healthy individuals, and production of these chemokines in the kidneys might promote the migration of CCR1$^+$ and CCR5$^+$ macrophages and T cells into the kidney[100].

CX_3CL1 is highly expressed in the glomeruli, the interstitial microvasculature, and the arterial regions of the kidneys in patients with lupus nephritis and in MRL/lpr mice. Levels of CX_3CL1 in the kidneys correlate with disease severity in patients with lupus nephritis, whereas CX_3CR1^+ monocyte infiltrates are detectable in the kidneys of patients with lupus nephritis and in MRL/lpr mice[101,102]. The role of XC chemokines in the pathogenesis of SLE is largely unknown and requires further investigation.

Chemokine receptors in SLE

The high expression of various chemokines in human SLE and in mouse models of SLE implicate specific chemokine receptors in SLE pathogenesis, including CXCR3, CXCR4, CXCR5, CCR1, CCR2, CCR5 and CX_3CR1. CXCR3 and CCR5 mediate T cell accumulation, CXCR5 promotes B cell accumulation, and CCR1, CCR2 and CX_3CR1 regulate monocyte recruitment, and these functions probably contribute to the pathogenesis of SLE and mouse models of SLE[99] (FIG. 2 and Supplementary Table 1). The role of ACKRs in the pathogenesis of SLE remains largely unexplored, although one study reported that the levels of *ACKR3* mRNA in peripheral blood mononuclear cells was lower in patients with SLE than in healthy individuals[103].

Targeting the chemokine system in SLE

Compounds that block chemokine and chemokine receptors have been tested in mouse models of SLE with promising results. Administration of CXCL13-blocking monoclonal antibodies considerably attenuated renal damage and reduced serum anti-dsDNA levels, renal immune complex deposition and inflammatory cytokine secretion in MRL/lpr mice[104]. Blockade of CXCL12 with a monoclonal antibody, or dual blockade of CXCL12 and CCL2 with neutralizing RNA aptamers that bind CXCL12 or CCL2, protected renal function in MRL/lpr mice and NZB/W mice[96,98]. Notably, dual blockade was more efficacious than blockade of either CXCL12 or CCL2 alone with the RNA aptamers in MRL/lpr mice[98]. A CX_3CL1 antagonist (an NH_2 terminally truncated CX_3CL1 analogue) also improved renal function in MRL/lpr mice via suppression of CX_3CR1^+ cell infiltration[105]. Finally, treatment with antagonists of either CCR1 or CXCR4 reduced renal injury and leukocyte recruitment into the kidney in NZB/W F1 mice[106,107]. These data suggest that chemokines and their receptors might be promising targets for new therapies for SLE (TABLE 1).

Compared with data from mice, data from clinical studies are more limited. In one study, inhibition of CCL2 with a small-molecule inhibitor (bindarit) for 24 weeks reduced urinary CCL2 concentrations by 50% and reduced proteinuria by 80–90% in patients with active lupus nephritis compared with treatment with placebo, although this compound probably also inhibits CCL7 and CCL8 as well as CCL2 (REF.[108]) (TABLE 2). Additional clinical trials are required to assess the potential beneficial effects of targeting chemokines and their receptors in SLE.

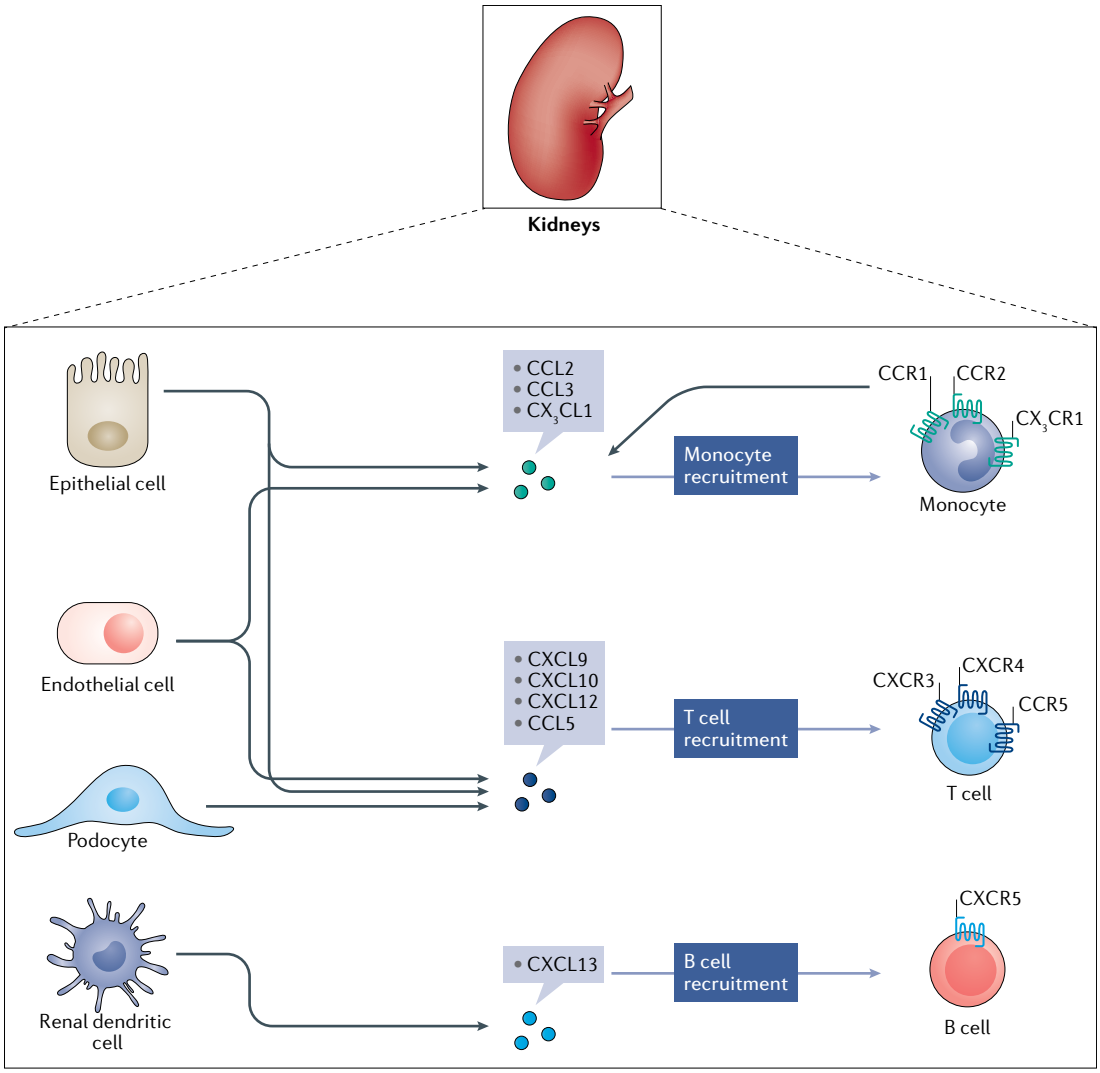

Fig. 2 | **Chemokines and chemokine receptors in systemic lupus erythematosus.** In the kidneys in patients with systemic lupus erythematosus, epithelial cells and endothelial cells produce CC-chemokine ligand 2 (CCL2), CCL3 and CX_3C-chemokine ligand 1 (CX_3CL1), which promote monocyte recruitment via CC-chemokine receptor 1 (CCR1), CCR2 and CX_3C-chemokine receptor 1 (CX_3CR1), respectively. Monocytes also produce these chemokines, which amplifies their recruitment in a feedforward manner. Epithelial cells, endothelial cells and podocytes can also produce chemokines that promote T cell recruitment through CXC-chemokine receptor 3 (CXCR3), CXCR4 and CCR5. Renal dendritic cells can promote B cell recruitment via CXC-chemokine ligand 13 (CXCL13)–CXCR5 signalling.

The chemokine system in SSc

SSc is an autoimmune disease characterized by inflammation, fibrosis and vasculopathy, which leads to a complex pattern of organ-based complications with high mortality and morbidity[109]. Existing treatments include the use of corticosteroids, methotrexate, cyclophosphamide, and mycophenolate mofetil for treating diffuse skin sclerosis and interstitial lung disease in patients with SSc; calcium channel blockers and phosphodiesterase type 5 inhibitors for Raynaud phenomenon (a common complication of SSc); and angiotensin-converting enzyme inhibitors for renal involvement[110]. However, the effectiveness of these therapies in patients with SSc is limited and there remains a pressing need for the development of new targets for the treatment of SSc.

Several mouse models, such as bleomycin-induced scleroderma, the tight-skin (Tsk-1) mouse model and the sclerodermatous chronic graft-versus-host disease mouse model, have been studied to better understand the pathophysiology of SSc, with bleomycin-induced scleroderma being the most commonly used model[111]. Bleomycin induces the production of extracellular matrix proteins and fibrogenic cytokines, such as transforming growth factor-β (TGFβ), by skin fibroblasts, which might contribute to the induction of fibrosis. Evidence suggests that chemokines and their receptors have an important function in leukocyte trafficking and in the development of fibrosis in these models and in SSc pathogenesis[110,111].

Chemokines in SSc

Several chemokines are highly expressed in patients with SSc (Supplementary Table 1). In particular, CCL2 is thought to have a prominent role in the pathogenesis

of SSc and is a promising therapeutic target[110]. CCL2 is highly expressed in the plasma and serum of patients with SSc compared with healthy individuals[112,113]. Notably, serum concentrations of CCL2 are higher in patients with early diffuse cutaneous SSc than in patients with late diffuse cutaneous SSc, suggesting that CCL2 has an important role in the early phase of SSc[112]. Probable sources of CCL2 in the early phase of SSc include local production by skin fibroblasts, endothelial cells and macrophages[114]. Interestingly, CCL2 can promote the production of matrix metalloproteinases by $CCR2^+$ skin fibroblasts in vitro, which, in turn, can promote the production of extracellular matrix proteins and exacerbate skin fibrosis[111,115].

Concentrations of CCL3 are increased in the dermal blister fluid, but not in the plasma, of patients with SSc compared with that in healthy individuals[4]. Other CCL chemokines (CCL4 and CCL5) are also increased in the plasma of patients with SSc compared with healthy individuals[4,116]. In this disease setting, monocytes and dendritic cells produce CCL4 whereas keratinocytes produce CCL5 (REFS[116,117]). CCL18 is also present at high levels in the serum of some patients with SSc-associated idiopathic lung disease, which correlates with progressive disease[118].

However, data on CC chemokines remains correlative, and how they contribute to the pathogenesis of SSc remains unknown. In mouse models of SSc, CCL1, CCL3, CCL8, CCL17, and CCL22 are highly expressed in the skin[119], but the function of these chemokines in SSc is still unclear. An important issue to address is which chemokines are important in the later phases of SSc and which chemokines (such as CCL2) are more important in the early phase of disease. Among the CXC family of chemokines, concentrations of CXCL3, CXCL4, CXCL6, CXCL8, CXCL9, CXCL10, CXCL11 and CXCL16 are increased in the serum or plasma of patients with SSc compared with healthy individuals[116,120–122]. In particular, CXCL4 is the predominant protein secreted by plasmacytoid dendritic cells in the circulation and in the inflamed skin of patients with SSc[122] and its plasma concentrations correlate with the presence and progression of lung fibrosis and pulmonary atrial hypertension[122]. Serum concentrations of CXCL10 and CXCL11 are increased in patients with early SSc compared with healthy individuals, and are strongly associated with disease activity[123]. In patients with SSc, the main sources of CXCL10 and CXCL8 are monocytes and dendritic cells[116] whereas CX_3CL1 is produced by skin endothelial cells, for which serum levels of CX_3CL1 are associated with the severity of sclerosis[124].

Chemokine receptors in SSc

Monocytes from the skin of patients with SSc express increased amounts of CCR2 and CX_3CR1 compared with those from healthy individuals, and this increased expression correlates with increased CCL2 production by skin fibroblasts and increased CX_3CL1 production by skin endothelial cells[115,125,126] (FIG. 3). In mouse models of SSc, CCR4 and CCR8 control $CD4^+$ T cell recruitment into the skin whereas CCR1 regulates $CD11b^+$ monocyte recruitment into the skin[119] (FIG. 3). In addition, endothelial cells in the skin of patients with SSc express high levels of CXCR2 and CXCR6 (REF.[120]). In general, CXCR2 and CXCR6 on endothelial cells are thought to be pro-angiogenic chemokine receptors. However, chemokine-induced signalling is impaired in endothelial cells from patients with SSc, suggesting that defective angiogenesis resulting from impaired chemokine receptor signalling might contribute to the vasculopathy seen in SSc[120].

Targeting the chemokine system in SSc

No clinical trials have yet been attempted to assess drugs that directly target chemokines and/or their receptors in patients with SSc. However, studies in mouse models of SSc have provided proof-of-principle evidence that targeting the CCL2–CCR2 or CX_3CL1–CX_3CR1 axes can reduce disease severity[126]. In fact, an anti-CCL2 monoclonal antibody, but not an anti-CCL5 monoclonal antibody, ameliorated skin fibrosis in mice with bleomycin-induced scleroderma[125]. Deletion of either CCR2 or CX_3CR1 also reduced skin fibrosis in a mouse model of TGFβ-induced SSc[126]. Hence, data from mouse models suggest that the CCL2–CCR2 and the CX_3CL1–CX_3CR1 chemokine axes promote TGFβ production and monocyte recruitment into the skin (FIG. 3), and blockade of these interactions could be a potential strategy for new therapies for SSc.

Other rheumatic diseases

The chemokine system in vasculitis

Vasculitis is a term used to describe a large group of disorders characterized by inflammation and destruction of the blood vessels. The diseases can be grouped according to the size of vessels affected: small vessels (such as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)), medium vessels (such as Kawasaki disease) and large vessels (such as Takayasu disease and giant cell arteritis (GCA))[127]. Commonly used treatments for vasculitis include corticosteroids and immunosuppressive drugs. However, immunosuppression can often lead to clinical complications and vasculitis in some patients is refractory to these drugs. Hence, new therapies for vasculitis are needed that are safer and more effective than currently used treatments.

Several experimental models of vasculitis, such as *Polyoma* virus infection-induced vasculitis, ANCA-induced vasculitis and *Lactobacillus casei*-induced vasculitis, are available, but these models of vasculitis can only be induced in mice with particular genetic backgrounds[128]. Injection of *Candida albicans* water-soluble fraction (CAWS) into mice leads to inflammation of the aortic root and coronary arteries and has been used as a model for Kawasaki disease. CAWS-induced vasculitis can be induced in a variety of genetic backgrounds and therefore is a more versatile model than other animal models for studying the pathogenesis of vasculitis[128,129]. In CAWS-induced vasculitis, $Ly6G^+$ neutrophils, $F4/80^+$ macrophages and $CD4^+$ T cells infiltrate sites of inflammation. Inhibition of neutrophil recruitment can ameliorate vasculitis, suggesting that neutrophils are important drivers of inflammation in this model[129].

Several chemokines are increased in the plasma and/or serum of patients with vasculitis and correlate with

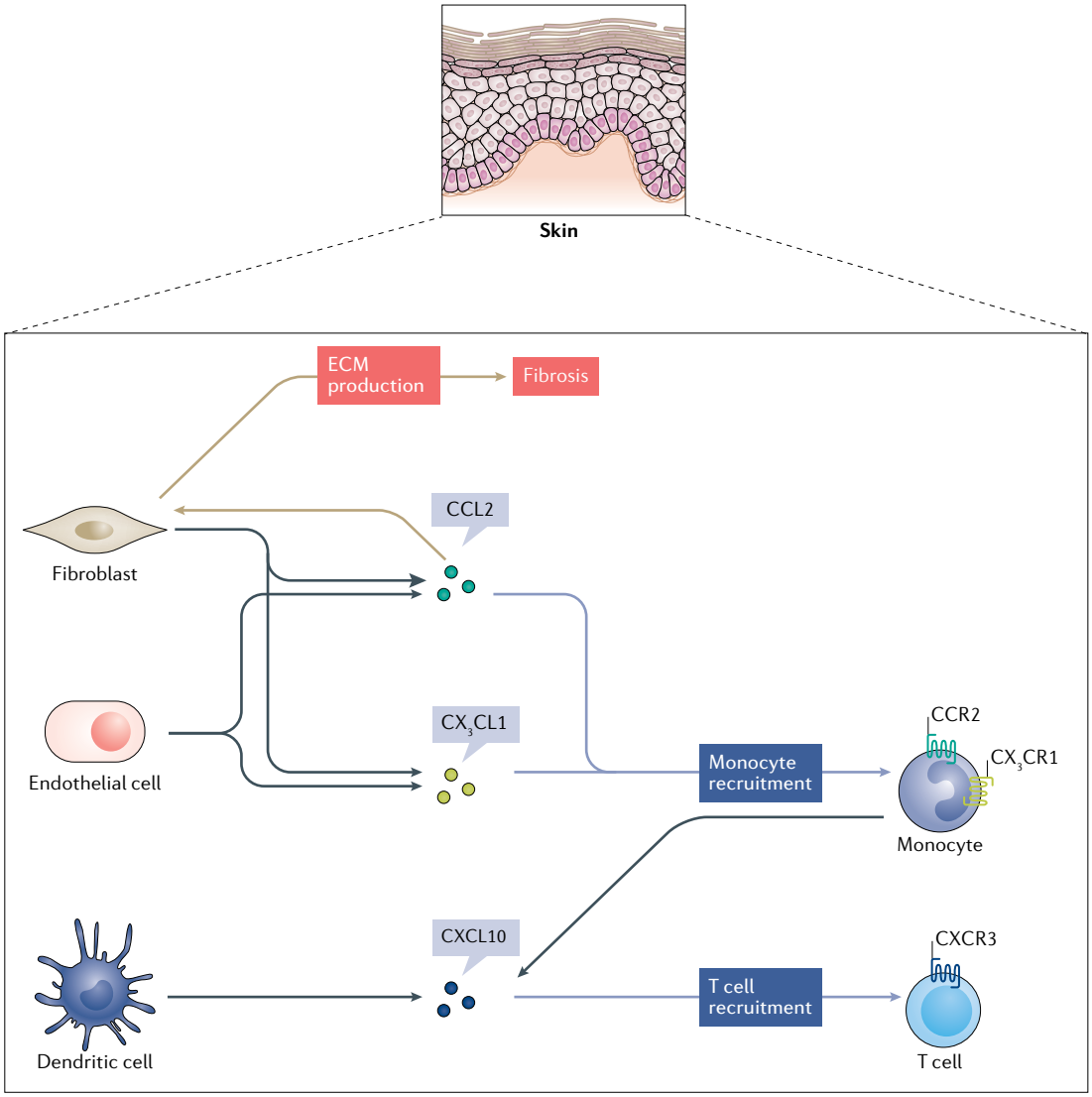

Fig. 3 | **Chemokines and chemokine receptors in systemic sclerosis.** In the skin in patients with systemic sclerosis, fibroblasts and endothelial cells release CC-chemokine ligand 2 (CCL2) and CX_3C-chemokine ligand 1 (CX_3CL1), which promotes monocyte recruitment through CC-chemokine receptor 2 (CCR2) and CX_3C-chemokine receptor 1 (CX_3CR1). Furthermore, monocytes and dendritic cells produce CXC-chemokine ligand 10 (CXCL10), which induces T cell infiltration via CXC-chemokine receptor 3 (CXCR3) signalling. Autocrine signalling in fibroblasts by CCL2 induces the production of matrix metalloproteinases leading to extracellular matrix (ECM) production and fibrosis.

disease activity (for example, CCL17 (REF.[130]), CCL18 (REF.[131]), CCL20 (REF.[132]), CXCL8 (REF.[5]), CXCL9 (REF.[5]), CXCL10 (REF.[5]), CXCL11 (REF.[5]), CX_3CL1 (REF.[133]) and XCL1 (REF.[134]) in AAV; CCL2 (REF.[135]) and CCL5 (REF.[136]) in GCA; CCL2 (REF.[136]), CXCL9 (REF.[137]), CXCL10 (REF.[137]), CXCL11 (REF.[137]) and CX_3CL1 (REF.[138]) in Takayasu disease; and CCL17 (REF.[139]), CXCL9 and CXCL10 (REFS[53,139]) in Kawasaki disease) (Supplementary Table 1). The serum concentrations of CX_3CL1 in patients with AAV, but not in patients with Takayasu disease or GCA, are increased compared with healthy individuals and are positively associated with disease activity[133]. In addition, CCL2, CCL7, CXCL2, CXCL3, CXCL9 and CXCL10 are highly expressed in the aortic root and coronary arteries of mice with CAWS-induced vasculitis[140,141]. In GCA, tissue-resident dendritic cells in the adventitia of affected arteries could be major producers of chemokines in the early phases of disease onset, whereas vascular smooth muscle cells and inflammatory monocytes recruited into the artery generate chemokines during the later phases[142] (FIG. 4). However, the main sources of chemokines in other forms of human vasculitis are unknown.

Pronounced levels of infiltrating $CCR2^+$ monocytes, CX_3CR1^+ monocytes and $CXCR3^+CCR6^+CD8^+$ T cells, along with high levels of the ligands for these receptors, are detectable in temporal artery biopsy samples derived from patients with active GCA[137,138] (FIG. 4). In addition, the expression of CXCR2 and CCR2 in the aortic root and coronary arteries are increased in mice with CAWS-induced vasculitis compared with control mice. In *Ccr2*-knockout mice, the development of CAWS-induced vasculitis is attenuated, suggesting that chemokines and their receptors are attractive targets for new vasculitis treatments[143,144]. Lessons might also be learnt from other

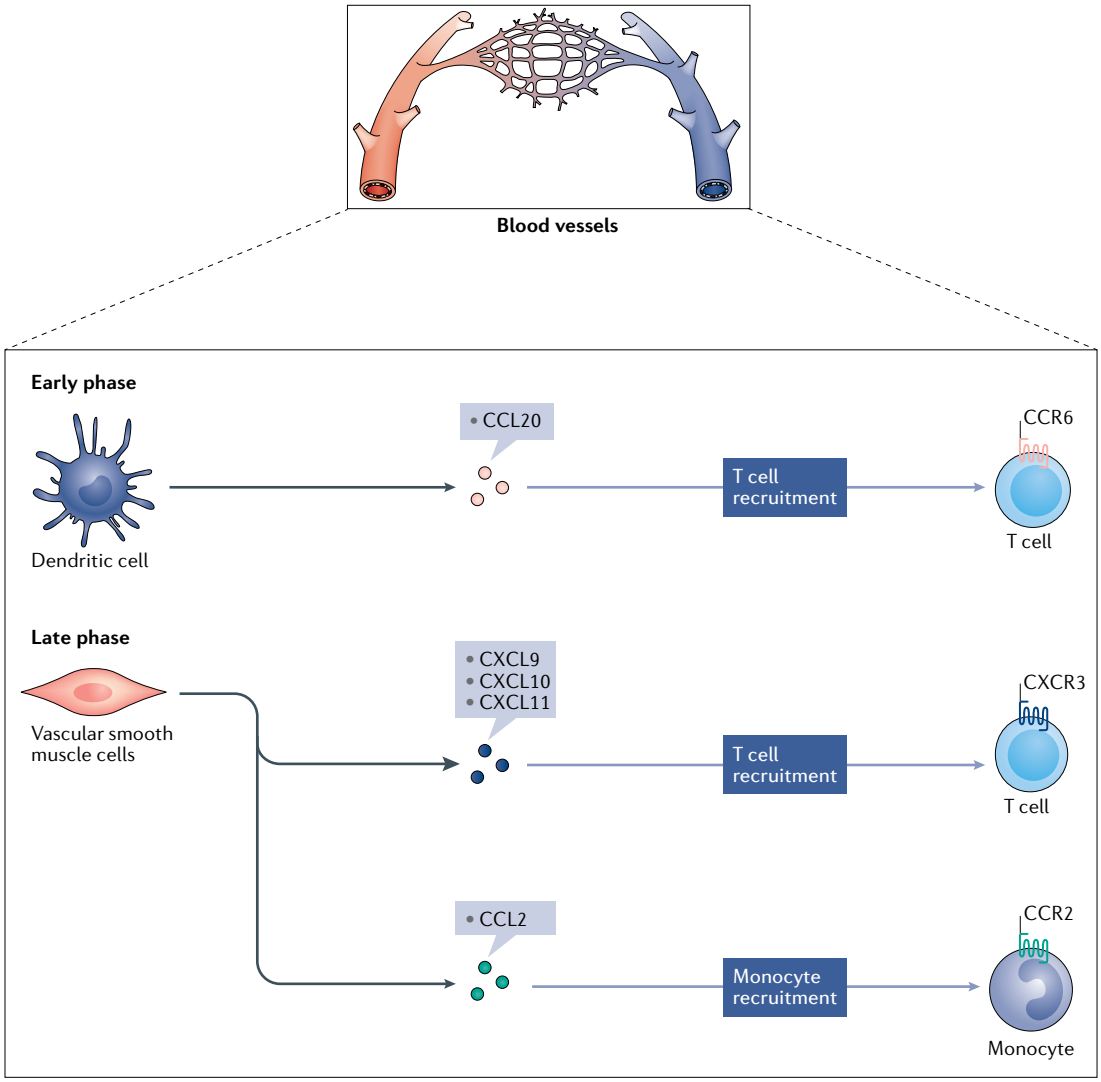

Fig. 4 | **Chemokines and chemokine receptors in giant cell arteritis.** In the early phase of giant cell arteritis, tissue-resident dendritic cells in the adventitia of the affected blood vessels produce CC-chemokine ligand 20 (CCL20), which promotes CC-chemokine receptor 6 (CCR6)$^+$ T cell infiltration. In the late phase, vascular smooth muscle cells in the tunica media produce CXC-chemokine ligand 9 (CXCL9), CXCL10 and CXCL11, which attracts CXC-chemokine receptor 3 (CXCR3)$^+$ T cells. Vascular smooth muscle cells also produce CCL2 resulting in CCR2$^+$ monocyte recruitment.

therapeutic approaches that can inform the development of chemokine-targeting therapies in vasculitis. For example, a small-molecule inhibitor of C5aR1 (CCX168) was effective in replacing high-dose glucocorticoids in the treatment of patients with AAV[145]; given the role of this receptor and its ligand (C5a) in regulating neutrophil function[146], targeting neutrophil recruitment through inhibition of chemokines and/or chemokine receptors, such as the CXCL8–CXCR1/2 axis, could be a promising therapy for AAV. However, the effect of inhibiting chemokines and chemokine receptors in human vasculitis or animal models has not been well tested, and additional studies are needed.

The chemokine system in IIM

IIM, a group of systemic autoimmune disorders that include polymyositis and dermatomyositis, is characterized by muscle inflammation and muscle weakness[147]. Despite sharing some histological features (such as the presence of T cells and macrophage infiltrates in muscle biopsy samples), polymyositis and dermatomyositis are histologically different. In polymyositis, CD8$^+$ T cells are the predominant muscle fibre infiltrates, whereas CD4$^+$ T cells predominantly infiltrate the muscle fibres in dermatomyositis[148,149]. Glucocorticoids and other immunosuppressive drugs are commonly used to treat dermatomyositis and polymyositis. Biologic drugs, such as TNF inhibitors, have also been tested for the treatment of patients with polymyositis and/or dermatomyositis, but the efficacy of other biologic agents requires testing and the development of new therapies is needed[147].

Experimental autoimmune myositis (EAM) and C protein-induced myositis (CIM) have been used as mouse models of myositis. SJL/J mice have a dysferlin gene mutation that causes spontaneous muscle necrosis and secondary muscle inflammation[150]. EAM is

inducible in SJL/J mice by repeated administration of muscle homogenate or partially purified myosin. CIM is induced in C57BL/6 mice with a single injection of recombinant skeletal muscle fast-type C protein[151]. In EAM, muscle T cell infiltrates are predominantly $CD4^+$ T cells whereas $CD8^+$ T cells predominantly infiltrate the muscle in CIM, suggesting that CIM is more similar to polymyositis than EAM[151,152].

Several chemokines are highly expressed in the muscles of patients with IIM (Supplementary Table 1), including CXCL9 and CXCL10. CXCL10 is abundantly expressed in the muscle in patients with polymyositis or dermatomyositis[153]; furthermore, in patients with dermatomyositis, serum concentrations of CXCL10 are associated with disease activity[154]. In polymyositis, CXCL10 is produced by $CD68^+$ macrophages, $CD4^+$ T cells and $CD8^+$ T cells, whereas in dermatomyositis $CD4^+$ T cells and $CD68^+$ macrophages produce CXCL10 (REF.[155]). In areas of severe inflammation in the muscles of patients with polymyositis (but rarely in patients with dermatomyositis), some myofibres express CXCL10 (REF.[156]). Furthermore, some $CD68^+$ macrophages and $CD8^+$ T cells also produce CXCL9 in polymyositis[155].

CCL2, CCL3, CCL4 and CX_3CL1 are also highly expressed in the muscles of patients with IIM[153,157]. In dermatomyositis, endothelial cells are the main CCL2-producing cell type whereas macrophages and cytotoxic T cells might be the major cellular sources of CCL2 and CX_3CL1 in patients with polymyositis[153]. CX_3CL1 is mainly produced by macrophages and endothelial cells in the muscles of patients with IIM[157]. High numbers of $CXCR3^+$ T cells, $CCR2^+$ monocytes and CX_3CR1^+ macrophages are present in the muscle of patients with IIM as well as mouse models of myositis[158–160] (FIG. 5). However, the main source of other chemokines in IIM remains unknown.

Therapies targeting chemokines and their receptors have yet to be tested in patients with IIM. In mouse models of myositis, an anti-CXCL10 monoclonal antibody reduced inflammatory cell infiltration into the muscle of mice with CIM[159], and an CX_3CL1 monoclonal antibody ameliorated disease in mice with EAM[158]. These results suggest that CXCL10 and CX_3CL1 might be promising targets for new therapies for IIM.

Conclusions

Numerous chemokines and their receptors are involved in the recruitment of leukocytes into inflamed organs in rheumatic diseases and are thus promising targets for therapeutic intervention. Blockade of the chemokine

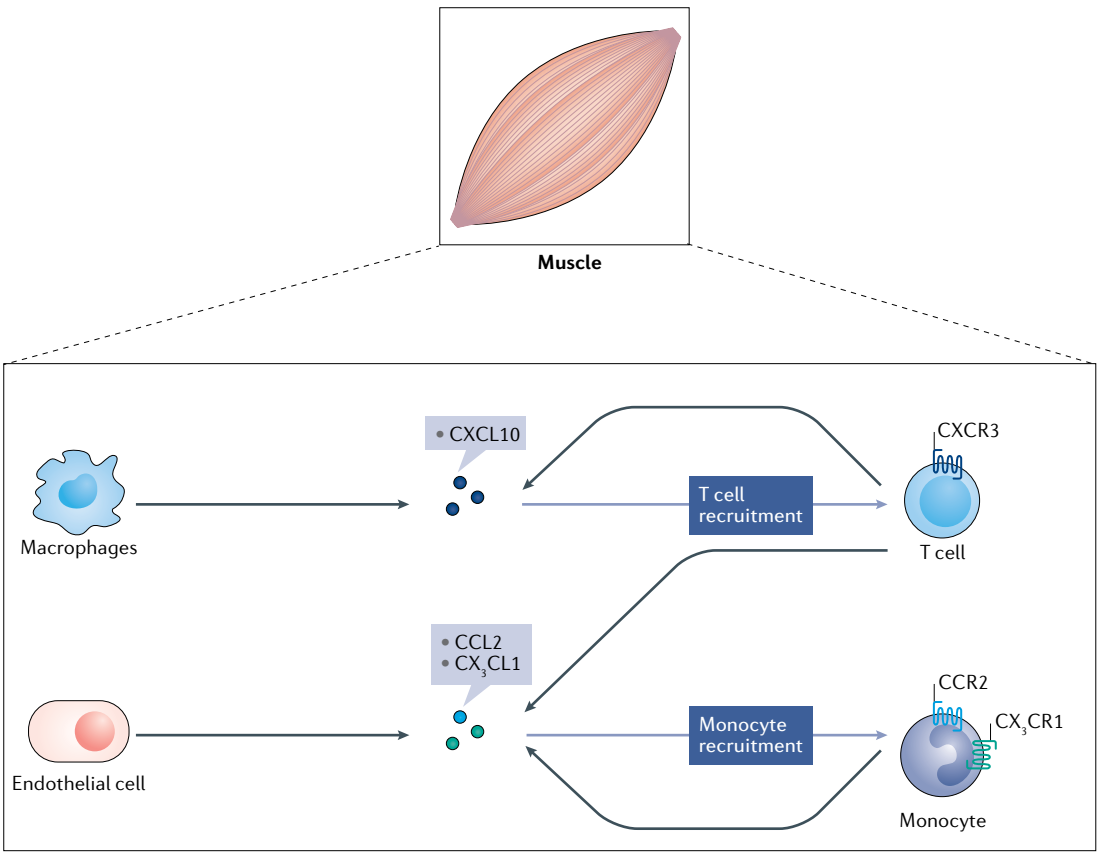

Fig. 5 | **Chemokines and chemokine receptors in idiopathic inflammatory myositis.** In idiopathic inflammatory myositis, CXC-chemokine receptor 3 $(CXCR3)^+$ T cells are recruited to the muscle in response to CXC-chemokine ligand 10 (CXCL10), which can be produced by T cells and macrophages. Furthermore, T cells and endothelial cells generate CC-chemokine ligand 2 (CCL2) and CX_3C-chemokine ligand 1 (CX_3CL1), which induce CC-chemokine receptor 2 $(CCR2)^+$ monocyte and CX_3C-chemokine receptor 1 $(CX_3CR1)^+$ monocyte recruitment into the muscle. Monocytes also produce these chemokines and promote additional recruitment in a feedforward manner.

system ameliorates inflammation in multiple animal models of rheumatic diseases, whereas in clinical trials of patients with rheumatic diseases (such as RA) targeting the most relevant receptor and ensuring high receptor occupancy at all times might be needed for therapeutic benefit. Functional overlap between many chemokine systems involved in leukocyte trafficking also adds to the challenge as inhibition of a single chemokine system alone might not be sufficient to completely suppress leukocyte recruitment. More effective approaches might involve targeting multiple chemokines and/or chemokine receptor systems, as indicated by studies in mouse models[25,63]. In addition, combining chemokine system blockade with therapies that target other pathways might be another approach worth evaluating for the treatment of rheumatic diseases. An important observation that requires further understanding is that different chemokines might be important at different stages of pathogenesis depending on the rheumatic disease. For example, one study that used multiphoton intravital microscopy to observe neutrophil entry into the joint in the K/BxN model found that, although both the classical C5a receptor, C5aR1, and atypical C5a receptor, C5aR2, are required for initial integrin-dependent arrest of neutrophils on the joint endothelium, these receptors were not involved in inducing neutrophil transendothelial migration[38,60]; by contrast, CXCR2 and ACKR1 were required for neutrophil diapedesis into the joint space. These studies point to a need to fully dissect the functional roles of chemokines and their receptors in the pathogenesis of rheumatic diseases. A considerable need remains for the treatment of rheumatic diseases. Promising results from clinical studies provide reasons to be optimistic and suggest that the development of more effective inhibitors of chemokines and their receptors has untapped therapeutic potential.

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The IL-23–IL-17 pathway as a therapeutic target in axial spondyloarthritis

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Abstract | The cytokines IL-23 and IL-17 have an important role in the pathogenesis of, and as a therapeutic target in, both animal models of chronic inflammation and some human chronic inflammatory diseases. The traditional view is that a main source of IL-17 is T cells and that IL-17 production is under the control of IL-23. IL-17 inhibition has shown good efficacy in clinical trials for ankylosing spondylitis (AS), a subtype of axial spondyloarthritis (axSpA) characterized by radiographic evidence of sacroiliitis. On the basis of data from animal models, genetic studies and the investigation of tissue and blood samples from patients with AS, IL-23 had also been predicted to be important in the pathogenesis of this disease and was therefore considered a potential therapeutic target for axSpA. However, two placebo-controlled, double-blind clinical trials in axSpA of monoclonal antibodies directed against either the p40 protein or the p19 protein of the IL-23 molecule had clear negative results. These findings indicate that IL-23 and IL-17 are at least partly uncoupled in axSpA. Reasons as to why, when and how such an uncoupling might occur are discussed in this Review, with special reference to the unique microenvironment of the subchondral bone marrow in axSpA.

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that predominantly affects the axial skeleton. The term axSpA covers both patients in the early phase of the disease who have inflammation but have not yet developed structural bony damage that is visible on X-ray imaging (termed non-radiographic axSpA), and patients with X-ray-detectable changes to the sacroiliac joints (SIJs) and possibly also to the spine (termed radiographic axSpA; also known as ankylosing spondylitis (AS))[1]. Most of the data on the IL-23–IL-17 pathway discussed in this Review come from patients with radiographic axSpA (or AS); therefore, the discussion will focus on AS. However, the discussion points can probably be extrapolated to all forms of axSpA.

Some evidence exists for a role for the interaction between genetic predisposition and exposure to bacteria in the pathogenesis of spondyloarthritis; for example, through damage to the barrier properties of the skin (psoriasis) or the mucosa (inflammatory bowel disease), or as a consequence of gastrointestinal or urogenital infections[1]. However, the strong association between musculoskeletal manifestations in axSpA and this barrier dysregulation might either be caused by altered exposure to bacteria or be because common genetic risk factors are responsible for inflammation in the skin, gut and joints. Despite some advances in our understanding of the genetic, cellular and molecular mechanisms involved in the pathogenesis of axSpA being made in the past few decades, none of the most recent insights has resulted in new treatments[2]. Therapeutic options for axSpA had previously been restricted to NSAIDs and TNF inhibitors[3], mostly as the result of clinical experience or clinical research and not on the basis of preclinical data. Interestingly, clinical trials targeting other elements of the immune system, such as IL-6, IL-1, T cells or B cells, have produced negative or unconvincing results in AS[3].

In the past few years, the cytokines IL-23 and IL-17 have been shown to have an important role in the pathogenesis of, and as therapeutic targets in, animal models of chronic inflammation and have also been investigated in great detail in human chronic inflammatory diseases[4,5]. IL-17 secretion was postulated to be under the control of IL-23; thus, similar responses to IL-17 inhibition and IL-23 inhibition were expected in human diseases. Indeed, inhibitors for each of these molecules had similar, somewhat negative, results in rheumatoid arthritis (RA)[6,7], moderately positive results in psoriatic arthritis (PsA)[8–10] and very good results in psoriasis[11,12]. Therefore, these trial results supported the idea of a coupling between IL-23 and IL-17. However, the idea that the relationship between IL-23 and IL-17 was not as straightforward as previously assumed had already been

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Key points

- The IL-17 pathway has an important role in the pathogenesis of axial spondyloarthritis (axSpA); IL-23 is thought to be involved too as it stimulates the production of IL-17.
- IL-17 inhibitors and TNF inhibitors are currently the only effective and approved biological DMARDs for axSpA.
- IL-23 inhibitors are effective against psoriasis and psoriatic arthritis, but not against axSpA; however, IL-23 inhibitors might have an effect on peripheral enthesitis in axSpA, which should be investigated further.
- On the basis of negative trial data, a role for IL-23 in the pathogenesis of axSpA is uncertain.
- Whether IL-17 inhibitors have an effect on new bone formation in axSpA has still to be clarified, but an effect of IL-23 inhibitors is unlikely.
- The discrepancy in efficacy between effective IL-17 inhibitors and non-effective IL-23 inhibitors in axSpA is unique among immune-mediated diseases and might be explained by an uncoupling of IL-17 and IL-23.

suggested by the moderate efficacy of IL-23 inhibition in Crohn's disease[13,14] and the poor efficacy, or even worsening of disease, if these patients were treated with an IL-17 inhibitor[15].

Given this background, it was a surprise when the results of two placebo-controlled trials in AS, one with ustekinumab (which binds to the p40 subunit of IL-23 that is shared with IL-12)[16] and one with risankizumab (which inhibits the p19 subunit of IL-23)[17], did not show effects for the IL-23 blocking agents above those of placebo on disease activity, despite IL-17 inhibitors being quite effective for the treatment of AS[18,19]. These findings have prompted the question of whether (and in what way) the IL-23–IL-17 pathway is a potential target for the treatment of axSpA, which is discussed in this Review.

Primary immunopathology of axSpA

The primary immunopathology in axSpA seems to be unique among inflammatory rheumatic diseases. The pathological processes related to the disease are particularly confined to the interfaces between tendons or ligaments and bone (known as entheses), and the interface between cartilage and bone, predominantly in the SIJs and the spine[20–22] (TABLE 1). However, in contrast to RA, the joint synovium is not an important site of inflammation in axSpA[1,22]. Detailed immunohistological data about local inflammation are difficult to obtain in axSpA because the affected structures are challenging to biopsy. Therefore, it cannot be excluded that the different affected structures (for example, not only bone but also tenosynovium and bursa can be involved in peripheral enthesitis) might behave differently in response to therapies such as IL-23 blockade.

Current evidence suggests that, in axSpA, subchondral bone marrow is replaced by an inflammatory fibroblast-rich granulation tissue that erodes the subchondral bone plate, but also has bone-forming capabilities[23]: signals for new bone formation are probably sent out from this kind of repair tissue. This process is currently best seen on MRI; subchondral inflammation in the bone marrow is visible on T2-weighted sequences with fat suppression (TABLE 1) and the further development of repair tissue is visible as so-called fatty tissue on T1-weighted sequences[1]. Peripheral arthritis and/or synovitis can also occur in axSpA, but are rarely dominant and often transient[24]. In terms of pathophysiology, PsA is located somewhere between axSpA and RA, presenting with a mixture of peripheral arthritis, enthesitis and spinal involvement[22].

Conventional treatments such as glucocorticoids and conventional synthetic DMARDs (csDMARDs; including methotrexate and sulfasalazine) are not effective for axial disease or enthesitis[25]. Therefore, the treatment recommendations for axSpA[25] and PsA[26,27] do not include the use of glucocorticoids or csDMARDs for axial manifestations or enthesitis, but recommend the use of biologic DMARDs once NSAID treatment has failed. Thus, histological results, MRI findings and the efficacy or inefficacy of certain treatments indicate that the inflammatory process in the subchondral bone marrow that occurs in axSpA (including peripheral enthesitis) is unique, a fact that should be taken into account when considering the discrepant efficacy of IL-23 inhibitors and IL-17 inhibitors in axSpA.

The IL-23–IL-17 pathway in axSpA

IL-17 and IL-23 biology

IL-17 was discovered in 1996, several years before IL-23, and was characterized by its effects on synoviocytes, which produced IL-6 and IL-8 in response to IL-17 (REF.[28]). The type of IL-17 that was first described, now called IL-17A, is the first member of the IL-17 family. The next closest family member is IL-17F, which shares 50% homology with IL-17A; IL-17A and IL-17F exist as homodimers but can also form an IL-17A–IL-17F heterodimer[29]. IL-17A is usually more potent than IL-17F, but both cytokines can increase the effects of other cytokines such as TNF[30].

IL-17 was first identified as a gene product of T cells[28], and it was later shown that IL-17 was produced by a subset of T cells, which were renamed T helper 17 (T_H17) cells[31]. Further studies showed that IL-17 can be produced by many types of cells, including other innate and adaptive immune cells such as $CD8^+$ T cells, γδ T cells, type 3 innate lymphoid cells (ILC3s) and natural killer T cells[32]. Although staining positively for IL-17, neutrophils and mast cells do not seem to express IL-17 mRNA, but instead can store exogenous IL-17 (REFS[33,34]).

IL-23 is a member of the IL-12 cytokine family. Both IL-23 and IL-12 are heterodimers, consisting of a common p40 chain with the addition of a p35 chain for IL-12 and a p19 chain for IL-23. IL-12 is important in T_H1-cell-mediated responses as it induces IFNγ production. By contrast, IL-23 is important in T_H17 cell-mediated responses as it induces IL-17A, IL-17F, IL-21 and IL-23 production[35]. Both IL-12 and IL-23 are produced in large amounts by all antigen-presenting cells, although most comes from dendritic cells, monocytes and macrophages. The contribution of IL-23 to any disease thus depends on the contribution and/or presence of these IL-23-producing cells[35].

Preclinical data in axSpA

Over the past few years, many reviews[2,36,37] on the pathogenesis of SpA have highlighted the IL-23–IL-17 pathway as relevant on the basis of preclinical data. For example, the number of IL-17-positive cells (only a small proportion of which were T cells) in subchondral bone marrow from the spine of patients with AS was

increased compared with tissue samples from the spines of patients with osteoarthritis[38]; similar results were also reported for IL-23-positive cells in spinal subchondral bone marrow in patients with AS[39]. Given the strong association of axSpA with HLA-B27 (REF.[1]), it is of interest that intracellular misfolding of the HLA-B27 molecule has also been reported to stimulate the IL-23–IL-17 pathway in vitro[40]. Furthermore, case–control genome-wide association studies demonstrated that, in addition to other polymorphisms (discussed in detail in REF.[41]), an *IL23R* polymorphism is associated with AS[42]. Polymorphisms in *IL23R* are also associated with psoriasis and Crohn's disease, and overexpression of IL-23 in mice induced a form of enthesitis that resembled the enthesitis that occurs in SpA[43].

The effects of the IL-23–IL-17 pathway on bone in SpA have been reviewed elsewhere[37]. Briefly, the direct effects of these two cytokines on osteoclasts and on bone resorption suggest that they might have a catabolic effect on bone. However, the potential effects of IL-17A on osteoblast differentiation probably depend on the cell type exposed, the differentiation stage of that cell and perhaps also the timing and duration of cytokine exposure. However, IL-23 does not seem to have an effect on osteoblast activation[37].

Clinical trial data in axSpA

IL-17 blockade. Clinical trials performed with the IL-17-blocking monoclonal antibodies secukinumab[18], ixekizumab[19], bimekizumab[44] and netakimab[45] have clearly shown the superiority of IL-17 inhibitors over placebo in TNF inhibitor-naive patients with axSpA, and secukinumab and ixekizumab were also superior to placebo in TNF inhibitor-experienced patients[46,47]. These trials[18,19,44,45] produced a difference in 40% improvement in Assessment of Spondyloarthritis International Society criteria (ASAS40) responses between treatment and placebo of about 25–30% in TNF inhibitor-naive patients, which is a response rate similar to that seen in previous TNF inhibitor trials[3] (TABLE 2). Furthermore, the COAST-V trial[19] of ixekizumab in TNF inhibitor-naive patients with AS included an active reference group of patients who received 40 mg of adalimumab. Although this trial was not powered to show the non-inferiority or superiority of ixekizumab versus adalimumab, the results showed that ixekizumab was at least as good as adalimumab in these individuals[19]. Similar to previous trials of TNF inhibitors, high dosages of some of the IL-17 inhibitors (as are often used in psoriasis) were not clearly better than moderate doses[19,47,48]. Overall, there can be no doubt that IL-17 inhibition is an effective treatment for AS. Studies in individuals with non-radiographic axSpA are currently ongoing[49,50].

IL-23 blockade. The good response of patients with AS to IL-17 inhibitors contrasted with the surprisingly negative outcomes of trials of IL-23 inhibitors (TABLE 2), which has raised questions as to whether there were any flaws in the study designs. The TNF inhibitor-naive patients with AS included in the ustekinumab (which also blocks IL-12)[16] and risankizumab[17] trials were well-selected with regard to baseline characteristics such as HLA-B27 positivity (>90% in the ustekinumab trial and 65–85% in the risankizumab trial) and high disease activity (including increased C-reactive protein). Furthermore, radiographs of the SIJs were read centrally for both studies, thereby reducing the risk of a wrong diagnosis. Yet, the results of these studies[16,17] were clearly negative (TABLE 2). Notably, however, the ustekinumab trial[16] had a relatively high placebo response rate compared with other trials.

In addition to the trial in TNF inhibitor-naive patients with AS, ustekinumab has also been investigated in patients with non-radiographic axSpA and in TNF inhibitor-experienced patients with AS (all three trials were reported in one article)[16]. Because the results in the study with TNF inhibitor-naive patients with AS were negative, the other two studies were discontinued before the end of recruitment; however, analysis of data obtained up until the studies were terminated confirmed the negative results of the first study[16]. Similarly, in the risankizumab trial[17], escape treatment with 180 mg of risankizumab up to 40 weeks in patients who had not achieved a 20% improvement in Assessment of Spondyloarthritis International Society criteria (ASAS20) response at week 24 on any dosing regimen did not improve disease activity. One study is still ongoing with the anti-IL-23 antibody tildrakizumab (which targets the p19 chain) in patients with AS[51]. It remains to be seen whether the negative results from the other trials[16,17] will also be confirmed in this trial[51].

The question has been raised as to whether ustekinumab and risankizumab might have been underdosed in these trials[16,17]. In a successful trial in psoriasis[52], 180 mg of risankizumab was given at weeks 0, 4 and 16, thereby providing a similar cumulative dose to the AS trial (in which 180 mg of risankizumab was given at weeks 0 and 8, and every 8 weeks thereafter)[17], albeit at different dosing intervals. Although this dosing regimen might have had some influence on the week 12 results in the AS trial[17], it cannot explain why there was no further improvement when treatment was continued up to week 40. Trial data on risankizumab in PsA has not yet been published; however, similar dosages of guselkumab (another selective inhibitor of the p19 chain of IL-23) were effective in psoriasis[11] and PsA[10]. In a trial in Crohn's disease[14], risankizumab was given at doses of 200 mg or 600 mg at weeks 0, 4 and 8. No clear difference in efficacy was reported between the two dosages of risankizumab, but both were substantially better than placebo at week 12 (REF.[14]). Thus, in Crohn's disease, the dose was slightly higher (200 mg instead of 180 mg) and one more injection was given in the first 8 weeks in comparison with the psoriasis[52] and AS[17] trials, in which only two doses were given during the first 8 weeks, either at weeks 0 and 4 or at weeks 0 and 8. Therefore, it cannot completely be excluded that a higher dose of risankizumab would also have produced an effect in patients with AS, but the lack of any difference in their effects between the lowest and highest dosages (90 mg and 180 mg) in the AS trial[17] argues against this premise. Ustekinumab has also been successfully tested in Crohn's disease against placebo, again with a slightly different dosing schedule to the trials in AS: in this study a single

Table 1 | **Axial and peripheral structures typically affected by inflammation in spondyloarthritis**

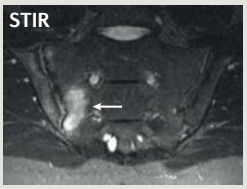
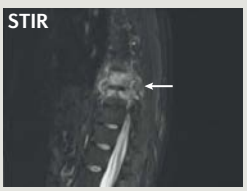
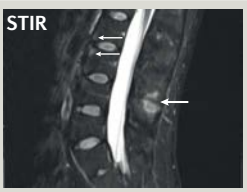
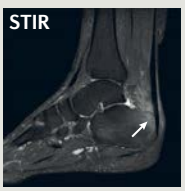
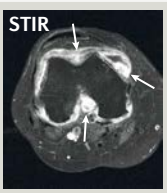
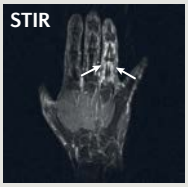
Anatomical structure	Tissues or structures involved	Typical pathological findings	Clinical manifestation	Example
Sacroiliac joints	Bone, hyaline cartilage, fibrocartilage, ligaments, joint capsule and synovial tissue	Bone marrow oedema (osteitis), capsulitis, enthesitis, bone erosions, fatty metaplasia of the bone marrow, subchondral sclerosis, joint space narrowing and bone proliferation resulting in ankylosis	Low (usually inflammatory) back pain and/or buttock pain	 MRI showing subchondral bone marrow oedema (osteitis, indicated by arrow) typical of active sacroiliitis as a manifestation of axSpA
Vertebral bodies	Bone in the areas of anulus fibrosus (fibrocartilage) and ligament attachments	Bone marrow oedema, bone erosions, fatty metaplasia of the bone marrow and bone proliferation resulting in syndesmophyte formation	Back pain	 MRI showing bone marrow oedema (osteitis) indicative of spondylitis (arrows); in severe cases, a larger area can be involved including an intervertebral disc (spondylodiscitis)
Facet joints Costovertebral and costotransverse joints	Bone, cartilage and synovial tissue	Bone marrow oedema, synovitis, bone erosions, fatty metaplasia of the bone marrow and bone proliferation resulting in ankylosis	Back pain (can be thoracic pain if the costovertebral and costotransverse joints are affected)	 MRI showing severe bone marrow oedema (osteitis, indicated by arrow) involving vertebral bodies (spondylitis) and posterior structures (pedicles, facet joints, costovertebral and costotransverse joints)
Spinal ligaments	Junction between ligaments and bone	Bone marrow oedema and ligament inflammation	Back pain	 MRI showing bone marrow oedema (osteitis) of the spinal processes indicative of enthesitis of the interspinous ligament (thick arrow) and spondylitis anterior (thin arrows)
Entheses	Junction between tendon or ligament and bone, with or without synovial tissue	Tendonitis, tenosynovitis, bone marrow oedema, bone erosions and osteoproliferation	Enthesitis	 MRI showing enthesitis of the Achilles tendon (arrow)[a]
Peripheral joints	Synovial tissue	Synovitis	Peripheral arthritis	 MRI showing arthritis of the right knee joint (arrows)[a]

Table 1 (cont.) | **Axial and peripheral structures typically affected by inflammation in spondyloarthritis**

Anatomical structure	Tissues or structures involved	Typical pathological findings	Clinical manifestation	Example
Fingers and toes	Tendons, ligaments and synovial tissue	Tendonitis, tenosynovitis and synovitis	Dactylitis	 MRI showing dactylitis (arrows)[a]

axSpA, axial spondyloarthritis; STIR, short-TI inversion recovery. [a]Images provided by Dr. K.-G. Hermann of Charité–Universitätsmedizin Berlin.

induction dose of 130 mg of ustekinumab was not better than a dose of 6 mg/kg (which is higher than 130 mg for most adults), and injections of a maintenance dose of 90 mg of ustekinumab every 8 weeks was not better than injections every 12 weeks[13]. This kind of dosing (starting with 130 mg and following up with 90 mg every 12 weeks) is in a similar cumulative dose range to the 90 mg given at weeks 0 and 4 and then every 12 weeks in trials in AS[16] or PsA[53]. Although higher dosages of IL-23 inhibitors than those given in the axSpA trials have not been directly tested, indirect evidence from clinical trials in psoriasis, PsA and Crohn's disease suggest that differences in dosing can probably not explain the negative results in clinical trials in AS. Additionally, it is unlikely that the anti-IL-23 antibodies are not reaching the site of inflammation in the bone, given the good efficacy of monoclonal antibodies against TNF or IL-17 for this indication (TABLE 2).

To explain the negative results from therapeutic trials with IL-23 inhibitors in AS, it has been speculated that IL-23 might have a pathogenic role in the initiation of AS (or axSpA) but not in maintaining established disease[37,54]. But even if this were the case, the gap of many years that often occurs between the first symptoms emerging and diagnosis of the disease[1] does not make a treatment that is effective only in the initiating phase of the disease a realistic option at this time.

Blockade of IL-23 or IL-17 and radiographic progression in the spine. Aside from the effect of a treatment on disease activity (measured by patient reported outcome parameters, acute phase reactants and MRI-detected inflammation in patients with axSpA), it is also of great interest whether a treatment has an effect on new bone formation[55] (normally measured by the formation or growth of syndesmophytes in the spine). Because the rate of such radiographic progression is slow, follow-up data for at least 2 years are mandatory for studies of new bone formation. Long-term data gathered over 2 years and 4 years of treatment with an IL-17 inhibitor or an IL-23 inhibitor are currently only available for secukinumab, which showed some syndesmophyte progression over this time, albeit at a relatively low level[56]. Whether this progression rate is lower than that achieved with treatment with non-biologic drugs[57] or TNF inhibitors[58] is currently still under investigation. No data on the effect of IL-23 inhibitors on radiographic progression in AS are currently available.

Blockade of IL-23 or IL-17 and enthesitis. Conventional therapies (probably with the exception of NSAIDs) for enthesitis often fail and data on the efficacy of biologic drugs are limited. To date, there have only been a few clinical trials of TNF inhibitors in patients with peripheral enthesitis[59,60]; however, in most trials of AS or PsA, the effect of TNF inhibitors on peripheral enthesitis has only been assessed as a secondary outcome. The efficacy of IL-17 inhibitors at treating enthesitis has also been analysed, and some favourable effects over placebo have been reported in the main PsA trials[9,61] and also in an open label extension[62]. In light of the negative trials of ustekinumab in axSpA[16], it is interesting to note that ustekinumab was better than placebo at treating PsA-associated enthesitis (reported as a secondary outcome parameter) in a double-blind clinical trial[53], was superior to a TNF inhibitor for treating peripheral enthesitis in a prospective randomized open-label study[63] and reduced enthesitis (as measured by ultrasonography) in patients with psoriasis who had subclinical enthesitis[64]. However, a prospective, double-blind controlled trial of an IL-23 inhibitor that includes patients with peripheral enthesitis and measures changes in enthesitis as a primary outcome criterion is urgently needed before a final statement can be made as to a possible effect of ustekinumab on enthesitis that is different from its effect on axial manifestations.

Are IL-23 and IL-17 uncoupled in axSpA?

To explain the discrepancy between the effects of therapies targeting IL-17 and therapies targeting IL-23 in axSpA, it is important to understand the differences and similarities between IL-17-mediated processes and IL-23-mediated processes in the context of the peculiarities of the local immune responses in axSpA. In particular, the effects of IL-23 on T_H17 cell differentiation and the effects of IL-17 and IL-23 on inflammation and bone destruction and formation are important, as discussed below.

IL-23 and T_H17 cell differentiation. The link between IL-23 and IL-17 was first shown in mice by studying the differentiation of T cells into T_H17 cells. In the presence of IL-6, transforming growth factor-β (TGFβ) induced the differentiation of T_H17 cells, whereas in the absence of IL-6, TGFβ induced the differentiation of regulatory T cells[65]. The addition of IL-23 increased production of IL-17 by T cells, but only once they had been activated[65].

Table 2 | **Clinical responses to blockade of TNF, IL-17 or IL-23 in radiographic axial spondyloarthritis**[a]

Target	Drug	Dosing regimen	Assessment time-point	Drug response ASAS20/ASAS40/ ASAS PR (%)	Placebo response ASAS20/ASAS40/ ASAS PR (%)	Refs
TNF	Adalimumab	40 mg s.c. Q2W	Week 12	58/40/21	21/13/4	95
	Certolizumab pegol[b]	200 mg s.c. Q2W	Week 12	58/43/23	38/18/4	96,97
		400 mg s.c. Q4W	Week 12	64/49/24	38/18/4	96,97
	Etanercept	25 mg s.c. twice weekly	Week 12	64/45/NA	29/16/NA	98
	Golimumab	50 mg s.c. Q4W	Week 14	59/45/23	22/15/5	99,100
	Infliximab	5 mg/kg i.v. at weeks 0, 2 and 6, and Q6W thereafter	Week 24	61/47/22	19/12/1	101
IL-17	Secukinumab[c]	150 mg s.c. Q4W after initial loading with 150 mg s.c. weekly from week 1 to week 4	Week 16	68/43/18	31/18/7	46
		150 mg s.c. Q4W after initial loading with 10 mg/kg i.v. at weeks 0, 2 and 4	Week 16	63/44/11	39/24/2	48
		300 mg s.c. Q4W after initial loading with 10 mg/kg i.v. at weeks 0, 2 and 4	Week 16	65/44/21	39/24/2	48
	Ixekizumab	80 mg s.c. Q2W	Week 16	69/52/NA	40/18/NA	19
		80 mg s.c. Q4W	Week 16	64/48/NA	40/18/NA	19
	Bimekizumab	64 mg s.c. Q4W	Week 12	62/43/NA	28/13/NA	44
		160 mg s.c. Q4W	Week 12	58/47/NA	28/13/NA	44
		320 mg s.c. Q4W	Week 12	72/46/NA	28/13/NA	44
	Netakimab[d]	40 mg s.c. at weeks 0, 1 and 2, and Q2W thereafter	Week 16	73/41/NA	43/14/NA	45
		80 mg s.c. at weeks 0, 1 and 2, and Q2W thereafter	Week 16	82/64/NA	43/14/NA	45
		120 mg s.c. at weeks 0, 1 and 2, and Q2W thereafter	Week 16	91/72/NA	43/14/NA	45
IL-23	Ustekinumab[e]	45 mg s.c. at weeks 0, 4 and 16	Week 24	55/31/NA	45/28/NA	16
		90 mg s.c. at weeks 0, 4 and 16	Week 24	50/28/NA	45/28/NA	16
	Risankizumab	18 mg s.c. single dose	Week 12	45/25/3	20/18/3	17
		90 mg s.c. at weeks 0 and 8	Week 12	33/21/3	20/18/3	17
		180 mg s.c. at weeks 0 and 8	Week 12	30/15/10	20/18/3	17

ASAS20, 20% improvement in Assessment of Spondyloarthritis International Society criteria; ASAS40, 40% improvement in Assessment of Spondyloarthritis International Society criteria; ASAS PR, Assessment of Spondyloarthritis International Society criteria partial remission; i.v., intravenous; NA, not available from the original publication(s); s.c., subcutaneous; Q2W, every other week; Q4W, every 4 weeks; Q6W, every 6 weeks; Q8W, every 8 weeks. [a]These trials are not head-to-head; therefore, comparison of results between trials is limited. [b]About 12% of patients in the certolizumab pegol group and 24% of patients in the placebo group were TNF inhibitor-experienced. Results are shown for the entire patient group as data for TNF inhibitor-naive patients are not available in the public domain. [c]About 40% of the patients in the MEASURE-2 trial[18] and about 25% of the patients in the MEASURE-3 trial[48] were TNF inhibitor-experienced. Results are shown for TNF inhibitor-naive patients. [d]About 14% of the patients treated with netakimab and 18% treated with placebo were TNF inhibitor-experienced. Results are shown for the entire patient group as data for TNF inhibitor-naive patients are not available in the public domain. [e]Also inhibits IL-12 by targeting the p40 subunit, which is common to IL-12 and IL-23. Results are shown for TNF inhibitor-naive patients.

At the time, the concern was that naive T cells do not express IL-23 receptor (IL-23R) and therefore should not be able to respond to IL-23. A further delineation of three steps involved in the differentiation of T_H17 cells in mice followed, including induction mediated by TGFβ and IL-6 (which leads to IL-23R expression), proliferation mediated by IL-21 and stabilization mediated by IL-23 (REF.[31]). Thus, it is important to bear in mind that the influence of IL-23 comes at a late stage of T_H17 cell differentiation, rather than at an early stage. Subsequent studies showed that, in the absence of IL-23, TGFβ and IL-6 induce the differentiation of non-pathogenic T_H17 cells in mice; the addition of IL-23 mediated the conversion of these non-pathogenic T_H17 cells into pathogenic T_H17 cells[66].

How this differentiation pathway applies to a disease situation in humans, such as axSpA, remains to be seen. Potentially, the first events of inflammation that are mediated by the pro-inflammatory cytokines IL-1 and IL-6 might cause the activation of innate or innate-like IL-17-producing cells, followed by an amplification of T_H17 cells and γδ T cells. The presence of IL-23 at a later stage could then induce a further step of maturation into pathogenic T_H17 cells. These cells have a high capacity for migration to inflamed sites, where they encounter local mesenchymal cells and are possibly further stimulated to produce IL-17, even in the absence of IL-23 (FIG. 1). Such interaction with mesenchymal cells is important for the switch from T_H17 cells that contain intracellular IL-17, to IL-17-secreting cells[67,68].

Effects of IL-17 on inflammation and bone. The effects of IL-17 on bone are important when considering targeting IL-17 in the context of arthritis (reviewed elsewhere[37]). Bone destruction and bone formation are often regulated in an opposing manner, leading to two main outcomes: an increase in bone destruction combined with a decrease in bone formation; or an increase

in bone formation combined with a decrease in bone destruction. This situation applies to the entire bone organ, in which many cell–cell interactions take place between bone cells, such as osteocytes, osteoblasts and osteoclasts, and immune cells from the bone marrow.

An increase in bone destruction combined with a decrease in bone formation typically occurs in RA and, to some extent, in PsA, although in PsA some degree of new bone formation also occurs[69]. The opposite is true of AS, in which new bone formation outweighs bone destruction, leading to the creation of syndesmophytes. In RA, IL-17 increases the production of cytokines such as IL-1, TNF and IL-6 and the release of the bone destruction-related biomarker C-telopeptide of type I collagen (CTX), as seen ex vivo in bone samples from patients with RA[70], eventually leading to bone destruction. Inhibition of IL-17 with blocking antibodies or soluble receptors (mimicking IL-17 blockade) reduces its destructive effect on bone structure, causing a decrease in ex vivo production of IL-6 and CTX[70]. The protective effect of IL-17 inhibitors on bone in RA is further increased when they are combined with inhibitors of TNF and IL-1, the best effects being obtained when all three inhibitors are used together[70].

The immunopathology of axSpA is different from that of RA in that inflammation at the cartilage–bone interface, including at the insertion sites of tendons and ligaments into the bone (entheses), occurs in axSpA, which can be followed by new bone formation. Mechanical stress is probably important for the site-specific location of inflammation at the entheses in axSpA[71]. At the site of tendon or ligament insertion into the bone, as well as in the synovium, tissue-resident cells can differentiate from mesenchymal cells[72]. Under inflammatory conditions, such as in axSpA, mesenchymal cells come into contact with immune cells that

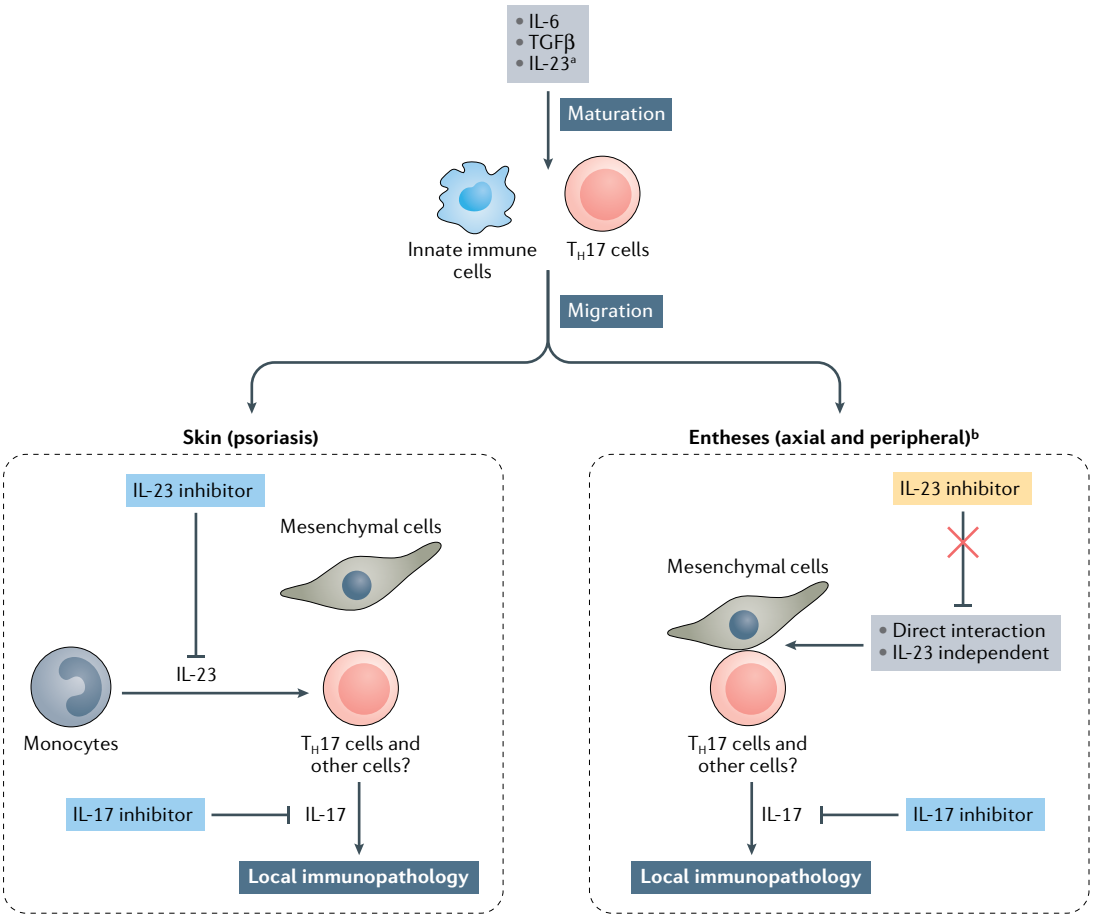
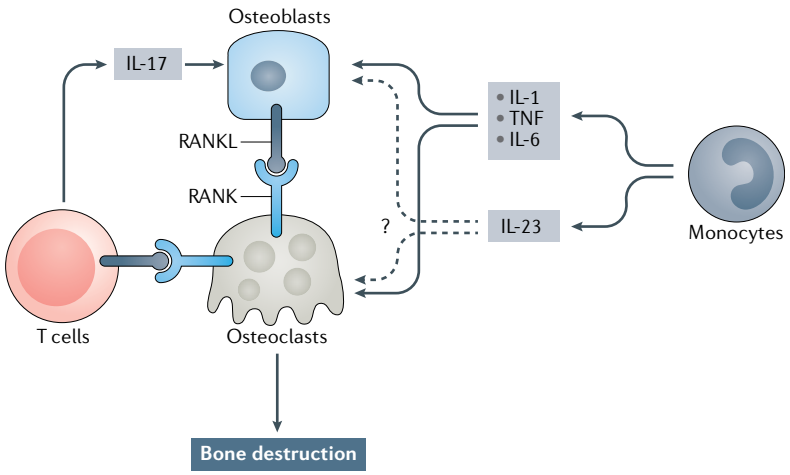


Fig. 1 | **Potential differences in the IL-23–IL-17 pathway in psoriasis and spondyloarthritis.** On the basis of the good efficacy of IL-17 inhibition in psoriasis and spondyloarthritis, it can be assumed that IL-17 has a relevant role in the pathogenesis of both diseases. However, the microenvironment of the tissue-specific inflammation in each disease differs: IL-17-secretion in the skin seems to be mediated by local IL-23 production, whereas IL-17 production at the entheses (both axial and peripheral) might be independent of IL-23, potentially mediated by direct mesenchymal cell interaction with local T cells. The exact role of non-T cells as a source of IL-17 and their involvement in local immunopathology is still to be defined. However, this theory provides one possible explanation for why the efficacy of IL-23 inhibitors differs between chronic inflammatory diseases that are all, at least partly, IL-17-mediated. TGFβ, transforming growth factor-β; T_H17, T helper 17. [a]IL-23 is involved at a late stage of T_H17 cell differentiation, mediating the conversion of non-pathogenic T_H17 cells into pathogenic T_H17 cells that can then migrate to local tissues. [b]The idea of IL-23 independency mostly refers to axial enthesitis: data on a potential independency from IL-23 for peripheral enthesitis is currently not sufficient to draw conclusions.

have migrated to the site of inflammation. These cells produce cytokines that affect the different cells in the entheses. When isolated mesenchymal cells are incubated under bone-forming conditions in vitro, bone formation increases in the presence of TNF alone and is further amplified by the addition of IL-17 (REF.[73]). This synergistic interaction leads to an increase in alkaline phosphatase and to the formation of calcium deposits[73].

Importantly, when mesenchymal cell-derived osteoblasts and osteoclasts interact and are activated, as occurs in inflamed bone, TNF and IL-17 induce bone destruction and cause a down-regulation of osteoblast function (FIG. 2a). By contrast, when there is no contact between osteoblasts and osteoclasts, the presence of TNF and IL-17 can lead to bone formation (FIG. 2b). Therefore, it is crucial to integrate the functional interactions that form a bridge between osteoblasts and osteoclasts into our understanding of the immunopathology of axSpA[37]. One such bridge is the receptor activator of NF-κB–receptor activator of NF-κB ligand (RANK–RANKL) interaction[74]. RANK is expressed by cells of monocyte lineage, such as osteoclasts and dendritic cells, whereas RANKL is expressed by cells of mesenchymal lineage, such as osteoblasts, fibroblasts and synoviocytes[37]. IL-17 and TNF increase RANKL expression on mesenchymal cells in vitro, as well as enhancing the effect of RANK on osteoclasts[73]. In addition, some T cells can accentuate this interaction by expressing RANKL and secreting IL-17 (REF.[37]). A potential bridging protein is the zinc finger adapter protein Schnurri-3, the loss of which alters the balance between osteoblast and osteoclast activity, leading to increased bone formation in mice[75,76]. In human mesenchymal cells, TNF and IL-17 synergistically increase the expression of Schnurri-3, in line with an increase in bone destruction[73]. Thus, it is important to consider the location and the nature of cell–cell interactions that occur when interpreting the effects of IL-17 on bone, and to bear in mind that osteoblasts are derived from mesenchymal cells.

a Bone cell interactions in destructive arthritis



b Bone cell interactions in axial spondyloarthritis

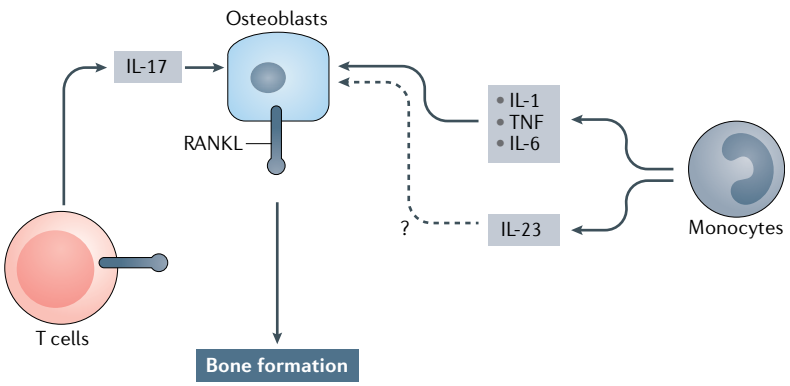


Fig. 2 | **Effects of cytokine exposure on bone and ligaments. a** | In the context of inflammation affecting the bone, such as occurs in rheumatoid arthritis, osteoblasts and osteoclasts interact via receptor activator of NF-κB (RANK) and receptor activator of NF-κB ligand (RANKL). Cytokines produced by monocytes, such as IL-1, TNF and IL-6, affect both osteoblasts and osteoclasts, resulting in bone destruction. Monocytes also produce IL-23, which induces the final maturation of pathogenic T helper 17 cells. These cells interact with osteoblasts and osteoclasts via direct and indirect mechanisms, thereby inhibiting bone formation and increasing bone destruction. The dotted line for the effects of IL-23 indicates that many of the assumed results still need to be confirmed. **b** | In the context of inflammation affecting the bone–cartilage or bone–fibrocartilage interface, such as occurs in spondyloarthritis, osteoclasts are not present; thus, interactions between osteoblasts and osteoclasts are absent. This lack of osteoclasts results in bone formation.

Differences in IL-23 and IL-17 function. Compared with IL-17, much less is known about the effects of IL-23 on bone. As described above, IL-23 is important for the production of IL-17, although the presence of IL-6 and IL-1 (which induces IL-6) is also important for the first steps in the differentiation of naive T cells into T_H17 cells and other IL-17-producing cells. An important question is whether the effects of IL-23 on inflammation and bone are direct or indirect; in vitro, the induction of osteoclastogenesis in peripheral blood cells by IL-23 seems to largely occur via the induction of IL-17 (REF.[77]).

As IL-23 is mostly produced by dendritic cells and monocytes, differences in the final effect of IL-23 inhibition on a given target, such as IL-17-producing cells, might be related to the presence and contribution of these IL-23-producing cells. An important difference has been observed in vitro in the interactions between mesenchymal cells of different origins and IL-17-secreting cells (FIG. 1). In the presence of bone marrow-derived or synovium-derived mesenchymal cells, the interaction between activated T cells and these mesenchymal cells is sufficient for the production of large amounts of IL-17 without an obvious need for monocytes or IL-23 (REF.[68]). By contrast, in interactions between skin-derived mesenchymal cells and activated T cells, the presence of monocytes and production of IL-23 is important for the production of IL-17 (REF.[78]). In the latter situation, the removal of monocytes (and consequently IL-23) resulted in reduced IL-17 production[78]. The results of these in vitro studies[68] suggest a differential contribution of monocytes (and IL-23) to the local production of IL-17 depending on the nature of cell–cell interactions present at different anatomical sites; however, more data are needed to reach a final conclusion on this hypothesis.

The different possible mechanisms of how to stimulate IL-17 production (described above) might indeed provide a potential explanation for the differences seen

in clinical responses when targeting IL-23 or IL-17 in psoriasis or PsA as opposed to axSpA. In psoriasis, monocytes produce IL-23 in the skin, thereby increasing local production of IL-17 by T cells[78]. In this situation, blocking either IL-23 or IL-17 will be effective at the effector site, as is observed in the clinic. By contrast, at sites in the bone or synovium, the requirement for monocytes (and IL-23) is limited because they are not needed for the local production of IL-17 (REF.[78]). Thus, blocking IL-23 would be ineffective in this situation, unlike blocking IL-17. This hypothesis might imply that the biological effects of IL-23 on various tissues are mostly indirect via IL-17 release[77].

In addition, data exist that suggest the possibility of a partial uncoupling of IL-23 and IL-17 secretion under certain circumstances (FIG. 1). In a study in mice, IL-17A regulated the tight junction protein occludin during epithelial injury in the gut and had a protective function[79]. In this investigation, IL-17 was produced by local γδ T cells and its production was independent from IL-23 (REF.[79]). However, IL-23-dependent production of IL-17 by γδ T cells has also been described[80]. In another study, a mouse model of colitis was worsened by IL-17 inhibition, whereas IL-23 inhibition decreased colonic inflammation and enhanced regulatory T cell accumulation[81], providing further evidence of a possible uncoupling of IL-23 and IL-17 function[82]. In a further mouse model of autoimmunity (experimental autoimmune encephalomyelitis), dual inhibition of IL-23 and IL-17 was more effective than targeting either cytokine alone[83], indicating again that IL-23 and IL-17 production might be partly uncoupled. A potential alternative source of IL-17 are ILC3s, which are important in barrier tissues such as the gut and skin for homeostasis, inflammation and repair[84]. IL-17 production by these cells might or might not be under the control of IL-23 (REF.[85]). Interestingly, ILC3s can be found in human entheses[86]. Furthermore, if the effects of IL-23 blockade in psoriasis were through inhibition of IL-17 production alone, one would expect to see safety signals similar to those seen in the IL-17 inhibitor trials; however, an increased rate of candidiasis and inflammatory bowel disease occurred in IL-17 inhibitor trials but not in IL-23 inhibitor trials[87], indicating that IL-23 might have a function outside of IL-17 production. Taken together, these data indicate that under certain circumstances, some cell types can produce IL-17 independently of IL-23, which might also be an explanation for the observed discrepancy in the efficacy of IL-17 inhibition and IL-23 inhibition in diseases such as AS and Crohn's disease.

Optimizing axSpA treatment

Conventional treatment in axSpA begins with physical therapy and NSAIDs[25]. The next step in the management recommendations is to treat with biologic agents[25]. TNF inhibitors and IL-17 inhibitors are currently the only biologic drugs that are effective for the treatment of axSpA[3] and have a similar efficacy, although data from head-to-head trials are not available. Starting with a TNF inhibitor as the first biologic is recommended[25], although this recommendation is formed on the basis of longer experience of using TNF inhibitors than IL-17 inhibitors, rather than on data showing the superiority of TNF inhibitors over IL-17 inhibitors. The relative roles of these two types of biologic drugs for the optimal management of axSpA still need to be defined: questions remain as to which biologic drug is best to start with; how to select patients who might respond better to one therapy than another; what the role of a combination therapy might be; and the effect of these two drugs on new bone formation[88]. Currently, it is not clear whether a hierarchy between TNF and IL-17 exists in the inflammatory process or whether both cytokines are equally important in all patients with axSpA.

Janus kinase (JAK) inhibitors are immune-modulating small molecules that target cytokines by blocking intracellular cytokine receptor signalling pathways and have been tested in therapeutic trials for several immune-mediated diseases[89]. The therapeutic effects of two JAK inhibitors, tofacitinib[90] (which targets JAK3 and JAK1) and filgotinib[91] (a selective JAK1 inhibitor) have been described in phase II trials in patients with active AS, in which they showed superiority over placebo treatment. Interestingly, however, inhibition of JAK1 and JAK3 does not preferentially target TNF or IL-17, the only two cytokines currently known to be crucial for the pathogenesis of axSpA[88]. Even if the effects of these JAK inhibitors (and others currently under investigation in clinical trials)[92] are confirmed in phase III trials[93] the exact mechanism by which they affect axSpA still needs to be defined[88]. In addition, the positive results of a phase II study of the selective inhibitor for tyrosine kinase 2 (TYK2; a fourth member of the JAK family), BMS-98616, in psoriasis[94] were reported in 2018; it would be interesting to investigate whether or not TYK2 blockade also has a clinical effect on PsA and axSpA.

Conclusions

Clinical trials have shown a good efficacy for IL-17 inhibitors in patients with axSpA, whereas IL-23 inhibitors failed to show such an effect. This discrepancy might be explained by the unique immunopathological microenvironment that occurs in axSpA, in which IL-17 secretion might take place in the absence of IL-23. Furthermore, many cell types other than conventional T cells are able to produce IL-17 in a manner that seems to be partially independent of IL-23. In fact, the dominant type of IL-17-secreting cell is yet to be defined in axSpA. The immunopathology of enthesitis is believed to be similar to that of axial manifestations of spondyloarthritis, but it is still to be shown whether IL-23 inhibitors are effective for the treatment of enthesitis or whether, similar to axial manifestations, they are not effective. The negative data from trials of IL-23 inhibitors in axSpA highlight the fact that it is difficult, if not impossible, to predict the efficacy (or inefficacy) of targeted therapies before a drug has been tested in clinical trials for the indication of interest. IL-17 inhibitors and TNF inhibitors are currently the only effective targeted therapies for axSpA; thus, future research needs to clarify how to identify the best therapy for each patient, and also whether the two treatments might be safely combined.

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