CLINICAL TRIALS

# Biopsy-driven trial a milestone towards precision medicine in RA

> direct assessment of synovial tissue pathology could be used to guide the choice of treatment for patients

The results of R4-RA, the first biopsy-driven, multicentre randomized controlled trial (RCT) in rheumatoid arthritis (RA), suggest that direct assessment of synovial tissue pathology could be used to guide the choice of treatment for patients with RA. If replicated and validated in independent cohorts, the findings could represent an important step forward in precision medicine for the disease.

"Precision medicine aims to tailor treatment to distinct features of an individual patient's condition," explains Laura Donlin, who was not involved in R4-RA. In oncology, for example, stratification of patients and tailoring of treatment according to tumour-specific molecular profiles is routine in clinical practice. "RA represents a promising test case for precision medicine amongst rheumatic diseases," says Donlin, pointing out that the range of medications available for RA have shown differential efficacy among patients with the disease, and that the past few years have seen major gains in knowledge of the molecular and cellular features of RA-affected joints. "The challenge now is in identifying which of these molecular features relates to the responsivity of an individual to a given medication," she adds.

Credit: S.Harris/Springer Nature Limited

The R4-RA investigators focused on the extent of B cell infiltration in the RA joint tissue (synovium) as a predictor of an individual patient's responsiveness to a treatment that targets B cells. "In prior studies, we identified that approximately 40% of patients have few B cells infiltrating the synovium while still displaying active arthritis," says co-author Felice Rivellese. "On this basis, we hypothesized that, in these patients, joint inflammation is driven by alternative cell types and/or pathways and they would be less likely to respond to the B cell depleting biologic rituximab and more likely to respond to a different biologic, such as tocilizumab." This hypothesis was supported by the results of a small pilot study of patients with established RA and an inadequate response to TNF inhibitor therapy, in which having few or no $CD20^+$ B cells was an independent predictor of non-response to rituximab.

In the phase IV R4-RA RCT, the presence of B cells in a patient's actively swollen joint was assessed by histological assessment of biopsy-obtained synovial tissue. The patients, who had all previously failed to respond to treatment with TNF inhibitors, were then classified as 'B cell rich' or 'B cell poor' and randomly assigned to receive either rituximab ($n=83$) or tocilizumab ($n=81$). Synovial tissue was also classified by B cell molecular signature using RNA sequencing.

Among patients histologically classified as B cell poor, there was no statistically significant difference in the proportion meeting the primary end point (improvement from baseline in clinical disease activity index score of 50% or more (CDAI50%) at week 16) between those treated with tocilizumab or rituximab (56% versus 45%). However, more patients in the tocilizumab group achieved a major treatment response (CDAI-MTR; defi ed as CDAI50% plus CDAI score <10.1) than in the rituximab group (46% versus 24%; $P=0.035$).

Notably, when joint tissue was assessed using RNA sequencing, the response rate was significantly higher in the tocilizumab group for both CDAI50% (63% versus 36%; $P=0.035$) and CDAI-MTR (50% versus 12%; $P=0.0012$).

Rituximab seemed to be as effective as tocilizumab for patients classified as B cell rich, although the study was not powered to evaluate their comparative efficacy.

"The ability to target biological therapies to the right patients would significantly impact the health economics of RA with reduced exposure of patients to expensive and potentially toxic drugs, while reducing suffering and disability for patients and huge costs to society," notes Costantino Pitzalis, co-author and chief investigator of R4-RA. Although the study has some limitations, the results suggest that assessing B cell expression signatures in synovial tissue could help identify patients who might not respond to treatment with rituximab. "This is an outstanding first step towards implementing evidence-based precision medicine approaches for patients with rheumatic diseases," notes Donlin.

*Sarah Onuora*

**ORIGINAL ARTICLE** Humby, F. et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. *Lancet* **397**, 305–317 (2021)

**RELATED ARTICLE** Pitzalis, C. et al. Transforming clinical trials in rheumatology: towards patient-centric precision medicine. *Nat. Rev. Rheumatol.* **16**, 590–599 (2020)

# IN BRIEF

## OSTEOARTHRITIS

### Antidepressant identified as potential DMOAD

New findings implicate the G protein-coupled receptor kinase GRK2 as a promoter of chondrocyte hypertrophy and a potential disease-modifying osteoarthritis drug (DMOAD) target. In a surgical model of osteoarthritis (OA), chondrocyte-specific deletion of GRK2, or pharmacological inhibition with the repurposed FDA-approved antidepressant drug paroxetine, attenuated chondrocyte hypertrophy, matrix degradation and OA progression. Paroxetine-mediated GRK2 inhibition also mitigated chondrocyte hypertrophy and cartilage degradation in human OA cartilage ex vivo.

**ORIGINAL ARTICLE** Carlson, E. L. et al. Paroxetine-mediated GRK2 inhibition is a disease-modifying treatment for osteoarthritis. *Sci. Transl Med.* **13**, eaau8491 (2021)

## RHEUMATOID ARTHRITIS

### RA remission attainable during pregnancy

Low disease activity (LDA) and remission are feasible treatment goals during pregnancy in patients with rheumatoid arthritis (RA) and can be achieved through following a modern treatment approach that includes treat-to-target and TNF inhibitor therapy, according to an analysis of the PreCARA cohort. Of the patients in this cohort, 75.4% were in a state of LDA or remission before pregnancy, which increased to 90.4% in the third trimester and remained stable post-partum. This proportion was higher than that of a historic reference cohort of pregnant patients with RA being treated according to standards of that time (2002–2010).

**ORIGINAL ARTICLE** Smeele, H. T. W. et al. Modern treatment approach results in low disease activity in 90% of pregnant rheumatoid arthritis patients: the PreCARA study. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-219547 (2021)

## SYSTEMIC LUPUS ERYTHEMATOSUS

### 2019 EULAR–ACR classification criteria for SLE performs well in children

In a retrospective analysis of the performance of the 2019 EULAR–ACR classification criteria for systemic lupus erythematosus (SLE) in paediatric patients (112 with juvenile SLE and 113 without SLE), the criteria had a high sensitivity (0.96, 95% CI 0.90–0.99) and high specificity (0.89, 95% CI 0.82–0.94), which was comparable to (or slightly improved from) that of the SLICC criteria. Notably, the sensitivity of the criteria improved over time (ranging from 0.83 one year after the onset of symptoms to 0.96 after more than two years). However, the specificity was lower than that reported for adults (0.93).

**ORIGINAL ARTICLE** Levinsky, Y. et al. Performance of 2019 EULAR/ACR classification criteria for systemic lupus erythematosus in a pediatric population – a multicenter study. *Rheumatology* https://doi.org/10.1093/rheumatology/keab140 (2021)

## SYSTEMIC SCLEROSIS

### Tocilizumab prevents ILD progression in early SSc

In a post-hoc analysis of the phase III focuSSed trial, tocilizumab treatment preserved lung function in patients with early systemic sclerosis (SSc) and progressive skin disease. Tocilizumab stabilized forced vital capacity (FVC) over 48 weeks, resulting in a least squared mean change in % predicted FVC of -0.1% compared with -6.3% in the placebo group. This effect was independent of the extent of lung involvement or fibrosis severity at baseline (as assessed by well-established quantitative high resolution chest CT measurements).

**ORIGINAL ARTICLE** Roofeh, D. et al. Tocilizumab prevents progression of early systemic sclerosis associated interstitial lung disease. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41668 (2021)

## VASCULITIS

# Different phenotypes identified for Behçet syndrome

Behçet syndrome is a heterogeneous condition, with diverse clinical manifestations and prognoses. Two papers published in *Arthritis Research & Therapy* have identified various phenotypes for Behçet syndrome, which could be a useful step in the road towards personalized medicine for this condition.

"In daily practice, we have noticed that major organ involvement in patients with Behçet syndrome can vary," states Jian-Long Guan, corresponding author on one paper. "For example, uveitis rarely overlaps with intestinal lesions in these patients and vice versa."

To explore these different phenotypes, the researchers performed a cross-sectional analysis of 860 patients with Behçet syndrome who had attended Huadong Hospital of Fudan University in China between September 2012 and January 2020.

Guan and colleagues found differing patterns of organ involvement between men and women, including a higher prevalence of papulopustular skin lesions, ocular disease, cardiovascular disease and central nervous system (CNS) involvement among male patients and a higher prevalence of genital ulcers among female patients.

Notably, a hierarchical cluster analysis revealed five subgroups of patients with differing organ involvement: a skin and mucosa subtype, an articular subtype, a gastrointestinal subtype, a uveitis subtype and a cardiovascular subtype with CNS involvement.

"Some patients with intestinal and vascular lesions have no obvious symptoms," reports Guan. "This cluster pattern could help us avoid unnecessary endoscopy screening for patients with uveitis without abdominal symptoms. We might also screen for vessel involvement in patients with CNS lesions."

A similar set of subgroups was identified by researchers in another study, investigating clinical clusters among patients with Behçet syndrome in Japan. "An important aspect of our paper is that we used two large registries for our analysis: about 600 patients with Behçet disease at Yokohama City University and about 6,000 patients at the Ministry of Health, Labor and Welfare in Japan," explains Yohei Kirino, corresponding author on this study. "This approach allowed us to validate the clustering analysis in two independent registries."

The five clinical clusters included a mucocutaneous subtype, a mucocutaneous with arthritis subtype, a gastrointestinal subtype, an eye subtype and a neurological subtype. Interestingly, Kirino and colleagues found that the proportion of each cluster varied over time, including an increase in frequency of patients with gastrointestinal involvement, in line with data from previous epidemiology studies in Japan and South Korean.

"Our study did not include important factors such as genetics and disease activity, so the 'resolution' of the clusters is blurry," explains Kirino. "We are currently conducting a publicly funded nationwide Behçet disease registry study, which will include more than 1,000 patients and will incorporate details of phenotype, treatment, prospectively tracked disease activity scores, GWAS and various biomarkers of Behçet disease. Through this analysis, we aim to identify more detailed subtypes of Behçet disease, optimize medical care and predict prognosis, and improve patient care," he concludes.

*Jessica McHugh*

**ORIGINAL ARTICLES** Zou, J. et al. Cluster analysis of phenotypes of patients with Behçet's syndrome: a large cohort study from a referral center in China. *Arthritis Res. Ther.* **23**, 45 (2021) | Soejima, Y. et al. Changes in the proportion of clinical clusters contribute to the phenotypic evolution of Behçet's disease in Japan. *Arthritis Res. Ther.* **23**, 49 (2021)

RHEUMATOID ARTHRITIS

# Cigarette smoke exacerbates joint damage via miR-132

Cigarette smoking is known to both increase the risk of developing rheumatoid arthritis (RA) and to exacerbate disease. Although some of the effects of cigarette smoke on RA have been discovered at a cellular level, the molecular mechanisms are largely unknown; a deficiency that a new study has aimed to address.

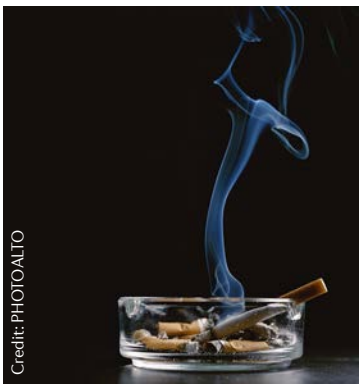
"We have previously shown that cigarette smoke can activate T helper 17 ($T_H17$) cells, which are closely associated with RA," explains first author Paula Donate. "In this new study, we therefore explored the molecular mechanism behind how cigarette smoke stimulates $T_H17$ cells."

Cigarette smoke contains several agonists for aryl hydrocarbon receptor (AhR), which is expressed by $T_H17$ cells, so the authors began by mapping microRNA expression in $T_H17$ cells upon stimulation with an AhR agonist. Of the microRNAs that were upregulated, miR-132 was taken forward for further study as the most promising candidate owing to its upregulation in $T_H17$ cells in mice with experimental arthritis following exposure to cigarette smoke and its high homology between mice and humans.

Interestingly, miR-132 was not only upregulated in $T_H17$ cells in response to cigarette smoke, but was also released by them in the form of extracellular vesicles. In vitro, these vesicles were able to induce osteoclastogenesis in pre-osteoclasts.

"Mechanistically, we found that the upregulation of miR-132 induces osteoclastogenesis via the suppression of COX2, which catalyses the synthesis of prostaglandins," states co-corresponding author Fernando Cunha. Prostaglandins are known inhibitors of osteoclastogenesis, so suppressing COX2 with miR-132 lifted this inhibition, leading to increased generation of osteoclasts.

> "exposure to cigarette smoke increased osteoclastogenesis …, which could be stopped by anti-miR-132"



Credit: PHOTOALTO

In mice with experimental arthritis, intra-articular injection of anti-miR-132 was sufficient to both block the expression of miR-132 in the synovium and to reduce arthritis. Furthermore, exposure to cigarette smoke increased osteoclastogenesis in these animals, which could be stopped by anti-miR-132.

"We also found that patients with RA express higher levels of miR-132 than do healthy individuals, and this increase was further elevated by cigarette smoking, suggesting that miR-132 is a potential target for therapeutic intervention for inflammatory disease in general, and RA in particular," adds co-corresponding author Foo Liew.

*Joanna Clarke*

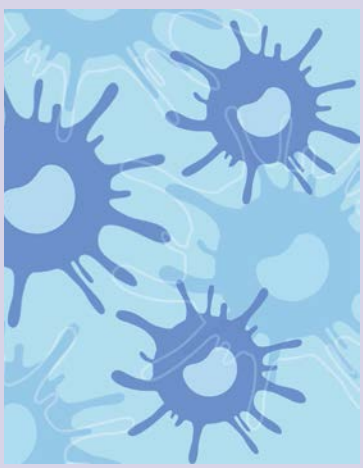
**ORIGINAL ARTICLE** Donate, P. B. et al. Cigarette smoke induces *miR-132* in Th17 cells that enhance osteoclastogenesis in inflammatory arthritis. *Proc. Natl Acad. Sci. USA* **118**, e2017120118 (2021)

SYSTEMIC SCLEROSIS

# Targeting pDCs in SSc

New research using a novel xenotransplant model provides evidence that human plasmacytoid dendritic cells (pDCs) have an important and direct role in skin inflammation and fibrosis, and highlights that specifically targeting these cells could be a viable therapeutic approach for systemic sclerosis (SSc).

> "specifically targeting these cells could be a viable therapeutic approach for [SSc]"



Credit: S.Harris/Springer Nature Limited

Previous research over the past decade has implicated pDCs in interferon (IFN)-induced responses, skin infiltration and fibrosis in the context of immune mediated inflammatory diseases (IMIDs), including SSc. "Nevertheless, all the evidence so far has focused only on indirect models using ex vivo human samples or mouse models with mouse pDCs," explains Rebecca Ross, co-corresponding author of the study published in *Annals of the Rheumatic Diseases*.

The novel in vivo model used in the latest study was devised by xenotransplantation of human pDCs into immunocompromised mice. "Xenotransplanted mice showed an increased IFN response to topical Toll-like receptor (TLR) agonist application and a strongly enhanced fibrotic and immune response to bleomycin," Ross notes. These effects were suppressed by administration of CBS004, a monoclonal antibody with high affinity for BDCA2, which is an inhibitory receptor expressed on pDCs and important for their function.

These in vivo findings were supported by experiments in organotypic skin rafts, which the researchers developed as a preclinical human skin model. In this in vitro model, expression of IFN-induced genes was increased in human skin cells including keratinocytes and fibroblasts following exposure to the supernatant of TLR-activated pDCs. Notably, this IFN gene signature was suppressed when the pDCs were co-cultured with CBS004.

"Altogether our data offer the first direct evidence supporting the development of BDCA2-targeting as a therapeutic application for pDC-mediated skin inflammation and fibrosis," highlights co-corresponding author Francesco Del Galdo. "We plan to define the clinical contexts in which a direct pDC targeting may lead to a therapeutic benefit and consider testing the performance of this antibody in the context of dedicated clinical trials."

*Sarah Onuora*

**ORIGINAL ARTICLE** Ross, R. L. et al. Targeting human plasmacytoid dendritic cells through BDCA2 prevents skin inflammation and fibrosis in a novel xenotransplant mouse model of scleroderma. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-218439 (2021)

GOUT

# How do dietary interventions affect serum urate and gout?

*Sarah L. Morgan and Jasvinder A. Singh*

An important question for patients and providers is whether and to what extent dietary interventions, diet supplements or weight loss can help to prevent incident gout or manage existing gout. Evidence is emerging, but randomized trials are still needed to fill this important knowledge gap.

*Refers to* Juraschek, S. P. et al. Effects of dietary patterns on serum urate: results from the DASH randomized trial. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41614 (2020).

Patients increasingly indicate that they prefer non-pharmacological management of gout[1], and dietary management and weight reduction are cornerstones of behavioural non-pharmacological interventions for managing gout. However, data from controlled trials about the effect of these interventions on disease activity in gout are mostly lacking. The results of a new study by Juraschek et al. suggest that adopting the Dietary Approaches to Stop Hypertension (DASH) diet could help to reduce serum urate levels in patients with gout, but also raises further questions about dietary interventions[2].

The DASH diet pattern emphasizes eating fruits, vegetables, low-fat dairy products, nuts, legumes and whole grains and lowering the intake of sodium, red and processed meats and sweetened beverages. The current study by Juraschek et al.[2] is a secondary analysis of the original DASH trial[3], a parallel arm 8-week study in adults with elevated blood pressure or hypertension in which the DASH diet was compared with a diet rich in fruit and vegetables and a typical American diet. In their new analysis, Juraschek et al. embrace the concept of food synergy (the additive, or more than additive, effects of nutrients, food constituents and foods on health outcomes[4]) by evaluating the effect of the DASH food pattern on serum urate levels in participants from the original DASH study[2]. No statistically significant difference was found between the DASH diet and a diet high in fruits and vegetables in the effect on serum urate levels (−0.25 mg/dl versus −0.17 mg/dl reduction over the typical American diet, respectively). However, because the DASH diet has additional components besides increased fruit and vegetable intake (such as increased intake of low-fat dairy and whole grains), it could be postulated that synergies with additional foods could confer additional benefits on hyperuricemia, and possibly on gout flares, that might be demonstrated in a future trial. The dietary interventions were carefully controlled by the use of isocaloric dietary patterns and meals that were either provided or consumed at the study site, assuring that dietary adherence was high[3]. Such a stringent design enabled the confounding variable of weight change to be controlled. Presumably, future studies of dietary interventions, weight loss and/or other behavioral interventions in gout will be completed with a similarly high degree of rigour.

Interestingly, in this study, the DASH diet was associated with greater serum urate reductions in adults without baseline hypertension, in those with higher baseline serum urate levels (above 6 mg/dl) and possibly in those without obesity (showed a trend towards statistical significance)[2]. These interesting and informative subgroup and stratified analyses are hypothesis generating and need to be confirmed with future studies. This finding of serum urate reduction was similar to another ancillary DASH diet study that showed greater lowering of serum urate in those with higher baseline serum urate levels than in those with lower levels[5]. Thus, adults in the general population with high serum urate levels who have borderline elevated blood pressure but do not meet the definition of hypertension might be the most likely to benefit from the urate-lowering effects of the DASH diet.

Because of the frequent coexistence of hyperuricemia and gout with other components of metabolic syndrome (such as central obesity, hypertension, insulin resistance and dyslipidemia)[6], and the beneficial effects of dietary interventions, it is not surprising that dietary interventions for metabolic syndrome (such as the DASH diet) have been postulated to have beneficial effects on hyperuricemia and gout. For example, insulin resistance is thought to modify the handling of urate by the kidney, thereby causing hyperuricemia, and hyperuricemia might exacerbate insulin resistance by impairing endothelial oxygen supply[6]. The DASH diet also lowers blood pressure and improves cardiovascular risk factors[3] — a potential added benefit for people with gout. However, with the exception of a few pilot trials[7–9], critical evidence about dietary interventions in the field of gout from well-powered, placebo-controlled randomized trials is lacking.

> “evidence about dietary interventions in the field of gout from well-powered, placebo-controlled randomized trials is lacking”

The currently available evidence can only address the effect of a DASH diet as a preventive strategy to reduce high serum urate levels (a biomarker for incident gout and symptomatic gout) in adults in the general population. A prospective cohort study of 44,444 men without a history of gout from the Health Professionals Follow-Up Study was carried out to investigate the rates of incident gout that met the preliminary ACR classification criteria for gout in individuals on a DASH dietary pattern compared with those on a Western dietary pattern (high in sweets, fried foods, red and processed meats and refined grains)[10]. In this study, a DASH dietary pattern score was assigned on the basis of data from food frequency questionnaires. During the 26 years of follow-up, those with higher DASH dietary pattern scores had a lower risk of incident gout than those who consumed a Western diet[10]. If empiric evidence or model-based analyses could be

provided to show the reductions in the 5-year or 10-year risks of incident gout that occur with the magnitude of serum urate reduction produced by the DASH diet (as reported by Juraschek et al.[2]), it would provide patients and providers with usable information about the magnitude of this benefit. Such knowledge would be helpful for patients with gout as they embark on life-long dietary changes that can be challenging to sustain.

> “Adherence is challenging for all nutritional intervention therapies”

Several questions remain regarding the reduction in serum urate of the magnitude of 0.25 with the DASH diet in the general population reported by Juraschek et al.[2]. Is this serum urate reduction clinically meaningful? Can it be sustained in an individual long-term? Are other cardiovascular risk reduction benefits sustainable? Patients with which clinical or genetic phenotype would benefit the most from this intervention? What are the interactions between medications used for the treatment of hypertension and/or hyperuricemia with this dietary intervention? The last question could be answered with a 2×2 factorial design trial, but additional trials or studies will be needed to answer the remaining questions.

If a future carefully controlled trial of the DASH diet in people with gout can demonstrate a beneficial effect on flares and disease activity (function, quality of life and joint swelling), an equally important challenge will be the translation of the DASH dietary pattern into everyday living. Given that patients with gout often prefer non-pharmacological approaches such as dietary management[1], the adoption of a DASH diet in everyday life will be an important consideration. Adherence is challenging for all nutritional intervention therapies; the implementation of strategies to remove barriers and facilitate dietary interventions, similar to those used to overcome challenges in adherence to urate-lowering therapy, will be vital.

How does diet modification fit into the overall management of gout? Pharmacological treatment with urate-lowering drugs such as allopurinol, febuxostat, probenecid or pegloticase is important for lowering serum urate to target levels. However, behavioural interventions that can be effective in lowering serum urate and improving gout outcomes, including diet modification, exercise and weight loss, are important adjuncts to the pharmacological management of gout. Even if the magnitude of serum urate reduction from behavioural interventions is lower than that resulting from the use of urate-lowering drugs, additional potential benefits from behavioural interventions, such as improvements in function and quality of life, could be helpful to patients with gout. Future controlled trials on behavioural interventions for lowering urate, as well as the effect of these dietary interventions on gout flares, will be an important addition to the toolkit to manage gout.

Credit: Javier Zayas Photography/Moment

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**Competing interests**

J.A.S. has received consultant fees from the ACR, Adept Field Solutions, Clearview Healthcare Partners, Clinical Care Options, Crealta/Horizon, Fidia, Focus Forward, Medisys, Medscape, Navigant Consulting, the National Institutes of Health, Practice Point Communications, Putnam Associates, Spherix, Trio health, UBM LLC and WebMD. J.A.S. owns stock options in Charlotte's Web Holdings, Inc., TPT Global Tech and Vaxart Pharmaceuticals. J.A.S. previously owned stock options in Amarin, Moderna Pharmaceuticals and Viking. J.A.S. is on the speaker's bureau of Simply Speaking. J.A.S. is a member of the executive of Outcomes Measures in Rheumatology (OMERACT), an organization that receives arms-length funding from 12 companies. S.L.M. declares no competing interests.

AUTOIMMUNITY

# Moving towards a molecular categorization of autoimmune disease

*Michael L. Whitfield*

The field of rheumatology is poised to categorize the phenotypes of systemic autoimmune diseases on the basis of measurable and quantifiable molecular signatures. Emerging efforts to identify similarities across diseases, predict clinical outcomes and predict response to therapy using quantitative, data-driven approaches could considerably change treatment paradigms.

*Refers to* Barturen, G. et al. Integrative analysis reveals a molecular stratification of systemic autoimmune diseases. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41610 (2020).

Systemic autoimmune diseases are heterogeneous conditions that have a wide range of clinical presentations and variable clinical courses. A hallmark of these conditions is their variable inflammatory responses and changes in disease activity over time. Although heterogeneity within particular systemic autoimmune diseases has been shown on a molecular level, comparisons among diseases have been limited, and the variability and similarities across systemic autoimmune diseases remains poorly defined. Efforts to stratify patients with systemic autoimmune diseases on the basis of molecular patterns, as attempted in a new study by Barturen et al.[1], could have important diagnostic and therapeutic implications in the movement towards precision medicine in rheumatology.

Genomic technologies have enabled systematic, data-driven approaches to improve our understanding of disease heterogeneity in a wide range of conditions. The most notable progress has been made in the field of cancer[2], where genomic technologies have resulted in diagnostic tools for breast cancer such as the MammaPrint and Oncotype DX gene expression tests, which are now widely used in the clinical setting[3]. In cancer, researchers have elucidated and characterized tumour heterogeneity using gene expression, epigenetics and genomic sequencing, and these successes culminated in efforts such as The Cancer Genome Atlas that catalogued multiple layers of genomic data, providing comprehensive views both across and within diseases. Similar studies of autoimmune diseases have not occurred, partially because of a lack of comprehensive genetic, genomic and epigenomic data from the same patients.

Barturen et al.[1] have taken steps toward mitigating this deficit and have shown data-driven groupings for multiple systemic autoimmune diseases using whole blood transcriptome, methylome and genetic cross-sectional data from 995 patients across seven different systemic autoimmune diseases and 267 healthy individuals. The diseases included were systemic lupus erythematosus (SLE), rheumatoid arthritis, systemic sclerosis (SSc), primary Sjögren syndrome, mixed connective tissue disease, primary antiphospholipid syndrome and undifferentiated connective tissue disease. The researchers identified four groups of phenotypes common across these diseases using data-driven analyses (FIG. 1). Three of these groups represented active pathogenic states and were referred to as 'inflammatory', 'lymphoid' and 'interferon' clusters. Patients with each of these seven diseases were found in each molecular group, suggesting commonality in the molecular processes across the spectrum of autoimmune disease.

One important finding from this study was the identification of a group of patients that had low disease activity and that grouped together with healthy individuals. This patient group seems similar to a 'normal-like' group identified elsewhere in analyses of patients with SSc, given the molecular similarity of the cohort to healthy individuals[4]. In the study by Barturen et al.[1], 74% of healthy individuals were part of this group compared with 3%, 11% and 12% for the other subgroups. These data suggest that measurable changes in disease activity might be detectable in peripheral blood samples of patients.

Importantly, this study also demonstrated that patients could move between states in a 'relapse–remission' pattern, using analysis of an independent cohort of patients that were followed over time. Intriguingly, although the researchers observed that some patients

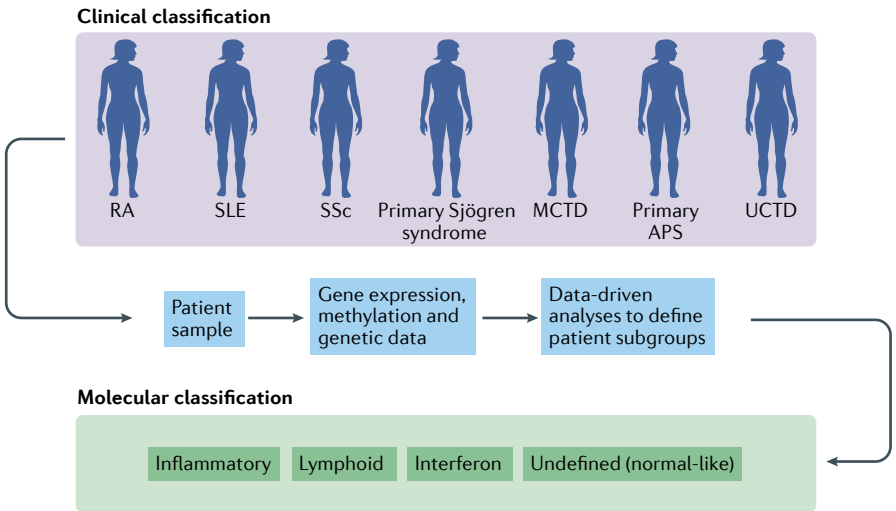


Fig. 1 | **Strategy for the molecular classification of systemic autoimmune diseases.** Patients with systemic autoimmune diseases might be categorized on the basis of molecular patterns, as attempted by Barturen et al.[1]. In this study, peripheral blood samples were collected from patients with one of seven different systemic autoimmune diseases — rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), primary Sjögren syndrome, mixed connective tissue disease (MCTD), primary antiphospholipid syndrome (APS) and undifferentiated connective tissue disease (UCTD) — and analysed for changes in gene expression and methylation. Data-driven analyses identified three major pathological subgroups, as well as one that resembled healthy individuals and that might represent a quiescent disease state.

switched between different pathogenic groups, the majority of patients tended to stick to a single pathogenic state, suggesting that most patients had a consistent molecular phenotype.

The work of Barturen et al.[1] and others using genomic approaches has slowly been creating 'molecular taxonomies' of autoimmune disease that complement, or potentially even supersede, current classification systems. Developing quantitative models for patient stratification (that is, the data science of diagnoses) on the basis of measurable molecular signatures might fundamentally change how we view these diseases. One must now ask the question, are we ready to add data-driven molecular classifications to the toolkit that we use to identify the clinical course of patients? If the answer is yes, then several things must occur. First, molecular subsets within specific autoimmune diseases that are identified by different groups must be compared and reconciled. Quantitative comparison of the groups identified by Barturen et al.[1] to those identified by others[4–7] is key and might require large meta-analyses of existing datasets. Molecular subsets have often been denoted by different names, but in many cases might have similar underlying molecular signatures. Barturen and colleagues note similarities between the methylome clusters in their work with those found in studies of patients with SLE[8]. Therefore, it is imperative that all data be released in the public domain with subgroup annotations and meta-data, so that cohorts can be aggregated, analysed and compared.

In addition, for molecular stratification to be valuable to patients and physicians, the results must be actionable. If we can determine that a patient with a systemic autoimmune disease falls into a consistent, pathology-associated molecular category, can we identify therapeutics that might target that specific group? To answer this question, we should push for the collection and analyses of molecular biomarkers on a genomic scale in clinical trials. Unbiased assays of gene expression, epigenetic changes and DNA polymorphisms, rather than targeted assays measuring only a few genes, are imperative to interrogate molecular phenotypes in different autoimmune diseases. The systematic collection of genomic data makes possible the unbiased analysis of almost any previously identified molecular signature and enables retrospective analyses of newly discovered signatures. Therefore, the researchers' conclusion that these results could have implications for clinical trials and the explanation for why some patients are non-responsive to therapy is a hypothesis that is supported by the literature and that can be tested experimentally. For example, molecular subsets in SSc, which are referred to by different names but might have analogous counterparts in the work of Barturen et al.[1], have been shown to predict response to hematopoietic stem cell transplantation[9] and treatment with abatacept[10].

The findings of Barturen et al.[1] suggest that systemic autoimmune diseases can be consistently classified into distinct molecular subsets that reflect the biology of the ongoing disease. This study raises the intriguing possibility that there is a finite and measurable number of molecular subsets across different systemic autoimmune diseases with common molecular pathologies. If the groups observed here can be reconciled with the groups identified in studies of individual systemic autoimmune diseases, and linked to either existing or experimental therapies, it might increase the likelihood of demonstrable patient responses, making precision medicine in rheumatology a reality. Finally, such insights might also identify therapeutic gaps, thus providing opportunities for novel therapeutic development.

The future of medicine and biology lies in the data. Embracing the data and the approaches embodied in the fields of computational biology, artificial intelligence and statistical and network-based approaches, that enable us to fully mine these datasets to create molecular classifications for use in the clinical setting, is critical. We often stand on the shoulders of giants, but we could reach higher by standing on the vast amounts of data within our reach, allowing us to rationally develop diagnostics and therapies that account for the molecular underpinnings of each individual's disease.

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**Competing interests**

M.L.W. is a Scientific Founder of Celdara Medical LLC. He has served a consultant for Bristol-Myers Squibb, Acceleron, Abbvie and Corbus Pharmaceuticals.

# Location, location, location: how the tissue microenvironment affects inflammation in RA

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Abstract | Current treatments for rheumatoid arthritis (RA) do not work well for a large proportion of patients, or at all in some individuals, and cannot cure or prevent this disease. One major obstacle to developing better drugs is a lack of complete understanding of how inflammatory joint disease arises and progresses. Emerging evidence indicates an important role for the tissue microenvironment in the pathogenesis of RA. Each tissue is made up of cells surrounded and supported by a unique extracellular matrix (ECM). These complex molecular networks define tissue architecture and provide environmental signals that programme site-specific cell behaviour. In the synovium, a main site of disease activity in RA, positional and disease stage-specific cellular diversity exist. Improved understanding of the architecture of the synovium from gross anatomy to the single-cell level, in parallel with evidence demonstrating how the synovial ECM is vital for synovial homeostasis and how dysregulated signals from the ECM promote chronic inflammation and tissue destruction in the RA joint, has opened up new ways of thinking about the pathogenesis of RA. These new ideas provide novel therapeutic approaches for patients with difficult-to-treat disease and could also be used in disease prevention.

Tissue specialization is essential for life; however, the fundamental principles that mediate tissue-specific cell behaviour are not fully understood. For example, why are fibroblasts in the gut so different from those in the skin, and why do brain-resident macrophages behave differently to those in the liver? Technologies that can interrogate tissues at the single-cell level are being used to generate an encyclopaedic inventory of the different cell populations comprising each tissue of the body[1], and are revealing extraordinary levels of cellular complexity and phenotypic plasticity. Mapping the anatomical location, and the interaction networks, of these newly discovered cell subsets will be the next essential step towards understanding tissue structure and function. Moreover, cells do not exist in a vacuum. The tissue microenvironment is an important determinant of cell behaviour, enabling cells to perform distinct roles dictated not only by their anatomical location, but also, more specifically, by their position within tissues. But what defines this microenvironment? Cells in tissues are surrounded and supported by an extracellular matrix (ECM). In each tissue, the ECM is made up of more than 1,000 different secreted molecules in a combination that is unique to that tissue, assembled into a complex 3D network that provides external cues that govern cell behaviour. Understanding how tissues function in health and disease, therefore, requires knowing both the identity of resident cell populations and how complex external microenvironments cohesively define cell phenotype in situ.

In this Review, we focus on the synovium and examine how changes in both the cellular and extracellular compartments of this tissue have a causal role in promoting chronic inflammation in rheumatoid arthritis (RA). We review how single-cell transcriptional analyses have revealed extraordinary microanatomical complexity within the RA synovium, leading to the identification of at least 18 distinct cell phenotypes that exhibit striking positional and functional segregation. These studies provide compelling new insights into the cellular basis of inflammatory joint disease. We also discuss how ECM networks create anatomically distinct sub-synovial niches that dictate behaviours that are specific to cells at certain locations within a tissue (referred to in this Review as site-specific behaviour). These networks directly contribute to chronic inflammation in the inflamed joint; information that is changing the way we think about how inflammatory joint disease arises and progresses, offering new methods of patient stratification, as well as novel classes of therapeutic drugs. We also highlight the main questions and challenges that remain related to the synovial tissue microenvironment.

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**Key points**

- All tissues are made up of cells surrounded by an extracellular matrix (ECM), an intricate 3D molecular network that is an important determinant of tissue architecture and cell behaviour.
- The synovium is a complex anatomical tissue comprising many cell (sub)populations that are located in distinct sub-synovial niches, each of which are specialized to perform unique roles in synovial homeostasis.
- In rheumatoid arthritis (RA), infiltrating immune cells join tissue-resident cells, leading to qualitative changes in cell phenotype that promote inflammation and tissue destruction, and suppress the resolution of inflammation.
- The ECM has an important role in dictating the organization of synovial cell networks and in programming synovial cell specialization.
- Changes in the synovial microenvironment start to occur early in the development of RA, and these aberrant extracellular cues shape pathogenic cell behaviour during the onset and progression of disease.
- Analysing localized changes in the synovium can improve disease classification and patient stratification, and targeting the ECM holds promise for the development of new strategies to treat and prevent RA.

## What is the tissue microenvironment?

All tissues consist of cells surrounded by an intricate ECM. This 3D network of secreted molecules provides structural support for cells and dictates their spatial organization within tissues. However, the ECM is not simply an inert scaffold, it is also an important determinant of cell phenotype, providing environmental cues that enable cells to move relative to each other and to perform distinct roles determined by their anatomical location[2,3]. ECMs are made from a selection of more than 1,000 molecules that are collectively known as the matrisome. Genes in the matrisome encode all of the proteins that can be secreted by cells and encompass core ECM molecules (such as collagens, proteoglycans and glycoproteins, including fibronectin, laminins, tenascins and thrombospondins), as well as matrix-associated proteins including: matrix-affiliated molecules (such as mucins, lectins, syndecans and galectins); matrix regulators (for example, crosslinking enzymes such as lysyl oxidases and transglutaminases, modifying enzymes such as kinases and sulfatases, proteases such as matrix metalloproteinases (MMPs) and cathepsins, and protease inhibitors such as tissue inhibitors of MMPs and cystatins); and soluble factors (such as growth factors, Wnts, cytokines and chemokines)[4,5].

The expression of site-specific combinations of matrisome molecules and their assembly into networks around cells creates unique tissue microenvironments, as well as local niches within tissues. Integrated mechanical and biochemical cues from each type of ECM provide essential context for cell behaviour, wherein distinct combinations of extracellular molecules cohesively define cell differentiation and specialization. For example, the components of a typical human synovial joint include tissues such as synovium, tendons, muscles, ligaments, bursae, menisci, articular cartilage and subchondral bone. Each constituent tissue of the joint is made up of a unique combination of matrisome molecules that confer the distinctive physical properties that, together, are necessary for effective joint function (BOX 1).

The ECM is as dynamic as it is complex, changing throughout development and ageing, as well as during inflammation and disease. However, for most human tissues (including the joint), a detailed understanding of the molecular and topological organization of the ECM networks surrounding cells is lacking. In addition, it is unclear how tissue architecture changes during inflammation and what the functional implications of these changes are. In the following sections, we review emerging data that highlight the importance of understanding the complex interactions between cells and their microenvironment for defining cell behaviour within the synovium and for controlling joint inflammation.

## Synovial tissue architecture

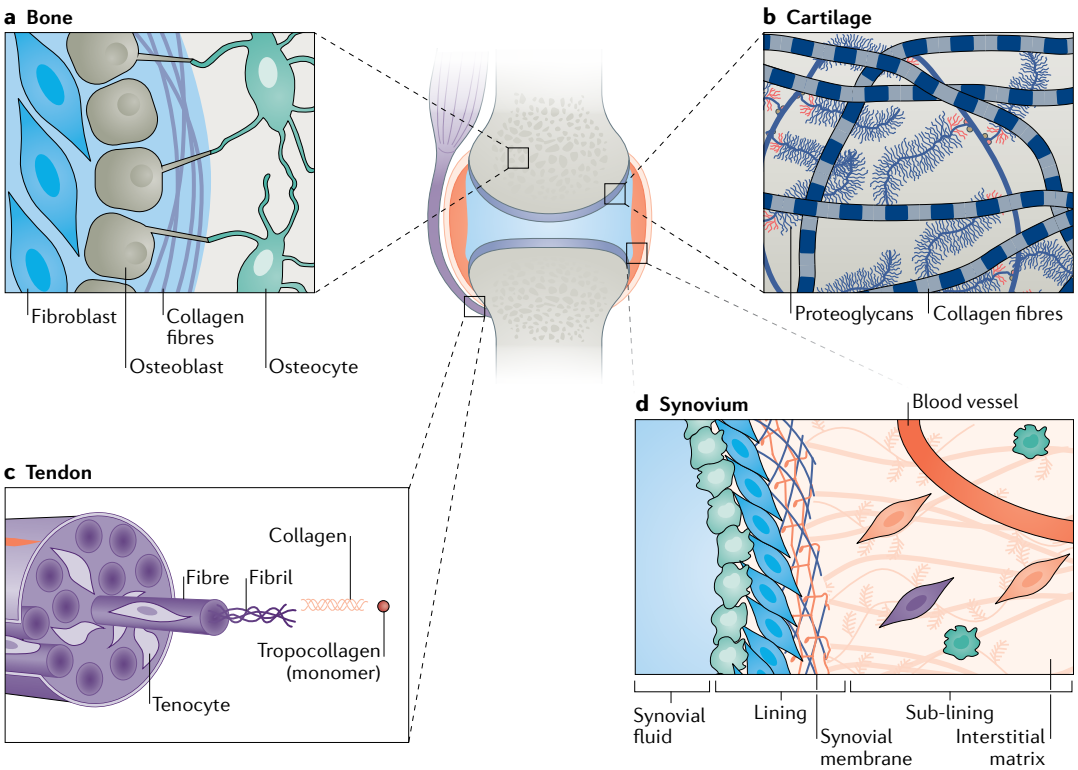
The synovium is an intricate tissue that is made up of a number of cell types including tissue-resident macrophages, fibroblasts, nerves and endothelial cells. Even at the gross histological level, subcellular compartmentalization within the synovium is evident, with the tissue forming two distinct zones: the lining layer and the sub-lining layer[6]. In a healthy joint, the lining is only 1–3 cells thick and is composed of tissue-resident macrophages and fibroblasts supported by a porous basement-like membrane (BOX 1). This zone of the synovium controls cellular and molecular ingress and egress between the synovium and the joint cavity and is important for maintaining joint integrity and the composition of synovial fluid, ensuring effective joint lubrication and nutrient exchange. The sub-lining, comprising fibroblasts and tissue-resident macrophages distributed throughout a looser collagenous ECM, contains blood and lymphatic vessels and nerves, serving to vascularize and enervate the synovium and provide transport routes for cells, nutrients and lymph into and out of synovial tissue[6].

The synovium becomes markedly expanded in RA, and the lining layer can increase to as much as 10–20 cells thick[7]. Infiltrating immune cells join tissue-resident macrophages and proliferating fibroblasts to cause synovial hyperplasia. This quantitative change in cellular populations is accompanied by qualitative changes in cell phenotype; lymphocytic, myeloid and fibroblast cellular subpopulations that promote inflammation and tissue destruction expand and are activated, whereas cell subsets that mediate the resolution of inflammation are suppressed, tipping the immune status of the joint towards chronic inflammation[7,8].

Changes in the organization of the synovial architecture are also evident in RA. The cellular influx and expansion that takes place is not random; only specific cells enter the joint, a process that is controlled by the chemokine repertoire of the synovium. Moreover, the synovium is markedly reorganized to create new compartmentalized niches, within which pathogenic cell behaviour is confined[7,8]. For example, ~40% of patients with RA develop ectopic (or tertiary) lymphoid structures in the synovium, of which 10–25% exhibit a germinal centre-like structure[9]. These aggregates of lymphocytes resemble secondary lymphoid organs, albeit with varying degrees of organization, and are characterized by a T cell-rich zone enclosing a central B cell-rich zone served by a network of high endothelial venules that enhance the recruitment of naive lymphocytes to

Box 1 | **Tissue-specific extracellular matrices in synovial joints**

Tissues are made up of cells and extracellular matrix (ECM), with each tissue being formed by the assembly of a unique selection of ECM molecules into a complex extracellular network. These networks confer different physical properties to tissues and dictate both cellular organization and cellular behaviour within tissues. Understanding tissue biology therefore requires understanding patterns of matrisomal gene expression, and how the resultant proteins are organized and modified to create distinct microenvironments. For example, in the human synovial joint, different extracellular networks contribute to the physical properties of bone, cartilage, tendons and synovium (see Figure). **a** | The subchondral bone consists of a layer of compact cortical bone and underlying cancellous bone. A hard, calcified, type I collagen-rich ECM enables bones to provide anatomical support to the body[194]. **b** | The articular surface of bone in synovial joints consists of a smooth layer of hyaline articular cartilage, which provides compressive resistance in the joint. An ECM rich in type II collagen and proteoglycans confers the shock absorbing capabilities of cartilage[194]. **c** | Tendons are the main functional anatomical bridges between muscle and bone. Tendons focus the force of muscle into localized areas on the bone (known as the entheses) and, by splitting to form a number of insertions, distribute the force of muscle contraction to different bones. An ECM comprising tightly packed parallel bundles of type I collagen fibrils confer tensile strength to tendons[195]. **d** | The synovium is a thin mesenchymal membrane that encapsulates the joint space and provides boundary layer lubrication to ensure frictionless movement. A healthy synovium is composed of two distinct layers; a lining layer and a fibrous-areolar sub-lining layer. The lining has a discontinuous ECM made up of types III, IV, V and VI collagen and laminin, which controls joint lubrication and nutrient exchange via the synovial fluid. The sub-lining has a looser, collagenous ECM[6].




the synovium (reviewed elsewhere[10]). Studies of synovial tissue have shown the existence of gradients of CXC-chemokine ligand 13 (CXCL13), CC-chemokine ligand 19 (CCL19) and CCL21, which support cellular segregation, and revealed where B cells differentiate in situ into plasma cells, thereby supporting autoantibody production[10]. Individuals with lymphoid cell-rich synovitis, which is defined by a distinct transcriptomic profile and by a high serum CXCL13 concentration, form a histologically distinct subset of patients with RA who have highly active disease that is difficult to treat[11]. Indeed, deep phenotyping of subtypes of early RA led to the identification of three distinct pathotypes, each characterized by distinct transcriptional signatures: those that lack substantial immune infiltrate (pauci-immune fibroid); those enriched for macrophages and monocytes but lacking B cells and which typically respond well to treatment (diffuse myeloid); and those with T cell and B cell aggregates accompanying a diverse immune cell infiltrate (lympho-myeloid)[12]. These data exemplify how disease pathotypes or endotypes can be categorized on the basis of synovial cell networks.

The pannus is also a well-described architectural feature of the inflamed synovium (FIG. 1a). Although used historically, the term pannus is likely to be replaced with the term 'activated aggressive RA synovium' in the future. This region of hypertrophic synovium, often called the aggressive front, is composed of macrophages and fibroblasts that release tissue-degrading enzymes that are responsible for the degradation of cartilage and bone[8]. Interestingly, RA synovial fibroblasts attach to the cartilage ECM and invade it progressively and

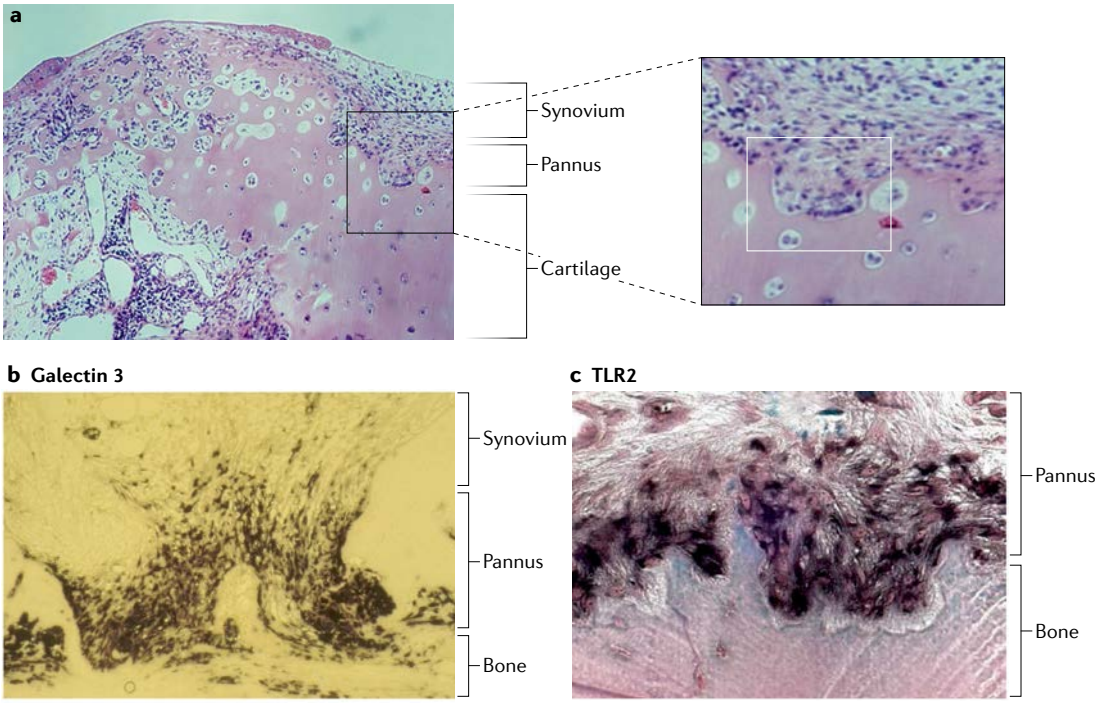



Fig. 1 | **The pannus as an architectural feature of the inflamed synovium.** The region in the inflamed joint where the hypertrophic synovium invades the adjacent cartilage and bone is called the pannus. In this region, synovial cells and chondrocytes are closely juxtaposed. **a** | The left-hand panel shows the overall architecture of the inflamed synovium, and the white boxed area in the right-hand panel highlights the specific zone of synovial–cartilage interaction. In this relatively small anatomical zone, exquisitely site-specific patterns of gene expression are observed. **b**,**c** | Examples of pannus-restricted biology include galectin 3 (part **b**) and Toll-like receptor 2 (TLR2) (part **c**) expression, which are upregulated specifically at the site of invasion into underlying bone and mediate localized synovial fibroblast activation and matrix metalloproteinase synthesis, as well as localized chemokine synthesis that contributes to the recruitment of infiltrating immune cells to the area. Images show alkaline phosphatase staining to visualize anti-galectin 3 monoclonal antibodies, and staining with digoxigenin-labelled anti-sense probes specific for TLR2, evident as areas of dark staining in each panel. Part **b** adapted with permission from Ohshima et al.[27], Wiley. Copyright © 2003 by the American College of Rheumatology. Part **c** adapted with permission from Seibl et al.[19], Elsevier.

destructively, a close relationship that has been observed in the MRL/*lpr* mouse model of arthritis[13], as well as in models in which human synovial tissue or isolated synovial fibroblasts are engrafted together with human cartilage into immunodeficient mice[14,15]. Areas of invasive pannus formation have been studied at a molecular level, revealing that this tissue niche is hypoxic[16] and displays a discreet pattern of gene expression. This pattern includes the upregulation of genes encoding proteins such as MMPs[17,18], Toll-like receptors (TLRs)[19], the transcription factor p53 (REFS[20,21]) and the ubiquitin-like protein SUMO1 (REF.[22]), and the downregulation of the tumour suppressor PTEN[23], which combine to create a destructive milieu in which aggressive pannus-resident cells are protected from apoptosis. Moreover, changes in epigenetic marks might also contribute to the aggressive phenotype of synovial fibroblasts at the site of invasion into cartilage[24]. The expression of tissue-degrading enzymes and apoptosis-inhibiting factors in RA synovial fibroblasts at the sites of cartilage destruction is associated with gene hypomethylation, which might explain why therapeutically targeting the progression of RA joint destruction is extremely difficult[25].

The tissue microenvironment itself also changes within the pannus, with altered extracellular protein expression having consequences for localized tissue invasion. For example, galectin 3, a secreted β-galactoside-binding protein that is present at increased concentrations at an early stage in RA pathogenesis, localizes almost exclusively to the pannus in the inflamed synovium[26,27] (FIG. 1b). In this regard, it is of interest that galectin 3 is found at sites of invasion, because this molecule is induced by RA synovial fibroblasts after adhesion to cartilage oligomeric matrix protein[28]. Galectin 3 directly activates synovial fibroblasts, stimulating the secretion of pro-inflammatory cytokines (such as IL-6) and chemokines (such as IL-8, CCL2, CCL3 and CCL5), as well as MMP3, via activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase signalling pathways[29]. Moreover, galectin 3 expression by RA synovial fibroblasts is required for IL-6 synthesis downstream of TLR2 (REF.[30]), a pattern recognition receptor that also localizes to the pannus in inflamed synovium[19] (FIG. 1c). Together, these data imply that local interactions between galectin 3 and TLR2 serve to activate pannus-resident synovial fibroblasts in a cytokine-independent manner, and to recruit immune cells to reinforce inflammation specifically at this important pathogenic site.

Thus, it becomes apparent how localized changes in the synovial tissue that occur in RA can direct site-specific

aspects of pathology. Such changes might also explain the fact that targeting cytokines in RA is not enough to cure this disease. However, a systematic cellular atlas that describes the spatiotemporal organization of synovial cells is missing, and little is known about how many different cell subsets make up this tissue or how they are organized into functional networks.

## The synovium at single-cell resolution

A step change in the ability to perform a census of the cell types present in synovial joints has occurred as a result of advances over the past 5 years in minimally invasive ultrasonography-guided biopsy, coupled with tissue digestion and single-cell RNA sequencing techniques[31–33]. Using these precision molecular analysis techniques, multi-parameter imaging and state-of-the-art bioinformatics, studies of tissue from inflamed joints have provided further insights into the complexity of the synovium, showing the RA synovium to comprise at least 18 distinct types of T cells, B cells, macrophages and fibroblasts[33], and enabling the first cellular map of the leukocyte and stromal cells in the synovium to be compiled for diseases such as RA[33,34] (FIG. 2).

Such single-cell resolution studies have revealed an unprecedented level of detail about the anatomical and functional specialization of synovial cells. For example, it has long been known that not only the number of T cells but also the balance of T cell subsets is an important determinant of immune status[35]. A novel pathogenic T cell population (termed T peripheral helper cells) that expresses high levels of programmed cell death protein 1 but not CXC-chemokine receptor 5 (CXCR5) was identified in the joints of patients with RA. These cells are not exhausted but, like T follicular helper cells, produce chemokines and cytokines that recruit B cells, including CXCL13 and IL-21, and aid plasma cell differentiation and IgG production. Unlike T follicular helper cells, T peripheral helper cells also possess the capacity to migrate into inflamed tissues such as the RA synovium[36]. This cell population was highly expanded in patients with seropositive RA but not in those with seronegative RA and might help to explain how ectopic lymphoid structures are formed[36]. These data indicate a complexity in the RA T cell compartment that has not been previously appreciated. Synovial fibroblasts also now clearly exhibit striking positional and phenotypic segregation (TABLE 1). Pro-inflammatory populations that are positive for the membrane glycoprotein THY1 predominate in the sub-lining layer and are substantially expanded in RA compared with osteoarthritis (OA), a process that is mediated by the cell surface receptor NOTCH3 (REF.[34]). By contrast, THY1-negative populations predominate in the lining layer and are responsible for cartilage and bone destruction during disease[34]. This degree of cellular resolution and functional delegation has helped researchers to unravel disease progression at a cellular level. However, the relationship between observations from single-cell analyses and the three histological pathotypes of RA synovium that have been described[12] remains to be fully determined.

New details are also emerging about macrophage populations in the RA joint (TABLE 1). Evidence from one study suggests that tissue-resident macrophages in the lining layer have a barrier function that maintains immune privilege in the joint[37]. This barrier becomes compromised in RA, allowing unrestricted infiltration of monocyte-derived cells into the synovium, whereas the barrier remains intact in OA, thereby preventing inflammation. In contrast to lining layer macrophages, sub-lining macrophages are thought to comprise heterogeneous populations of monocyte-derived and tissue-resident cells, among which pro-inflammatory phenotypes

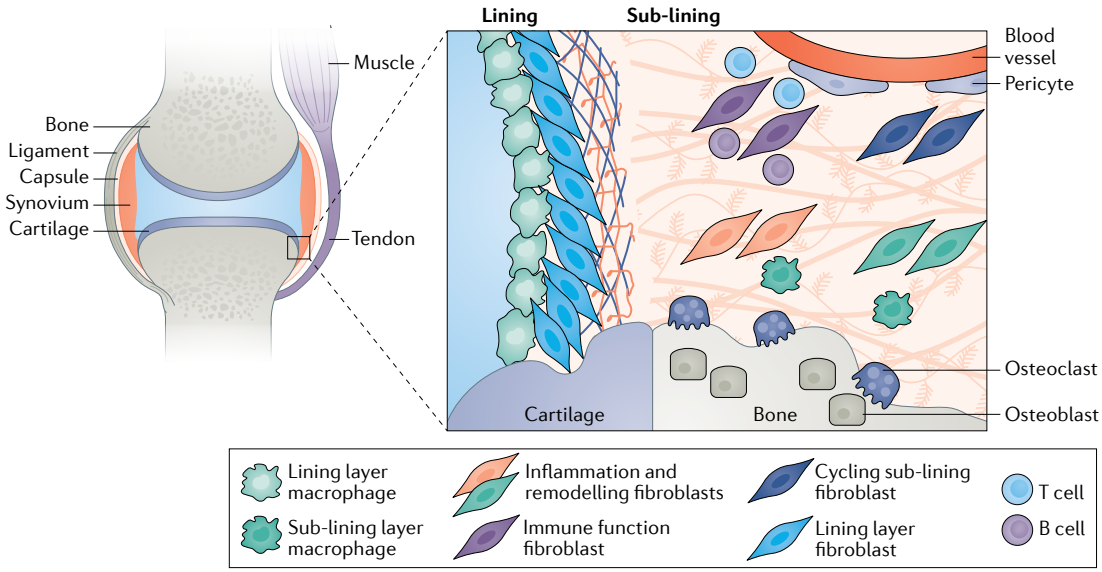



Fig. 2 | **Distinct fibroblast populations in rheumatoid arthritis synovium inhabit distinct tissue niches.** Single-cell transcriptional analyses have revealed five different fibroblast populations in the inflamed mouse synovium, three of which are conserved in human tissue. Although single-cell RNA sequencing-defined subsets might represent extremes of differentiation, fixed phenotypes or cell types on a continuum of activation, the observation that these subsets are located in different anatomical domains of the synovium suggests that different subsets mediate distinct functions.

Table 1 | **Conserved cell populations in rheumatoid arthritis joints**

| Cell subsets | Marker genes (human) | Marker genes (mouse) | Activation marker or effector genes |
|---|---|---|---|
| ***Fibroblasts*** | | | |
| Lining layer | Negative (*CD90*); positive (*CD55* and *PGR4*) | Negative (*Cd90*); positive (*Pgr4*) | *RANKL*:*OPG* ratio, *CCL9*, *CLIC5*, *MMP1*, *MMP2*, *MMP3*, *MMP9*, *MMP13*, *HAS1*, *HTRA4* and *DNASE1L3* |
| Sub-lining layer (immunomodulatory) | Positive (*CD90* and *CD34*) | Positive (*Cd90* and *Cd34*) | *IL6*, *IL33*, *IL34*, *IFI30*, *LIF*, *CXCL9*, *CXCL12*, *CXCL13*, *CCL2*, *CCL19* and *CCL21* |
| | Negative (*CD34*); positive (*CD90* and *DKK*) | | |
| Sub-lining layer (perivascular) | Negative (*CD34*); positive (*CD90* and *HLA-DRA*) | Negative (*Cd34*); positive (*Cd90*) | |
| ***Macrophages*** | | | |
| Lining layer | Not reported | Negative (*Cfsr1*); positive (*Cx3cr1*) | *TREM2*, *VSIG4*, *AXL*, *MFGE8*, *JAM1*, *ZO1*, *CLDN5*, *FAT4* and *VANGL2* |
| Interstitial | Negative (*CD11C* and *CD38*); positive (*NURP1*) | Negative (*Cx3cr1*); positive (*Cfsr1*, MHC class II genes and *Aqp1*) | *MERTK*, *CTSK*, *HTRA1*, *GPNMB* and *ITGB5* |
| | Positive (*C1QA*, *CD11C* and *CD38*) | Negative (*Cx3cr1*); positive (*Cfsr1* and *Relma*) | *MRC1*, *CD163* and *MARCO* |
| Monocyte-derived (infiltrating) | Positive (*SPP1*, *CD11C*, *CCR2* and *CD38*) when activated by interferon | Negative (*Ly6c2*); positive (*Ccr2* and *Arg1*) | *ARG1*, *IFI6*, *IFI44L*, *LY6E* and *SPP1* |
| | Positive (*IL1B*, *CD11C*, *CCR2* and *CD38*) | Negative (*Ly6c2*); positive (*Ccr2* and *Il1b*) | *NR4A2*, *HBEGF*, *PLAUR*, *RGS2*, *IL1B*, *HTF3*, *CXCL2* and *EREG* |

Data in table summarized from REFS[31–34,37,39].

dominate in RA[37]. In a study of RA synovial macrophage heterogeneity with a focus on comparative analysis of disease remission and disease flare, four distinct subpopulations were identified that comprised nine discrete phenotypic states[38]. Two of these subpopulations ($MerTK^{+}TREM2^{hi}$ macrophages and $MerTK^{+}LYVE1^{+}$ macrophages) were enriched in patients with RA whose disease was in remission compared with those with active disease. A reduction in these cell subpopulations was associated with an increased risk of disease flare and, ex vivo, these macrophages produced inflammation-resolving lipid mediators, suggesting a role in synovial repair responses[38]. In addition, another study has revealed the existence of macrophages and fibroblasts in the RA synovium that are positive for the growth factor HBEGF and that induce fibroblast invasiveness, providing insight into functional, pathogenic cellular interaction networks across subpopulations from different lineages[39].

Together, these studies demonstrate how our understanding of the architecture of the joint has progressed from gross anatomy, through to sub-synovial structures (including pannus tissue and tertiary lymphoid structures) to the single-cell level, and how this progression has enabled the emergence of a more complete cell atlas of the joint. These data have also shown how changes in the balance of synovial cell subpopulations underpin chronic inflammation during the onset and progression of RA, as compared with OA. Some of the underlying mediators of these changes are beginning to emerge; for example, a NOTCH3-dependent expansion of THY1-positive fibroblasts occurs in the sub-lining layer in active disease[40], whereas the numbers of THY1-negative fibroblasts and MerTK-positive macrophages in the lining layer contract. Moreover, an increase in the ratio of MerTK-positive to MerTK-negative macrophages in the RA synovium in patients in remission suggests that lining layer macrophages can regulate remission in RA[38].

These data could aid the development of therapeutic strategies that target pathogenic cell populations in RA. For example, functional subclasses of fibroblasts have proven difficult to define, characterize and study in health and disease. Consequently, there are no approved drugs that specifically target fibroblasts in human diseases. The identification of so-called 'pathogenic' fibroblast subpopulations[34] offers an attractive new target for therapies that would not suppress the immune system. However, as fibroblasts are a functionally heterogeneous group of cells that support discrete biological functions within the joint tissue, knowing which fibroblast subsets should be targeted and suppressed and which should be retained and augmented is challenging. A clear understanding of the biology and clinical relevance of fibroblast heterogeneity is therefore essential to provide a coherent rationale for their therapeutic targeting in the treatment of diseases such as RA. The selective targeting of pathogenic fibroblast subsets using anti-fibroblast monoclonal antibodies, analogous to B cell depletion using rituximab, could complement other targeted therapies commonly used against leukocytes and their cell products[41,42]. Another strategy might be to target epigenetically modified fibroblast subsets[24,25], for example, by targeting the aggressive phenotype of synovial fibroblasts that is characterized by epigenetic modifications such as

acetylation and methylation[43,44]. Improved understanding of RA synovial macrophage subsets is also offering the potential for additional methods of modulating pathogenic myeloid cell behaviour. MerTK-positive macrophage subsets, or the anti-inflammatory mediators released by these cells during disease remission, could be tractable targets for boosting synovial repair processes[38]. However, despite a clearer picture of the cellular networks inhabiting the RA synovium, uncertainty remains around what initiates and maintains pathogenic behaviour in different cell subsets in RA.

## Immunological landscape of the ECM

Research from the past few years has clearly shown that synovial cell networks compartmentalize into distinct micro-domains within healthy joints and that distinct sub-synovial niches arise in the synovium in RA compared with OA. However, synovial cells do not exist in a vacuum; thus, an understanding of the microenvironmental cues that shape their phenotype should provide insight into joint tissue homeostasis and disease. The ECM can affect cell behaviour via a diverse range of mechanisms[45], all of which contribute to synovial tissue biology (TABLE 2 and FIG. 3).

***Physical properties and mechanical cues.*** The ECM defines the physical properties of tissues. In the body, synovial fluid is the richest source of hyaluronic acid, a glycosaminoglycan comprising polymeric disaccharide repeats, which protects cartilage from frictional damage[46] (FIG. 3a). Similarly, a coating of lubricin (a mucinous glycoprotein also found in synovial fluid as well; also known as proteoglycan 4) on articular surfaces is the main method of effective joint lubrication[47]. ECM molecules also bind to other ECM molecules to form complex multicomponent structural networks. For example, the thin basement-like membrane of the synovial lining layer contains a mixture of collagens (types III, IV, V and VI) and laminin, and both support lining layer cells and act as a molecular sieve, controlling bidirectional solute transfer between the synovium and synovial fluid[6,48] (FIG. 3b). The specific ECM architecture of the joint is therefore vital to allow controlled, bidirectional flow of cells and molecules between the synovium

Table 2 | **How the tissue microenvironment can affect cell behaviour in synovial joints**

| Matrix molecule or network | Effect and location | Refs |
|---|---|---|
| ***Physical properties and mechanical cues*** | | |
| Hyaluronic acid | High concentrations in synovial fluid prevent friction | 46 |
| Lubricin | Distributed on the articular surface to lubricate the joint | 47 |
| Synovial lining membrane | Maintains synovial integrity and immune privilege by regulating and restricting a molecular and cellular exchange that is lost in RA | 6,37,49 |
| Synovial sub-lining interstitial matrix | Controls ECM alignment, porosity and tissue micromechanics to regulate stromal cell adhesion and movement | 51 |
| | Dictates tissue stiffness, which affects macrophage polarization and activation | 52 |
| ***Spatial positioning*** | | |
| Hyaluronic acid and lubricin | High concentrations in synovial fluid prevent cell adhesion at the cartilage surface to facilitate unimpeded joint articulation | 6 |
| Fibronectin | Within the synovial lining membrane, fibronectin promotes cell adhesion to create a cohesive barrier | 55 |
| | Ectopic expression in the RA pannus stabilizes cell invasion machinery | 58 |
| | Upregulation in the endothelial basement membrane in RA provides permissive tracks that support T cell infiltration | 56,57 |
| ***Soluble factor patterning and activity*** | | |
| Glycosaminoglycans | High concentrations at the endothelial basement membrane in RA create chemokine gradients that enhance cell infiltration | 60–64 |
| HSPGs | Serve as co-receptors at the cell surface for chemokines and growth factors, potentiating signalling | 68–71 |
| ***Direct signalling to cells*** | | |
| Tenascin C | Upregulation in the RA synovial sub-lining activates TLR4-mediated inflammation | 78,80,81 |
| Hyaluronic acid fragments | In RA synovial fluid, low-molecular-weight fragments activate TLR2-mediated pro-inflammatory signalling | 106 |
| Osteopontin fragments | In RA synovial fluid, C-terminal fragments induce macrophage chemotaxis, and phosphorylated N-terminal fragments enhance macrophage spreading and activation | 107–109 |
| Damaged collagen | In the pannus, degradation of cartilage collagen increases localized MT1-MMP expression by synovial fibroblasts | 104 |

ECM, extracellular matrix; HSPG, heparan sulfate proteoglycan; MT1-MMP, membrane type 1-matrix metalloproteinase; RA, rheumatoid arthritis; TLR, Toll-like receptor.

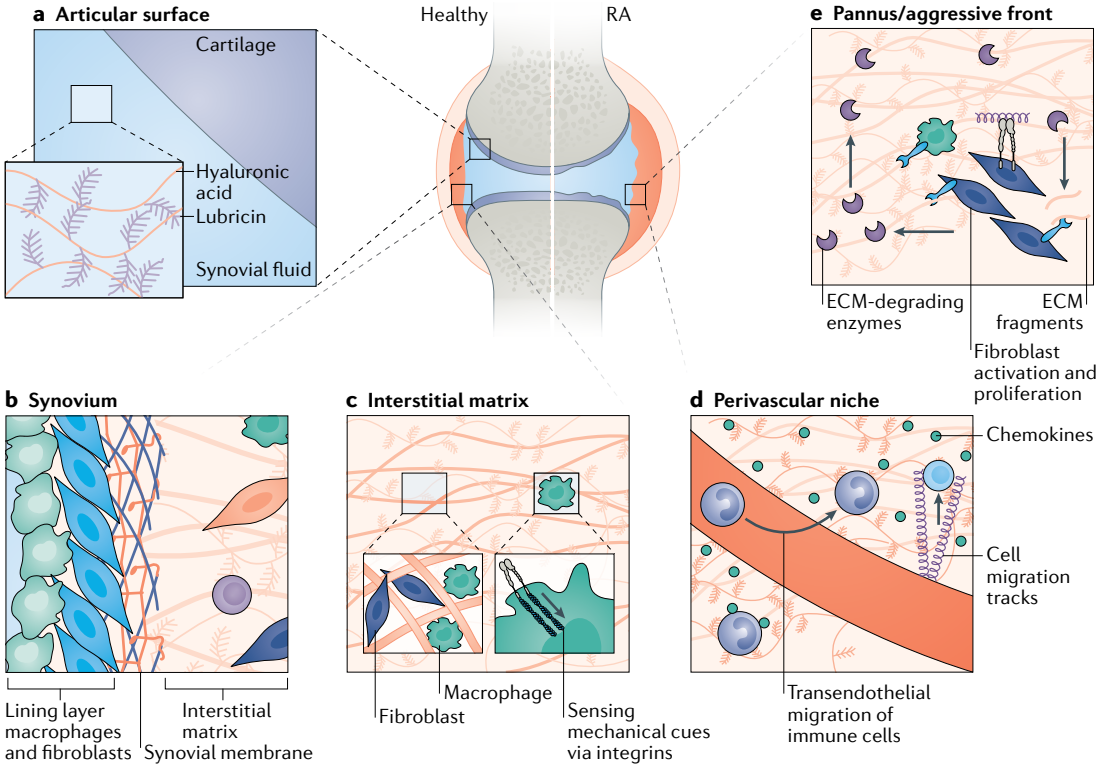



Fig. 3 | **Tissue microarchitecture in the healthy and RA joint.** Within the joint, distinct combinations of extracellular matrix (ECM) molecules define local tissue structure and function. The ECM confers physical properties to tissues. **a** | At the articular surface, proteoglycans and glycosaminoglycans ensure frictionless joint articulation, a property that is diminished in rheumatoid arthritis (RA) as these molecules become degraded to create pro-inflammatory ECM fragments. **b** | The synovial lining layer contains a discontinuous basement-like membrane that forms a porous meshwork, comprising points of anchorage that organize lining layer cells into a cohesive network to create a barrier that restricts cell movement, the integrity of which is lost in RA. **c** | The ECM provides mechanical cues that directly control cell phenotype; these become altered during synovial hyperplasia and fibrosis, in which changes in the organization of the fibrous interstitial matrix dictate stromal cell movement. ECM stiffness also affects macrophage phenotype. Changes in the biophysical properties of tissues are directly sensed by cells via receptors including integrins, which signal to alert the cell to altered external biomechanics. **d** | As well as controlling the spatial positioning of cells by providing points of adhesion and migration barriers, the matrix also creates tracks that are permissive for cell migration, such as those in and around the endothelial basement membrane. In RA, increased expression of proteoglycans creates patterns of gradients of soluble factors around blood vessels and serves as chemokine co-receptors, thereby orchestrating enhanced cell infiltration via the perivascular niche. **e** | The ECM is a rich source of biochemical signals that are directly sensed by cell surface receptors and dictate cell behaviour. These signals can derive from complex multicomponent networks of extracellular molecules or from fragments of ECM molecules that are generated during tissue remodelling. Both are exemplified in the pannus, where ectopic ECM deposition provides a cell substrate permissive for fibroblast activation, spreading and invasion, and damaged ECM sustains signalling loops that perpetuate tissue destruction via the production of matrix-degrading enzymes.

and the joint cavity, maintain tissue structure and integrity, control synovial fluid content and volume, clear up debris and maintain immunological homeostasis[49].

In addition to structural functionalization, the mechanical properties of the ECM also provide environmental cues to tissue-resident cells. In this way, not only is the molecular content of the ECM involved in dictating cell behaviour but the physical structure of the ECM network itself also defines the mechanical cues derived from the tissue[50] (FIG. 3c). Interstitial cell migration within the fibrous synovial microenvironment is regulated both by tissue microstructure (such as ECM alignment and porosity) and tissue micromechanics (such as tensile, compressive and shear moduli), which cells use to directly sense biophysical cues via integrin receptors[51]. Emerging data also show how changes in tissue mechanics control immune cell plasticity and polarization. For example, spatial confinement restricts late events in the activation of pro-inflammatory macrophages[52], which might have implications for how immune responses are modulated as tissue stiffness changes during synovial hyperplasia and fibrosis. In a manner analogous to ECM stiffness within the tumour microenvironment emerging as an important determinant of cancer progression and treatment response[53,54], so too should the influence of the mechanical properties of the synovium, derived from the ECM content and higher-order organization, be considered for disease progression in RA.

***Tissue architecture and spatial positioning.*** The ECM controls the spatial positioning of cells within tissues in many ways. For example, both lubricin and hyaluronic acid exert anti-adhesive properties that prevent cell adhesion at smooth articulated surfaces within joints

that would otherwise be impeded by cell occupancy[6]. Conversely, deposition of the pro-adhesive ECM molecule fibronectin within the synovial lining layer membrane helps to maintain cellular interaction networks by anchoring synovial fibroblasts to their surrounding matrix[55] (FIG. 3c). Expression of fibronectin at the basal lamina and at the endothelial surface in inflamed synovium has been proposed to serve as a permissive migration track for infiltrating lymphocytes, enabling T cells to cross the endothelial basement membrane in RA[56,57] (FIG. 3d). Ectopic expression of fibronectin in the RA joint also enables aberrant cell adhesion; for example, large amounts of fibronectin in the pannus enhances synovial fibroblast adhesion to cartilage, stabilizing invadopodia (actin-rich protrusions of the plasma membrane that are associated with tissue degradation) by promoting coherent points of anchorage that facilitate cartilage invasion[58] (FIG. 3e). The matrix also has an important role in restricting cell migration, as the synovial membrane serves as a barrier to maintain immune privilege in the synovium, which is disrupted in RA[37].

***Patterning of soluble factors.*** Soluble factors, such as cytokines, chemokines and growth factors, by virtue of their being secreted by cells, are part of the matrisome. The role of several of these pro-inflammatory mediators in RA is well documented and forms the basis for a number of the current biological therapies that are used to treat people with RA[59]. However, within tissues, these molecules often require interaction with other matrisomal components to signal, and their presentation, concentration and bio-availability throughout the synovium provides context for their function (FIG. 3d). Indeed, core matrisomal molecules control the localization of soluble factors in tissues and can determine their activity. Chemokine immobilization by glycosaminoglycans (particularly heparan sulfate proteoglycans (HSPGs)) at the luminal endothelial surface of blood vessels establishes chemokine gradients for migrating leukocytes[60], as well as protecting these soluble factors from degradation[61] and facilitating oligomerization required for optimal activity[62]. For example, in the RA synovium, increased expression of the HSPG syndecan 3 tethers CXCL8 in the endothelial lumen, and this interaction promotes leukocyte trafficking into the inflamed tissue in mice with antigen-induced arthritis[63,64].

The ECM is also an essential reservoir for other soluble factors, including cytokines, bone morphogenetic proteins, Wnts and growth factors, the binding of which is both promiscuous and specific. The ECM molecules fibronectin, vitronectin, tenascin C, osteopontin, type I collagen and fibrinogen each bind to several soluble factors from among the vascular endothelial growth factor (VEGF), platelet-derived growth factor, fibroblast growth factor (FGF), transforming growth factor, insulin-like growth factor and bone morphogenetic protein families. However, each ECM molecule has a distinct set of soluble binding partners. Moreover, these molecules bind with different affinities across each family of growth factors; for example, tenascin C binds to VEGFB but not to VEGFA, vitronectin binds to FGF18 but tenascin C does not, and neither bind to FGF1 or FGF6 (REF.[65]). These interactions not only control the concentrations and locations of soluble factors within tissues, but are also essential for their function by serving as co-receptors. The binding of core matrix molecules to soluble factors can also be complex and involve higher order interactions between multiple matrix-resident molecules to enable exact location-specific patterning. For example, the binding of vasculature-associated type V collagen to heparin sulfate[66], which in turn controls growth factor networks, has an important role in creating a signalling-rich perivascular niche[67]. Proteoglycans are well documented accessory molecules[68], and syndecans in particular have important roles in cartilage breakdown and synovial inflammation[69]. A good example of the use of ECM molecules as co-receptors is in FGF2 signalling. FGF2 is a growth factor that is upregulated in RA and contributes to promoting fibroblast activation during disease progression[70]. Optimal activity of FGF2 requires the formation of a ternary complex between the heparin sulfate chains of syndecan 4 and the FGF receptor, as well as signalling via the cytoplasmic domain of syndecan 4 to strengthen the duration and intensity of downstream signalling upon ligand binding[71]. Clearly, the role of this, and many other, soluble factors might not be fully understood without examining how they interact with other extracellular tissue components. Moreover, simply targeting the activity of individual soluble factors in RA might not represent the most effective, or tissue-specific, means of modulating their activity.

***Direct signalling to cells.*** ECM molecules provide important biochemical signals directly to cells. By virtue of their ability to interact with a large repertoire of cell surface receptors, including integrins, ECM molecules can influence a range of cellular behaviours, including proliferation, survival, cell death and differentiation[45]. Small soluble effector molecules tend to evoke relatively simple signalling pathways; for example, at 17 kDa, TNF activates just two receptors, TNFR1 and TNFR2 (REF.[72]). By contrast, ECM molecules are large multi-modular molecules; thus, they have more complex interaction partners. Thrombospondin 1, for example, is a 450-kDa secreted glycoprotein with seven modular domains that is present at increased concentrations in RA serum and synovium[73,74]. This ECM molecule has at least 83 different binding partners, including other ECM molecules and soluble factors, as well as a plethora of cell surface receptors[75].

Direct cues from the tissue microenvironment have an important role in maintaining tissue homeostasis. Endogenous danger signals are immunologically silent in healthy tissues but can trigger pro-inflammatory responses upon cellular stress or tissue damage. These danger signals include alarmins (intracellular molecules that are released into the extracellular milieu during cell activation or death[76]) and ECM molecules that are upregulated or modulated upon tissue injury or that undergo post-translation modification[77]. These damage-associated molecular patterns are sensed by pattern recognition receptors such as TLRs and integrins, triggering innate immune responses and shaping

adaptive immune responses designed to restore homeostasis and activate tissue repair[77]. In the joints of people who do not have RA, these signals are essential in order for cells to detect and respond to injury and insult. However, dysregulation of these pathways is emerging as a major cause of chronic inflammation and tissue destruction in RA. For example, tenascin C is an ECM molecule that is not expressed in most healthy tissues, including the joint, but that is transiently upregulated following tissue injury, upon which it activates TLR4-mediated inflammation[78]. Typically downregulated and cleared from tissues following repair, tenascin C accumulates in large amounts in the synovium of people with RA[79]. Expression of this pro-inflammatory ECM molecule is required for the persistence of joint inflammation and tissue destruction in several different experimental models of arthritis[78,80,81]. These studies collectively exemplify how the ECM surrounding and supporting cells has a vital role in dictating site-specific behaviour via directly signalling to cells.

## ECM in the pathogenesis of RA

Emerging data indicate that dysregulated signals from the ECM might promote chronic inflammation in the joint during the pathogenesis of RA, and that targeting these signals might provide an effective means of restoring immune control. Whole-exome sequencing has revealed new gene variants associated with RA susceptibility, among which variants in genes involved in ECM and ECM receptor signalling pathways (such as *COL4A4*, *COL6A5*, *COL11A1*, *COL11A2*, *HSPG2*, *ITGB5*, *LAMC1*, *THBS1*, *RASGRF1*, *FLNB* and *MYL5*) were highly enriched[82]. Microarray analysis comparing healthy synovium with RA synovium also revealed differentially expressed genes involved in cell adhesion and organization of the ECM (such as *PTPRC*, *SDC1*, *CD8A*, *CD2*, *HLA-DPA1*, *ITGA4*, *HLA-DMB*, *CD6*, *HLA-DOB*, *PDCD1LG2*, *COL3A1*, *SDC1*, *COL1A2* and *INTGB2*)[83]. Although the effects of sequence variation or upregulation of such genes in people with RA is unknown, these data implicate changes in the ECM and the tissue microenvironment in RA pathogenesis.

Altered tissue turnover has long been a pathological hallmark of RA[7,8,84,85], and serum concentrations of ECM metabolites are commonly used as biomarkers of joint remodelling and bone degradation[86,87]. To assess bone turnover, the C-telopeptide fragment of type I collagen (which is generated by osteoclast-derived cathepsin K) is used as a biomarker of bone resorption[88], whereas osteocalcin (which is produced by mature osteoblasts) and the N-terminal type I procollagen propeptide (which is released during collagen fibril synthesis) are used as biomarkers of bone formation[89]. Cartilage degradation is assayed by examining serum concentrations of cartilage oligomeric matrix protein[90], the C-terminal telopeptide of type II collagen[91] and C2M (a fragment of type II collagen)[92]. Similarly, synovial remodelling is reflected by high circulating concentrations of C1M, C3M and C4M (fragments of type I, type III and type IV collagen, respectively), which are generated by MMP cleavage[93–96], or by high concentrations of proteases implicated in tissue destruction, such as total MMP3 or activated MMP3 (REFS[97,98]). A reduction in serum concentrations of ECM metabolites accompanies positive responses to therapies including tocilizumab, methotrexate, adalimumab and tofacitinib[95,99–102]. Analysis of such biomarkers at baseline can also be used to predict who will respond well to tocilizumab[99], and to predict a lack of efficacy of Syk inhibition via fostamatinib on structural end points[103]. These serological markers therefore serve as reliable surrogates of tissue destruction in RA and might prove useful in stratifying the responses of patients to treatments.

Emerging data also show that ECM metabolites are not simply inert molecules that are released from joint tissue as collateral damage as disease progresses but are actively involved in RA pathogenesis. Expression of the tissue-degrading enzyme membrane type 1-matrix metalloproteinase (MT1-MMP) is increased in the joints of people with RA at sites of pannus invasion into cartilage[18]. Upregulation of MT1-MMP via activation of the cell surface receptor DDR2 on synovial fibroblasts is more pronounced when induced by collagen variants that are missing non-helical telopeptides than when induced by intact collagen fibrils, and is enhanced in response to damaged cartilage[104], suggesting a positive feedback loop in which collagen degradation reinforces further tissue destruction. Fragments of hyaluronic acid are also detected in RA synovial fluid[105]. The size of hyaluronic acid fragments dictates the function of this glycan; for example, low-molecular-weight fragments activate TLR2-mediated inflammation in macrophages, whereas high-molecular-weight fragments do not[106]. These data suggest that increased amounts of ECM metabolites contribute to both tissue remodelling and inflammation in RA.

Fragments of osteopontin are also present at increased concentrations in synovial fluid from people with RA[107]. Cleavage of this ECM molecule by thrombin creates a C-terminal fragment that induces CD44-dependent macrophage chemotaxis, and an N-terminal fragment that promotes β3-integrin-mediated macrophage spreading and activation[108,109]. The pro-inflammatory activity of osteopontin fragments is further regulated by phosphorylation; whereas the chemotactic activity of the C-terminal fragment is independent of modification, the N-terminal fragment requires phosphorylation to induce macrophage activation, leading to cytokine and MMP release[108,109]. Higher amounts of phosphorylated osteopontin and phosphorylated osteopontin fragments are found in synovial fluid from patients with RA than in synovial fluid from patients with OA, but there are no differences in total osteopontin concentrations between RA and OA[110], suggesting that both proteolytic processing and post-translational modification of ECM molecules contribute to disease activity. Indeed, autoantibodies that recognize citrullinated proteins (which are created by the post-translational conversion of arginine to citrulline via peptidylarginine deiminases) are gold-standard diagnostic markers for RA[111]. Anti-citrullinated protein antibodies recognize a number of modified ECM molecules[112,113] including: citrullinated epitopes in type II collagen[114], which are well-established pathogenic mediators of joint disease in vivo[115,116]; citrullinated fibrinogen[117], increased concentrations of which are predictive of higher disease

activity scores[118]; citrullinated tenascin C[119], which might have a role in delineating different disease aetiologies[120]; citrullinated aggrecan, concentrations of which correlate with frequencies of citrullinated-aggrecan-specific T cells in people with RA[121]; and citrullinated fibronectin[122]. Intra-articular injection of citrullinated collagen or citrullinated fibrinogen enhances their arthritogenic potential compared with their unmodified forms[123–125]. Moreover, citrullination of fibrinogen, fibrin or fibronectin in vitro enhances their pro-inflammatory capabilities[126–128], whereas citrullination of collagen or fibronectin alters their integrin binding repertoires and capacities to support synovial cell adhesion[122,129]. Citrullinated fibronectin effectively promotes cell survival, in contrast to unmodified fibronectin, which induces apoptosis[55,126]. Citrullinated fibronectin also exhibits increased affinity for VEGF but is less effective at binding to and inhibiting the aggrecanase ADAMTS4 than unmodified fibronectin[130,131]. As such, ECM molecule modification can not only break tolerance (create novel antigen epitopes that lead to the generation of T cell and B cell responses against endogenous molecules), it can also generate pathological protein variants that can exacerbate inflammation in the RA joint.

## Diagnosis — the truth is in the tissue

One question that arises from the study of circulating ECM metabolites and antibodies that recognize modified ECM molecules is how well these biomarkers reflect tissue pathology in the joint. Examination of collagen, fibrinogen and fibronectin via immunohistochemistry in biopsy-obtained synovial tissue samples has been used to assess the degree of fibrosis in RA synovium[132]. This approach, although more invasive than serological analysis, takes into account the idea that synovial pathology is compartmentalized and enables the examination of RA pathogenesis in the context of synovial anatomy. These details of tissue architecture are likely to be important in enabling a full understanding of synovial pathology. For example, microfibrillar-associated protein 4 (MFAP4), an ECM molecule that binds to elastin and collagen, is implicated in stromal hyperplasia and fibrosis in liver and lung diseases[133]. MFAP4 is found at similarly high concentrations in the serum and synovial fluid of patients with RA and patients with OA, compared with the low concentrations that occur in healthy individuals[129]. In the synovium, MFAP4 is detected in synovial sub-lining arteriole vessel walls and in adventitial tissue at sites of immune cell infiltration; however, it is absent from the internal elastic membrane of vessels in RA synovium, while present at high concentrations at this site in OA synovium[134]. The consequences of the differential distribution of MFAP4 in OA and RA synovial tissues are not yet clear, but these data highlight the fact that alterations in local tissue architecture are not always reflected in 'bulk' serum or tissue analysis.

Although circulating biomarkers can correlate with tissue pathology, they are not always causal, and it is clear that changes to biomarkers in the serum do not mirror the totality of changes in the synovium. Work examining the distribution of tenascin C in the joint exemplifies how important mechanistic detail can be lost without the context of tissue anatomy. Concentrations of this pro-inflammatory ECM molecule are increased in the serum and synovial fluid of patients with RA[135,136], correlating with the amount of bone erosion that occurs in these patients and predicting poor improvements in measures of pain in response to TNF inhibition[136]. In the RA synovium, tenascin C is found predominantly in the sub-lining layer, where it is restricted to two specific niches: a dense ECM surrounding CD34-negative fibroblast populations and close to CD34-positive perivascular fibroblasts that are located underneath blood vessels at sites of lymphocyte infiltration[137]. This detailed mapping reveals specific cellular targets for tenascin C in the RA joint, which might have remained obscured without anatomical analysis, and directs further mechanistic investigation into the potential role of tenascin C in promoting prolonged activation of pro-inflammatory signalling in fibroblasts[78,138] or in modulating pericyte adhesion, migration[139] or differentiation[140] during RA.

Considering the advances that have taken place in our knowledge of the cellular and molecular basis of synovial inflammation, it is clear that analysis of cell subset interaction networks in the tissue (for example, the presence of inflammatory versus destructive fibroblasts, or the numbers of T peripheral helper cells or HBEFG$^+$ macrophages), together with the microenvironmental cues that instruct their behaviour, will probably be the most accurate way of assessing the events that underlie RA. Such analysis will enable more precise disease classification, leading to process-driven patient stratification and improved targeted therapeutic interventions. However, although advances in synovial tissue biopsy methodology have enabled safer and more practicable tissue acquisition (sometimes involving two or more repeat samples)[141], by design, such interrogation of tissue micro-niches might be subject to sampling heterogeneity, and approaches designed to image the synovium in vivo could provide a useful complement to tissue harvest. PET scanning using targeted radiotracers to visualize specific ECM components such as collagen[142] or fibronectin[143] is being developed as a viable method of imaging tissue fibrosis in vivo (reviewed elsewhere[144,145]). PET imaging of GPVI-Fc, a fusion protein comprising the soluble human IgG1 Fc domain and the extracellular domain of platelet glycoprotein VI (a trans-membrane platelet glycoprotein that binds with high affinity to ECM molecules including collagen, fibronectin and fibrinogen), is also emerging as a means of visualizing changes in the synovium in vivo. This chimeric molecule has been used to image nascent exposure of ECM during tissue damage and synthesis of new fibrous tissue in anti-glucose-6-phosphate isomerase serum-induced experimental arthritis[146]. These approaches constitute the first steps towards detailed molecular analysis of the synovial ECM in real time in vivo.

## Exploiting the tissue microenvironment

Understanding the cells of the synovium and the tissue microenvironment at unparalleled resolution has not only illuminated our understanding of the biology of the joint and provided insight into disease status and disease mechanisms, it is also paving the way for new

Table 3 | **ECM-targeting strategies in development for the treatment of RA**

| Approach | Example | Mode of action | Development phase | Refs |
|---|---|---|---|---|
| ***Drug delivery*** | | | | |
| Immunocytokine | Cytokine–antibody fusion protein dekavil (F8-IL10) | Mediates delivery of an anti-inflammatory cytokine to inflamed joints via recognition of an ECM molecule | Phase Ib | 151 |
| Chimeric antibodies | Anti-TNF antibodies fused to the heparin-binding domain of PlGF2, or to the collagen-binding domain of decorin | Antibodies are preferentially retained in the inflamed joint | Preclinical | 156,157 |
| ***Drug activity*** | | | | |
| Chimeric cytokine receptors | Soluble TNFR fused to an MMP-cleavable adiponectin-derived cap | Creates controllable receptor–cytokine binding that is activated at sites of high protease activity, acting as a sink for free cytokine | In vitro | 158 |
| ***Inhibition of pathological processes*** | | | | |
| Tissue destruction | Therapeutic monoclonal antibodies that recognize MMPs | Blocks the tissue degrading activity of specific proteases | Phase Ib (anti-MMP9 antibodies) | 161 |
| | | | Preclinical (anti-MT1-MMP antibodies) | 162 |
| Leukocyte infiltration | Decoy chemokines, such as signalling-incompetent variants of CXCL8 with high heparin sulfate affinity, or peptides comprising the CXCL8 heparin-binding domain | Displaces endogenous chemokines from tissue glycosaminoglycans | Preclinical | 163,164 |
| | Decoy glycosaminoglycans, such as soluble syndecan 3 | Competes for chemokine binding to endogenous glycosaminoglycans | Preclinical | 165 |
| Synovial inflammation | Therapeutic monoclonal antibodies that block osteopontin–fibronectin interactions or that prevent the activation of TLR4 by the fibrinogen-like globe domain of tenascin C | Block interactions between ECM molecules or cell activation via ECM molecules | Preclinical | 137,167 |

CXCL8, CXC-chemokine ligand 8; ECM, extracellular matrix; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1-matrix metalloproteinase; PlGF2, placenta growth factor 2; RA, rheumatoid arthritis; TLR, Toll-like receptor; TNFR, TNF receptor.

therapeutic strategies to be developed. The ECM is being used as a target in the development of a wide variety of new treatments[147], which are being applied to RA in a number of different ways (TABLE 3).

***Advances in drug delivery.*** Exploiting the tissue specificity of ECM molecule expression has led to new approaches in drug delivery. Linking established anti-inflammatory agents to antibodies that recognize ECM molecules that are not found in healthy tissue but which are upregulated at disease sites has created a new class of immunomodulatory agent that can home in on areas of disease and deliver localized, site-specific treatment. This approach has been comprehensively reviewed elsewhere[148] and is most recently exemplified by F8-IL10 (also known as dekavil), a cytokine–antibody fusion protein comprising a single-chain variable domain fragment of antibody F8 and the anti-inflammatory cytokine IL-10. F8 recognizes the extra domain A of fibronectin, a fetally restricted splice variant of this ECM molecule, which is re-expressed in adults at sites of inflammation and in cancer[149]. F8-IL10 exhibits targeted delivery of IL-10 to the inflamed synovium in murine models of arthritis, and to both clinically and sub-clinically inflamed joints in patients with RA[150]. Although PET–CT imaging revealed the unexpected localization of F8-IL10 to the liver and spleen in patients with RA, no safety issues were reported in phase Ib clinical trials in RA[151]. This approach might effectively overcome the lack of efficacy of systemically administered IL-10 in patients with RA[152]. Indeed, F8-IL10 inhibited the progression of established disease in mice with collagen-induced arthritis when tested alone and in combination with methotrexate[153], and showed early signs of therapeutic benefit in over half of the participants in a phase Ib study[151]. F8-IL10, and other fusion proteins designed to deliver anti-inflammatory agents directly to inflamed sites, represent a novel class of therapeutic agents that selectively block antigens at the site of inflammation by targeting the ECM[148].

Engineering the ECM-binding capabilities of anti-TNF antibodies also shows promise for improving the efficacy of targeting TNF using intra-articular injection as a delivery method. Systemic TNF blockade can induce generalized immunosuppression, whereas intra-articular administration of anti-TNF antibodies reduces the risk of systemic immunosuppression but is limited by rapid drug clearance from inflamed joints[154,155]. Chemical conjugation of the heparin-binding domain of placenta growth factor 2 (which binds with high affinity to many different ECM molecules) to rat monoclonal antibodies that recognize mouse TNF increased antibody retention times in the joint and substantially improved clinical scores in mice with collagen antibody-induced arthritis, compared with unconjugated anti-TNF antibodies[156]. Similarly, conjugating anti-TNF antibodies to the collagen-binding domain of decorin improved antibody accumulation in inflamed paws during collagen

antibody-induced arthritis and suppressed disease progression more effectively that unmodified antibody[157]. This approach might make intra-articular drug administration feasible for monoarthritis and help to limit the off-target effects of systemic immune suppression.

TNF blockade has also been re-engineered using MMP-cleavable inhibitory peptides. The construction of a chimeric TNFR in which the trimerization domain of adiponectin is linked to the N-terminus of the extracellular domain of TNFR2 via a substrate sequence for MMPs 2 and 9 creates a cap that blocks the access of TNF to the TNFR, which can be released by MMP cleavage[158]. In vitro, this approach successfully allowed controlled binding of the chimeric TNFR to TNF[158]. If these results can be recapitulated in vivo, then increased MMP activation at sites of inflammation could be used to enable TNF to bind to soluble chimeric receptors, thereby precluding the activation of cellular TNFRs and providing a powerful means of conferring inflamed tissue-selective TNF blockade.

***Preventing ECM degradation.*** An altogether different strategy for treating RA is to directly target ECM degradation to prevent excessive joint tissue destruction (reviewed eslewhere[159,160]). Although early approaches using broad-spectrum small molecule MMP inhibitors were fraught with unacceptable adverse effects, attempts with specific protease inhibitors show more promise. A phase Ib trial of anti-MMP9 monoclonal antibodies showed that this approach is safe and well tolerated[161], and preclinical data have shown that combining TNF and MT1-MMP blockade confers long-term protection from inflammation and tissue damage in mice with collagen-induced arthritis[162]. These data highlight how inhibiting both inflammatory and tissue-destructive processes at the same time can exert synergistic effects in established disease. However, targeting these mediators affects comparatively late events in RA pathogenesis.

***Manipulating the binding of soluble factors to the ECM.*** New data have begun to reveal the possibility of intervening early on in the disease process, before deregulated cytokine networks and tissue destruction are evident. One elegant way of intervening at the point of leukocyte invasion into the inflamed synovium could be to use decoy chemokines. Engineered to have a higher affinity for glycosaminoglycan interaction sites, but to be incapable of competent signalling via chemokine receptors, these agents can effectively displace wild-type chemokines from essential ECM binding sites, thereby acting as powerful dominant negative chemokine inhibitors. For example, CXCL8 variants with enhanced HSPG binding and ablated CXCR1 or CXCR2 binding reduced peri-articular neutrophil infiltration and inhibited leukocyte adhesion to the venules at the site of joint inflammation in mice with methylated bovine serum albumin-induced experimental arthritis, resulting in inhibited leukocyte transmigration into the knee cavity[163]. Similarly, short-chain basic peptides, representing the glycosaminoglycan-binding region of chemokines such as CXCL8, bind to HSPG with high affinity, reduce leukocyte migration through an endothelial cell layer in vitro, compete with intact CXCL8 for binding around the endothelium in synovial tissue from patients with RA and reduce inflammation and neutrophil infiltration in mice with antigen-induced arthritis[164]. Alternatively, administration of the soluble extracellular domain of syndecan 3 has been used to sequester unwanted chemokines in the joint. Soluble syndecan 3 inhibited the migration of CCL7-activated leukocytes in vitro and ameliorated histological disease severity in experimental models of RA, concomitantly reducing the number of blood vessels that stained positive for CCL7 in the inflamed synovium[165].

***Targeting chronic pro-inflammatory signals from the ECM.*** ECM molecules are more than just postcode proteins that can be used to deliver existing drugs, placeholders for chemokines or substrates for proteolytic degradation; they also have an important role in promoting disease. By creating distinct niches within the RA joint, the ECM delivers aberrant pro-inflammatory signals to resident cell networks. Targeting these networks can be useful in modulating disease at an early stage. For example, the binding of thrombin-cleaved osteopontin to fibronectin at the cell surface of synovial fibroblasts aids B cell adhesion and stimulates the production of pro-inflammatory cytokines in vitro[166]. A single-chain variable fragment antibody that recognizes osteopontin and blocks its interaction with fibronectin effectively reduced synovial fibroblast migration and adhesion to B cells in vitro and improved the clinical score, amount of synovial hyperplasia and cartilage damage, and cytokine concentrations when given at an early time point to mice with collagen antibody-induced arthritis[167]. These data show how targeting important ECM molecule interactions during disease onset can be useful in preventing the formation of immune permissive environments.

Increasingly, it is becoming apparent that changes in the synovial microenvironment take place long before any overt clinical symptoms emerge. For example, serum concentrations of tenascin C and ficolin 1, both of which are secreted endogenous TLR4 agonists[81], are raised in people with synovitis who go on to develop RA compared with in people with synovitis that spontaneously resolves[168,169]. Interestingly, baseline concentrations of ficolin 1 are predictive of disease remission in RA[168]. Moreover, therapeutic monoclonal antibodies that inhibit TLR4 activation by the fibrinogen-like globe of tenascin C prevent chronic inflammation and halt disease progression when given at an early time point during collagen-induced arthritis[137]. These data suggest that identifying and targeting events that precede disease development might pave the way for better outcomes via early intervention, and even raise the possibility of disease prevention in pre-symptomatic individuals. This new ECM-modifying class of drugs functions by blocking signals from the inflamed synovium, and therefore also offers the advantage of selective blockade of tissue-specific and disease-specific cues, rather than systemic immune suppression, by suppressing the true mediators of disease but leaving intact a patient's ability to respond to infection.

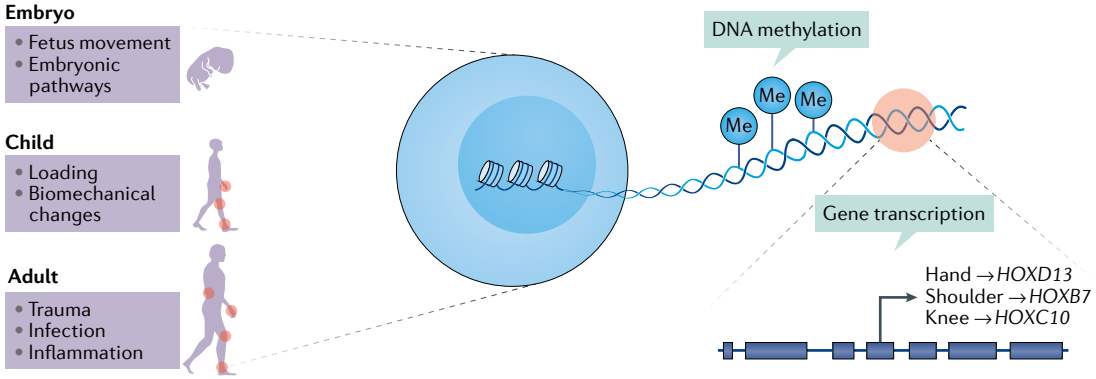



Fig. 4 | **Shaping joint-specific cellular phenotypes.** Positional memory in joint stromal cells can be modified at all stages of life. During embryonic development, joint-specific pathways and stimulatory signals such as fetal movement work in concert with joint-specific homeobox (HOX) gene expression to shape the different joint regions. In early childhood, the transition to walking upright is associated with substantial adaptation of motor and biomechanical processes that shape gene expression in the tissues involved. Later in life, non-physiological loading, trauma and other environmental factors such as infection and inflammation (for example, rheumatoid arthritis) can lead to joint-specific changes.

## Challenges and future perspectives

Although the therapeutic approaches discussed in the previous section seem promising, with some already in early-phase clinical trials[150] and others opening up potential windows for very early disease intervention or even prevention[170], many questions remain. At the most fundamental level, we do not yet have a full picture of what combination of the >1,000 matrisomal genes are expressed in the synovium, or how the resultant proteins and proteoglycans are organized at the sub-synovial level. Advances in proteomic analysis techniques are providing a much greater depth of interrogation of ECM constituents in various tissues[171,172]; however, proteomic deconstruction is challenging for the synovium because large amounts of tissue are rarely available, particularly from healthy individuals or people with early RA.

RNA sequencing of single cells from RA joints has provided striking resolution of gene expression at the cellular subpopulation level. However, this approach alone does not capture the full complexity of the tissue microenvironment, which necessitates an understanding not only of gene expression but also of post-transcriptional processing and protein post-translational modification, all of which are important factors in dictating ECM assembly and function. Furthermore, high-resolution cellular analysis at a single point in time makes it difficult to discern whether cell populations identified in this way represent distinct cell types (and lineages) or the same cell types at distinct points on a spectrum of phenotypic polarization.

An additional challenge lies in understanding precisely how target cells respond to the integrated biochemical and mechanical signals provided by multicomponent 3D tissue microenvironments. Many approaches to assessing cell phenotype require the isolation of cells from tissues; however, the process of cell isolation has a profound effect on the cell phenotype itself, accounting for as much as 40% of the transcriptome of processed cells[173,174]. This effect makes it difficult to differentiate cell behaviour instructed in situ from that caused by the stress of cell purification processes. Technologies such as NICHE-seq[175] or spatial transcriptomics[176] can now provide information about localized gene expression programmes, whereas matrix-assisted laser desorption/ionization mass spectrometry imaging can enable the visualization of the spatial distribution of molecules, such as glycans, peptides or proteins, by their molecular masses[177]. Used in parallel with multiplex imaging and the improved capabilities in optical sectioning provided by light sheet microscopy, which enables imaging of intact tissues and organs at a good resolution[178], these methods can now be applied to better resolve the content of the ECM of the joints and its organization at the single-cell level in situ. Such approaches could yield a potentially rich source of tractable new targets with which to diagnose and treat inflammatory joint diseases.

Another important consideration is how external cues contribute both to programming cell identity and to orchestrating the transient cellular activation states required to respond to dynamically fluctuating tissue conditions. In tissue-resident macrophages from different organs, the tissue environment is crucial in the creation and maintenance of organ-specific macrophage functions[179], although the full extent of how integrated external signals programme this positional memory remains to be completely unravelled. Tissue-derived signals potentially also shape the phenotypes of fibroblasts from different organs; for example, differences exist in the epigenetic landscape, gene expression and response to stimuli of cultured synovial and dermal fibroblasts, suggesting a stable imprinting of organ-specific gene expression, even when cells are dissociated from tissue architecture[180–182]. In synovial[183], dermal[184] and intestinal fibroblasts[185], the expression of homeobox genes (which govern positional cellular identities during embryonic development) differs between anatomical regions within tissues, showing that the anatomical site also shapes cellular gene expression. This site-specific expression is illustrated nicely by the differences found between tissues in hip, knee and ankle joints[183,186–190]. Mechanical stimulation of joint cells is another well-established mediator of cell identity during embryonic development[191], as well as postnatally, and also influences the composition of

the ECM[192,193]. Together, these data imply that, at different anatomical sites, differences in embryonic development and environmental cues induce changes in the content and structure of the synovial microenvironment and define cell behaviour at transcriptomic and epigenetic levels, which could, at least partially, explain the specific pattern of joint involvement seen in many joint diseases (FIG. 4).

## Conclusions

The interrogation of synovial cell populations using single-cell transcriptomics and spatial mapping of the cell subsets identified by this approach within tissues is revealing the detailed anatomical complexity of the synovium. Our understanding of the cellular basis of synovial health and disease has been accelerated by examination of how specialized cell networks function within discreet synovial neighbourhoods. In parallel, analysis of the role of the tissue microenvironment in defining synovial tissue structure and function is starting to reveal how extracellular cues are essential in organizing cellular networks and in directing niche-specific cell behaviour. These data are also changing our thinking about how inflammatory joint disease arises and progresses, supporting more holistic consideration of synovial cell networks, wherein communication between different cell types and their surrounding ECM within discreet but interconnected neighbourhoods in the synovium is essential for tissue homeostasis. Perturbations in any aspect of these symbiotic networks are deleterious to synovial tissue homeostasis and can be pathogenic. We are already starting to see how this new perspective has the potential to change clinical practice, both in terms of disease diagnosis and classification (for example, in efforts to use local changes in synovial tissue to better assess a patient's disease status) and in offering new treatment options that can either improve the efficacy or specificity of drugs currently used to treat patients with RA, or offer completely novel approaches to ameliorating disease.

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# TNF in the era of immune checkpoint inhibitors: friend or foe?

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Abstract | Immune checkpoint inhibitors (ICIs) are effective in the treatment of patients with advanced cancer and have emerged as a pillar of standard cancer care. However, their use is complicated by adverse effects known as immune-related adverse events (irAEs), including ICI-induced inflammatory arthritis. ICI-induced inflammatory arthritis is distinguished from other irAEs by its persistence and requirement for long-term treatment. TNF inhibitors are commonly used to treat inflammatory diseases such as rheumatoid arthritis, spondyloarthropathies and inflammatory bowel disease, and have also been adopted as second-line agents to treat irAEs refractory to glucocorticoid treatment. Experiencing an irAE is associated with a better antitumour response after ICI treatment. However, whether TNF inhibition can be safely used to treat irAEs without promoting cancer progression, either by compromising ICI therapy efficacy or via another route, remains an open question. In this Review, we discuss clinical and preclinical studies that address the relationship between TNF, TNF inhibition and cancer. The bulk of the evidence suggests that at least short courses of TNF inhibitors are safe for the treatment of irAEs in patients with cancer undergoing ICI therapy. Data from preclinical studies hint that TNF inhibition might augment the antitumour effect of ICI therapy while simultaneously ameliorating irAEs.

Immunotherapy is now a standard approach to cancer treatment alongside surgery, radiation, chemotherapy and targeted therapies. Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that augment the pre-existing host antitumour response by blocking down-regulators of the immune system including cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD1) and programmed cell death ligand 1 (PDL1). However, in augmenting host immune responses, ICIs cause autoimmune adverse effects, termed immune-related adverse events (irAEs), in >80% of treated patients, including high-grade irAEs in ~60% of patients being treated with a combination of ICIs, ~30% of patients being treated with a CTLA4 inhibitor and ~20% of patients being treated with an inhibitor of the PD1 pathway[1–4]. Almost any organ of the body can be affected by irAEs, but different ICIs tend to target different organs. For example, rash and colitis are common with anti-CTLA4, whereas arthritis and pneumonitis are more characteristic of anti-PDL1 and anti-PD1 therapy[1].

Approximately 4% of patients with cancer undergoing ICI therapy develop inflammatory arthritis[5], the majority of whom present with either a rheumatoid arthritis (RA) or a polymyalgia rheumatica phenotype[5–7]. Rheumatoid factor and anti-cyclic citrullinated peptide antibodies can be present in such patients but are less common than in patients with RA[6,7]. Guidelines for the management of ICI-induced inflammatory arthritis are based on expert consensus and borrow heavily from treatments that were developed for RA[8]. Most patients are initially treated with corticosteroids at doses determined by the severity of arthritis, and steroid-sparing agents including hydroxychloroquine, sulfasalazine and methotrexate. TNF inhibitors and occasionally IL-6R blockers are used in patients with steroid-refractory or persistently steroid-dependent arthritis[9–12].

Data from various studies show that patients undergoing ICI therapy who develop irAEs have improved progression-free survival and overall survival[13–15], suggesting that ICIs augment shared immune pathways that promote both irAEs and antitumour activity. This finding raises the logical question as to whether immunosuppressive agents used to treat irAEs also promote cancer progression, whether by interfering with the antitumour activity of ICIs or via another route. Although studies have documented the oncological safety of using corticosteroids to control irAEs[16], treatment of irAEs with high-dose corticosteroids, although life-saving for patients with severe irAEs such as myocarditis or colitis[17,18], was noted to reduce the overall survival of patients with hypophysitis[19]. Survival was also reduced in those patients who were receiving corticosteroid treatment at the time of initiation of ICI therapy[20]. This finding implies a need to identify targeted therapies

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**Key points**

- Different arms of the immune response are important for autoimmune versus anticancer activities, and TNF inhibitors restrain some of these arms while promoting or having a neutral effect on others.
- Preclinical studies provide evidence that short courses of TNF inhibitors, despite their efficacy in ameliorating immune-related adverse events (irAEs), do not restrain the anticancer effects of immune checkpoint inhibitors (ICIs).
- TNF inhibitor treatment of rheumatic diseases does not seem to increase the risk of cancer, except for non-melanoma skin cancer and possibly lymphoma.
- Short courses of TNF inhibitors are likely to be safe in the treatment of ICI-associated irAEs, but data on the safety of long-term TNF inhibitor use for irAEs are lacking.
- Clinical studies that directly assess the effect of TNF inhibitor treatment on ICI efficacy are required to draw conclusions regarding the safety of TNF inhibitor treatment for irAEs.

that block pathways contributing to irAE pathogenesis but that spare those pathways contributing to cancer survival.

The question of whether a treatment for irAEs promotes cancer progression is particularly relevant to rheumatologists because ICI-induced inflammatory arthritis often persists and can require long-term treatment with DMARDs such as TNF inhibitors[10]. In this Review, we address this question for TNF inhibitors by drawing from literature on the link between TNF and cancer, the link between TNF inhibitors and cancer both within and outside the context of ICIs, and the role of TNF in the tumour microenvironment.

## The multifaceted effects of TNF

When TNF was first isolated in 1975 by Carswell, Old and colleagues, it was identified as the factor responsible for endotoxin-induced haemorrhagic necrosis of experimental tumours[21]. The line of research leading to the isolation of TNF can be traced back to William Coley's use of bacterial extracts to treat patients with cancer starting in 1896 (REF.[22]). Although the validity of the clinical case series reported by Coley was controversial, his work motivated subsequent preclinical studies in animal models. Further research in 1944 showed that lipopolysaccharide endotoxin was the active agent in bacterial extracts inducing haemorrhagic tumour necrosis in a mouse model of benzopyrene-induced skin tumours[23]. In 1962, researchers found that serum from endotoxin-treated animals also induced tumour necrosis, implying that bacterial endotoxin acts indirectly, inducing an intermediary 'tumour necrosing factor' that acted on tumours[24]. It was this factor that was isolated by Carswell et al. in 1975 (REF.[21]). The gene encoding human TNF was cloned in 1984 (REF.[25]), and the gene encoding mouse TNF was cloned in 1985 (REF.[26]). Ascertaining the sequence of TNF led to the discovery that this protein is the same protein molecule as cachectin[27], a factor found to mediate acute shock and chronic cachexia during infection. Development of anti-TNF antibodies led to the discovery that TNF has an important role in RA synovial inflammation[28], corroborated by studies showing that human TNF transgenically overexpressed in mice induces synovial inflammation[29]. Thus, TNF was found to have roles in the pathophysiology of cancer, sepsis and inflammatory disease.

In this Review, we discuss the role of TNF in cancer as is relevant to the safety of TNF inhibitors in the treatment of irAEs. We also discuss the role of TNF in inflammatory disease as is relevant to the efficacy of TNF inhibitors in the treatment of irAEs, with the caveat that irAEs are iatrogenic disease entities whose aetiology is not well understood and might be different from that of spontaneous inflammatory diseases. Although irAEs are known to be caused by ICIs, it is not yet known which of the cell types that are modulated by ICIs mediate these autoimmune toxicities, or why TNF inhibition is an effective treatment, although previous work on the mechanism of action of TNF inhibitors will be a valuable guide. The roles of TNF in cancer and inflammatory diseases are summarized in BOX 1.

### *Role of TNF in cancer*

Early optimism that TNF would be a useful anticancer therapy was tempered by the realization that it has a narrow therapeutic window. In clinical trials, systemically administered TNF caused acute shock but without the antitumour responses originally reported by Coley[30]. The physiological serum concentration of TNF in humans is to the order of 10 pg/ml[31], whereas the doses of TNF used in these clinical trials corresponded to TNF serum concentrations to the order of 10 ng/ml[30]. It is now thought that haemorrhagic tumour necrosis induced by high-dose TNF is largely mediated by the pro-coagulant effects of TNF that lead to thrombosis within the tumour vasculature[32]. TNF has been shown to induce endothelial cell apoptosis in vitro[33]. If this process occurs in vivo it could be another mechanism by which high-dose TNF induces haemorrhagic tumour necrosis. The current consensus is that soluble TNF alone, at levels tolerated by patients, is not directly cytotoxic to cancer cells[34]. However, non-soluble membrane-bound TNF or TNF in conjunction with a second effector molecule can be directly cytotoxic. TNF does have direct cytotoxic effects on cancer cells when used together with small molecules that oppose inhibitor of apoptosis proteins (IAPs)[35]. In addition, membrane-bound TNF (which serves as a ligand to TNF receptors on adjacent cells) has been shown, in vitro, to have a direct cytotoxic effect on target cells, including the KYM-1D4 cancer cell line[36,37].

In parallel with studies of TNF as a potential antitumour therapy, evidence began to emerge in the late 1980s that TNF could in fact be a tumour-promoting factor. Patient-derived juvenile chronic myelogenous leukaemia cells were found to produce TNF and use it as an autocrine growth factor[38]. Another study, in a rabbit cornea model, showed that low-dose TNF induces angiogenesis, an unexpected finding given that high-dose TNF causes destruction of tumour vascular beds[39]. Later studies found that TNF stimulates tumour growth and does so in part by promoting angiogenesis[40].

Work since the late 1990s has shown that TNF at physiological levels (as opposed to the supraphysiological levels of TNF used in antitumour therapy) has a major role in tumorigenesis. In the 1990s, methods of generating gene knockout mice provided powerful tools to elucidate the role of specific genes in mammalian biology. The development of TNF knockout mice

led to the discovery that lack of TNF had a protective effect against skin tumours induced by the carcinogen DMBA[41]. Similarly, TNF receptor knockout mice were also protected from UVB-induced skin tumours[42]. Administration of TNF inhibitors to mice had a protective effect in urethane-induced pulmonary tumours and colonic tumours associated with chemically induced colitis[43,44].

Additional evidence for a pro-tumorigenic role for TNF came from studies of gastric cancer associated with *Helicobacter pylori* infection. Gastric mucosal tissue samples from patients with chronic gastritis, gastric intestinal metaplasia, gastric dysplasia or gastric adenocarcinoma all showed higher expression levels of TNF than samples from healthy individuals. Moreover, in the same individuals, a higher expression of TNF was associated with positivity for *H. pylori*, suggesting that the association of gastric cancer with *H. pylori* infection might in part be mediated by the induction of host TNF production by *H. pylori*[45]. Indeed, *H. pylori* can produce Tip-α, a protein that induces host TNF production and functions as a pro-tumorigenic factor in a manner possibly mediated by TNF signalling[46].

Another line of evidence indicating that TNF is pro-tumorigenic comes from genetic studies of human populations. The -308G/A polymorphism in the promoter of *TNF* modulates *TNF* transcription[47]; the less common A variant is a stronger transcriptional activator of the gene[47]. As a result, individuals with the heterozygous G/A genotype have a twofold increase in TNF production over individuals with the G/G genotype (as shown in a whole blood lipopolysaccharide stimulation assay[48]). Evidence from a number of case–control studies show that individuals with this promoter polymorphism are at an increased risk of cancer. For instance, a study of 9,986 patients with gastrointestinal cancer (colorectal, oesophageal, gastric, hepatocellular or pancreatic cancer) and 15,511 healthy individuals showed that the A/A and G/A genotypes taken together confer 1.2-fold odds of gastrointestinal cancer compared with the G/G genotype (95% CI 1.1–1.4)[49]. Another study of 5,757 patients with prostate cancer and 6,137 healthy individuals showed that the A/A and G/A genotypes taken together confer 1.5-fold odds of prostate cancer compared with the G/G genotype (95% CI 1.1–2.1)[50].

Box 1 | **TNF in cancer and inflammatory disease**

**Pro-cancer effects**
- Induces tumour angiogenesis[40]
- Promotes cancer cell survival and proliferation[137]
- Helps cancer cells to evade immune surveillance[146,148]

**Anticancer effects**
- Induces haemorrhagic tumour necrosis via a pro-coagulant effect[32], by inducing endothelial cell death[33]
- Membrane-bound TNF has direct cytotoxic activity against cancer cells[37]

**Pro-inflammatory effects**
- Upregulates endothelial cell expression of leukocyte adhesion molecules[53]
- Induces synovial fibroblast production of IL-6, IL-8 and prostaglandin[61]
- Induces a pro-inflammatory macrophage phenotype[65]

**Anti-inflammatory effects**
- Ameliorates T cell-driven experimental autoimmune encephalomyelitis[55]
- Promotes regulatory T cell suppressive function[66]
- Chronic TNF attenuates T cell response to antigen stimulation[58]

### *Role of TNF in inflammatory disease*

The first randomized double-blind trial of a TNF inhibitor for the treatment of inflammatory disease was a study in RA in which TNF inhibition showed considerable efficacy[51]. This study demonstrated the therapeutic potential of TNF inhibitors for inflammatory disease and spurred further studies that expanded clinical indications to psoriatic arthritis, psoriasis, ankylosing spondylitis, juvenile idiopathic arthritis, Crohn's disease and ulcerative colitis[52].

The clinical promise of TNF inhibitors also motivated studies into its mechanism of action and the role of TNF in inflammatory disease. TNF has a gatekeeping role in local tissue inflammation through its effects on vascular endothelial cells; it upregulates endothelial cell expression of surface adhesion molecules that recruit circulating leukocytes into the local tissue[53]. TNF also induces endothelial cell expression of cyclooxygenase 2 and subsequent prostaglandin release, resulting in vasodilation[53].

TNF is a cytokine that both promotes and restrains inflammatory processes through opposing functional consequences of signalling through its two surface receptors: TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2). TNFR1 is expressed on all cells, whereas TNFR2 is expressed on a restricted subset of cell types including immune cells, endothelial cells and neurons[54]. In general, TNF activates innate immune responses via TNFR1 signalling, while suppressing adaptive immune responses via TNFR2 signalling[54]. TNFR1 signalling in mesenchymal cells activates an innate immune response that promotes disease in RA, spondyloarthropathies (SpAs) and inflammatory bowel disease (IBD)[55]. By contrast, TNFR2 signalling seems sufficient to ameliorate T cell-driven experimental autoimmune encephalomyelitis[55], although the T cell subsets that mediate this effect are unknown. The suppressive effect of TNFR2 signalling on the adaptive immune response is thought to explain the unexpected aggravation of disease by TNF inhibition in a trial in patients with multiple sclerosis, as well as the sporadic occurrence of demyelinating disease in patients receiving a TNF inhibitor for other diseases[54].

The duration, or chronicity, of TNF stimulation helps to determine its effect on inflammatory pathology. Administration of TNF three times a week in NZB/W F1 lupus-prone mice delayed the onset of renal disease and led to improved survival at 3 months[56]. Similarly, TNF administered recurrently over 3 months protected non-obese diabetic mice against the development of diabetes[57]. In mice with T cells expressing a transgenic T cell receptor (TCR) specifically for the influenza

haemagglutinin antigen, repeated administration of TNF over 3 weeks yielded T cells that had attenuated responses to TCR stimulation; such T cells had a reduced capacity to proliferate and produce cytokines after TCR stimulation with the specific antigen[58]. Thus, chronic TNF stimulation might ameliorate autoimmune inflammation in part by reducing T cell responsiveness to antigen stimulation.

How TNF modulates cells in inflammatory disease tissue has been most closely studied for RA and IBD. Synovial samples from patients with RA show robust immunohistochemistry staining for TNF[59], and synovial fibroblasts and macrophages from patients with RA express TNF receptors[60]. TNF signals through TNFR1 on synovial fibroblasts to induce the production of IL-6, IL-8 and prostaglandin E2 (REF.[61]), all of which have pro-inflammatory properties. TNF has also been shown to signal through TNFR1 on $CD4^+$ T cells to inhibit T helper 1 ($T_H1$) and $T_H17$ cell differentiation and expansion in a mouse model of collagen-induced arthritis[62]. Correspondingly, TNF inhibitor treatment, which is expected to attenuate TNFR1 signalling, increased the numbers of lymph node $T_H1$ and $T_H17$ cells[62]. Although $T_H1$ and $T_H17$ cells are considered pathogenic, TNF inhibitor treatment also reduced the accumulation of these cells in the joint[62]. This finding concurs with the observation in patients with RA that TNF inhibition can increase numbers of peripheral blood pathogenic T cells while ameliorating arthritis[62,63]; TNF inhibitors have been shown to reduce inflammatory cell infiltration into the joint[64]. In studies of IBD, TNF inhibition induced macrophage differentiation to a regulatory phenotype that ameliorates intestinal inflammation[65]. TNF signalling through TNFR2 in various T cell subsets have different effects on experimental colitis. For example, TNFR2 signalling in regulatory T ($T_{reg}$) cells and $CD8^+$ T cells ameliorates colitis[66,67], whereas in $CD4^+$ effector T cells this signalling exacerbates colitis[68].

ICI-induced inflammatory arthritis and ICI-induced colitis share molecular features with RA and IBD, respectively. Synovial biopsy samples from a patient with ICI-induced inflammatory arthritis showed robust staining for TNF[69], similar to synovial samples from patients with RA[59]. Similarly, colonic biopsy samples from patients with ICI-induced colitis showed evidence of increased TNF signalling in myeloid cells compared with cells from healthy individuals[70], which is in line with the finding that TNF signalling in macrophages helps to mediate IBD[65]. These shared molecular features provide support for borrowing TNF inhibitors from the RA and IBD armamentarium to treat ICI-induced inflammatory arthritis and ICI-induced colitis.

## TNF inhibitor use and cancer risk

In this section, we review data from clinical studies investigating the link between TNF inhibitor use and the risk of cancer development and recurrence, and use this information to make inferences regarding the safety of TNF inhibitors during irAE treatment. Currently, limited data are available regarding the effect of TNF inhibitor treatment on the efficacy of ICI therapy (that is, the effect on cancer survival), but we can also draw from a large body of data on the link between TNF inhibition and risk of cancer development and recurrence accumulated over many patient years of experience using TNF inhibitors for a wide range of indications.

### *Effects on ICI efficacy*

In a study of 27 ICI-treated patients with melanoma who developed colitis and underwent TNF inhibitor treatment, the patients had a median progression-free survival of 3 months, which is comparable to that reported in previous studies of ICI-treated patients with melanoma who did not undergo TNF inhibitor treatment[71]. Similarly, a retrospective study of patients with ICI-induced colitis found no difference in overall survival in those patients treated with corticosteroids alone ($n = 38$) compared with those patients treated with corticosteroids plus a TNF inhibitor ($n = 23$) ($P = 0.263$)[72]. By contrast, in a study published in 2020, in which Verheijden and colleagues[73] examined patients with grade ≥3 irAEs of various types (65 of whom received a TNF inhibitor and 157 of whom received corticosteroids only), the median overall survival was lower in the TNF inhibitor-treated group than in the corticosteroid-only group (17 versus 27 months; adjusted HR 1.61; 95% CI 1.03–2.51). However, the use of overall survival as the end point might have introduced confounders as some high-grade irAEs (such as colitis) have a higher mortality than others (such as high-grade endocrine toxicity, which can be treated with hormone replacement); 61 of the 65 patients in the TNF inhibitor group had ICI-induced colitis, whereas the breakdown of irAE subtypes was not reported for the corticosteroid-only group[73]. This study also failed to account for the time to irAE onset, which, as a rule, is shorter for colitis than for endocrinopathies[74,75] and is shorter for more severe than for less severe irAEs[74–76]. This difference could have biased the results because of the different follow-up times and treatment exposures between patients who did versus patients who did not require a TNF inhibitor[77]. For example, patients who did not require a TNF inhibitor might also be the ones who developed later-onset irAEs; to be included in this group, patients had to have first survived long enough to acquire a later-onset irAE.

Empirically, it is notable that despite extensive efforts to identify tumour biomarkers to predict a clinical response to ICI therapy, TNF has not emerged as one such biomarker[78–80]. This finding suggests that TNF signalling in the tumour microenvironment has a neutral net effect on pathways that promote or inhibit the antitumour activity of ICI therapy, implying that TNF inhibition does not diminish this activity.

### *Effects on risk of cancer*

Post-marketing surveillance of TNF inhibitor treatment in patients with RA, SpA, IBD and psoriasis provides data on the effects of decreasing levels of TNF on cancer risk. However, these studies might be biased by the fact that patients with autoimmune disease have a higher baseline risk of cancer than the general population, including a higher risk of lymphoma and lung cancer for patients with RA and a higher risk of non-melanoma

skin cancer for patients with psoriasis[81–83]. This elevated risk is thought to be caused by the presence of chronic inflammation, but shared genetic and environmental risk factors might also have a role[81]. Thus, disease severity (which is associated with the degree of inflammation) could confound the analysis of TNF inhibitor-associated cancer risk, at least in observational studies, if TNF inhibitor treatment is given preferentially to patients with more active disease. Risk assessment is also confounded by concomitant medications taken by patients on a TNF inhibitor, as some conventional DMARDS are also associated with risk of cancer. Examples include an elevated risk of hepatosplenic T cell lymphoma in TNF inhibitor-treated patients with IBD taking concomitant thiopurines (for example, azathioprine or 6-mercaptopurine)[84] and an increased incidence of non-melanoma skin cancer in patients taking methotrexate[85]. Other factors that bias some studies of cancer risk in TNF inhibitor-treated patients include the use of self-reported cancer diagnoses, a lack of an active comparator group (for example, patients being treated with other DMARDs), a lack of adjustment for other treatments and comorbidities and inconsistent definitions of the time of TNF inhibitor exposure[86].

***Solid tumour malignancies.*** The above methodological limitations could, if anything, introduce spurious associations between TNF inhibition and cancer where there is none. It is therefore reassuring that most observational studies of patients with RA, SpA, IBD and psoriasis have failed to show any increased risk of cancer in patients being treated with a TNF inhibitor[87–91]. For example, a systematic literature review and meta-analysis of nine large RA registries (87,018 patient years in the TNF inhibitor treatment group and 50,734 patient years in the untreated control group) found no increased risk of solid tumours in patients undergoing TNF inhibitor treatment (risk ratio 0.84; 95% CI 0.60–1.18)[90]. An early meta-analysis of clinical trials did show an elevated risk of cancer in patients undergoing TNF inhibitor treatment (odds ratio 3.3; 95% CI, 1.2–9.1)[92], but was criticized for not using individual level data and for using an average follow-up period, even though the patients being treated with a TNF inhibitor had a longer follow-up period[86,93]. Subsequent meta-analyses of clinical trials have failed to show an increased cancer risk in patients being treated with a TNF inhibitor[94–97], and studies using large administrative datasets have been similarly negative[98,99]. One exception is the Wegener's Granulomatosis Etanercept Trial (WGET), which compared cyclophosphamide alone with cyclophosphamide plus a TNF inhibitor (etanercept) for the treatment of granulomatosis with polyangiitis, and found a higher incidence of solid tumours in patients who received combination therapy[100]. This finding suggests that there might be synergistic toxicity when TNF inhibitors are used together with cytotoxic agents, similar to that observed when TNF inhibitors are combined with thiopurines[84]. Such synergistic effects might have relevance for patients with cancer receiving ICI therapy in combination with chemotherapy who require irAE management.

***Lymphoma.*** A small number of studies have suggested that patients treated with a TNF inhibitor are at an increased risk of developing a lymphoma. One published series described 48 cases of malignancy reported to the FDA in children on a TNF inhibitor, half of which were lymphomas[101]. Even though this case series did not control for confounding factors such as the risk of cancer associated with the underlying condition or concomitant medications, the FDA issued a black box warning of cancer risk in children being treated with a TNF inhibitor and later warned of an excess risk of developing especially rare hepatosplenic T cell lymphomas for children with IBD being treated with a combination of a TNF inhibitor and thiopurine[102]. An analysis of TNF inhibitor-treated patients with IBD in the French National Health insurance database also showed a higher rate of lymphoma (HR 2.41, 95% CI 1.60–3.64)[103] compared with patients with IBD who had no TNF inhibitor exposure; furthermore, a study of patients with juvenile idiopathic arthritis, IBD or psoriasis that used a Medicaid database hinted at a similar, albeit non-significant, increase in risk of lymphoma in those patients receiving TNF inhibitor treatment (adjusted HR 2.64, 95% CI 0.93–7.51)[98]. However, administrative datasets lack important information about confounders such as disease phenotype and severity, and many other prospective rheumatic disease registries have failed to show any increased risk of lymphoma in TNF inhibitor-treated patients[87,104–107].

***Skin cancer.*** One study of a Swedish RA registry found an increased risk of melanoma in TNF inhibitor-treated patients with RA[108]. By contrast, no increased risk was found in a larger study that combined 11 European RA registries[109], in a Scandinavian SpA registry study[110] or in a large meta-analysis of RA clinical trials[111]. However, patients with psoriasis or RA undergoing TNF inhibitor treatment might be at an increased risk of non-melanoma skin cancer[112,113]. With the possible exception of non-melanoma skin cancer[114], studies have also failed to show an increased risk of cancer recurrence in patients with cancer being treated with a TNF inhibitor[115,116].

In summary, although the assessment of TNF inhibitor-associated cancer risk is confounded by the excess background risk in patients with rheumatic diseases, available evidence suggests that these inhibitors do not increase the risk of solid tumours. The risk might be increased for lymphoma, particularly in patients with IBD being treated with a TNF inhibitor plus thiopurines, or for non-melanoma skin cancer in patients with psoriasis being treated with a TNF inhibitor. These data provide reassurance about the safety of TNF inhibition for the treatment of irAEs in the setting of ICI use, except perhaps in patients being treated for lymphoma.

## irAE therapy: is TNF inhibition safe?

In this section, we review preclinical studies of the effects of TNF and TNF inhibition in the tumour microenvironment, and synthesize this information to make supporting inferences regarding the safety of TNF inhibitors in the treatment of irAEs. We summarize the immune and non-immune cell types present in the tumour microenvironment (TABLE 1), and discuss studies that address the

Table 1 | **Activities of various cell types in tumours and their effects on tumours**

| Effect on tumour | Cell type | Mechanism | Refs |
|---|---|---|---|
| **Antitumour effects** | CTLs | Kill cancer cells that display tumour-associated antigens; produce IFNγ, which has antitumour effects | 123,154 |
| | NK cells | Kill cancer cells that over-express ligands recognized by NK cell receptors | 155 |
| | $T_H$ cells | Maintain adequate numbers of CTLs; promote CTL tumour infiltration | 156 |
| | $T_H1$ cells | Promote the development of tumour antigen-specific CTLs; produce IFNγ, which has antitumour effects | 154,157 |
| | Dendritic cells | Present tumour antigens to naive antigen-specific T cells to induce their effector differentiation, including into $T_H1$ cells and CTLs | 157 |
| **Mixed effects** | $T_H2$ cells | Can be pro-tumorigenic or antitumorigenic depending on the context | 158 |
| | $T_H17$ cells | Can be pro-tumorigenic or antitumorigenic depending on the context | 140–142, 158 |
| | Endothelial cells | Regulate immune cell infiltration into tumours via adhesion molecules | 159 |
| | MSCs | Regulate immune cell infiltration into tumours via chemokines | 145 |
| **Pro-tumour effects** | Follicular helper-like T cells | Follicular helper-like $CD4^+$ $Foxp3^-$ $PD1^{hi}$ T cells are pro-tumorigenic | 121 |
| | $T_{reg}$ cells | Inhibit $T_H$ cell proliferation; inhibit differentiation of naive $CD4^+$ T cells into $T_H1$ and $T_H2$ cells; inhibit CTL cytotoxicity | 160,161 |
| | MDSCs | Decrease the numbers of CTLs, $T_H$ cells and NK cells; increase the numbers of $T_{reg}$ cells; inhibit the activity of CTLs by inactivating TCRs | 123,124 |
| | Tumour-associated macrophages | Produce CCL22, which recruits $T_{reg}$ cells; produce IL-10 and TGFβ, which are immunosuppressive cytokines | 162 |

CTLs, $CD8^+$ cytotoxic T lymphocytes; MDSCs, myeloid-derived suppressor cells; MSCs, mesenchymal stromal cells; NK cells, natural killer cells; $T_H$ cells, $CD4^+$ T helper cells; $T_{reg}$ cells, $CD4^+$ regulatory T cells.

effect of TNF, TNF inhibitors or ICIs on relevant cell types. We synthesize information from these studies into a model of interactions between cell types that also incorporates TNF inhibition and immune checkpoint inhibition (FIG. 1). This model enables us to systematically analyse the different paths by which TNF inhibitors and ICIs regulate cancer cell proliferation, and to make a prediction regarding the net effect of TNF inhibitors on tumour growth. We also discuss two preclinical studies that test this prediction.

The general pattern that emerges from this model is that TNF inhibition promotes the activity of antitumour immune cell types (such as $CD8^+$ cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells) while restraining the activity of immunosuppressive cell types (such as $T_{reg}$ cells and myeloid-derived suppressor cells (MDSCs)), with an expected net antitumour effect that augments the benefits of ICIs. The effect of TNF inhibition on dendritic cells and naive $CD8^+$ T cells is an exception that might be pertinent in the setting of prolonged TNF inhibitor use. A further caveat is that although two preclinical studies have tested the net effect of TNF inhibition on tumour growth, studies in patients will be needed to validate this model in the clinical setting.

### *ICIs, TNF inhibitors and tumorigenesis*

ICIs that target CTLA4, PD1, or PDL1 inhibit signalling through co-inhibitory[117] pathways in tumour-infiltrating T cells[118]. Both inhibition of CTLA4 signalling and inhibition of PD1 signalling promote proliferation of exhausted PD1-positive CTLs, which subsequently become 'reinvigorated' to take on an effector-like phenotype associated with antitumour activity[119]. These CTLs continue to express PD1 but proliferate and express the activation surface markers HLA-DR and CD38 (REF.[120]). ICIs have differing effects on different subtypes of $T_H$ cells[119,121]. Overall, ICIs promote the function of tumour-infiltrating T cells with a net antitumour effect[118,119]. PD1 signalling in myeloid precursor cells promotes the development of MDSCs[122], which are pro-tumorigenic[123,124]; thus, ICI might also exert their antitumour effect in part by inhibiting MDSC development[122]. The interactions between ICIs and tumour microenvironment immune cells, including $T_{reg}$ cells, tumour-associated macrophages and MDSCs, have been comprehensively reviewed elsewhere[78].

***Effect of TNF on CTLs and NKs.*** TNF signals through TNFR2 on CTLs to promote activation-induced cell death[125,126], thereby depleting the pool of antitumorigenic CTLs. TNF also signals through TNFR1 on CTLs to inhibit CTL infiltration into mouse B16K1 melanoma tumours, whereas TNF inhibition promotes CTL infiltration into tumours[127]. Moreover, TNF signalling in NK cells induces the expression of the co-inhibitory receptor TIM-3 (REF.[128]) and decreases the expression of the activating cytotoxicity receptor NKp46 (REF.[129]), thereby impairing NK cell antitumour activity. As TNF signalling restrains the function of these two antitumour immune cell types, it can be inferred that TNF inhibition would promote their function.

***Effect of TNF on $CD4^+$ T cells.*** TNF signals through TNFR2 on $T_{reg}$ cells to promote $T_{reg}$ cell proliferation[130] and thereby increases their suppressive function as a population[130]. TNF might also inhibit the suppressive function of $T_{reg}$ cells in vitro[131,132], but this effect seems to be outweighed in vivo by the effect of TNF on $T_{reg}$ cell population expansion[130,133]. TNFR2-positive $T_{reg}$ cells (defined as $CD4^+$ $CD25^+$ $TNFR2^+$ cells) suppress the proliferation of non-$T_{reg}$ cells (defined as $CD4^+$ $CD25^-$ cells)[134] to a greater extent than TNFR2-negative $T_{reg}$ cells, and are found at a high density in Lewis lung carcinoma tumours[134], as well as in human ovarian cancer ascites[135]. In a B16F10 mouse model of melanoma

lung metastasis, administering TNF promotes pulmonary $T_{reg}$ cell proliferation and increases metastatic tumour growth[136]. A TNFR2 blocking antibody inhibits proliferation of $T_{reg}$ cells isolated from human peripheral blood, and kills $T_{reg}$ cells isolated from ascites of patients with ovarian cancer[137], as well as from the blood of patients with cutaneous T cell lymphoma[138]. It can be inferred that TNF inhibition, by exerting the opposite effect to TNF, would restrain the function of immunosuppressive $T_{reg}$ cells.

TNF signals through TNFR2 on $T_H$ cells to promote $T_H$ cell proliferation and $T_H1$ pro-inflammatory cytokine production[68], and to promote their resistance to suppression by $T_{reg}$ cell[139]. The effect of TNF inhibitors on $T_H1$ cells might then be an exception to the general pattern seen for other immune cell types, in that TNF inhibition would restrain the function of these antitumour immune cells. Nevertheless, as TNF inhibitors restrain $T_{reg}$ cells, which suppress $T_H1$ cells, TNF inhibitors might indirectly promote $T_H1$ cell function. TNF also signals through TNFR1 on $T_H$ cells to increase the relative number of $T_H17$ cells and $T_H17$ cytokine production[140]. Evidence suggests that $T_H17$ cells can have both pro-tumour and antitumour effects: for example, $T_H17$ cells recruit pro-tumour myeloid cells[140] and antitumour $T_H1$ cells[141], and promote activation of antitumour CTLs[142].

***Effect of TNF on MDSCs and MSCs.*** TNF signals through TNFR2 on MDSCs to promote MDSC survival[143] and their suppressive activity[123,144]. TNF inhibition impairs the growth of mouse FB61 fibrosarcoma tumours and simultaneously impairs peripheral accumulation of MDSCs, suggesting a correlation between an increased number of MDSCs and tumour growth in this model[143]. TNF also promotes mesenchymal stromal cell (MSC) accumulation in tumours in a mouse lymphoma model and induces MSCs to produce high levels of chemokine ligands for the chemokine receptor CCR2 (REF.[145]). These chemokines recruit CCR2-expressing tumour-associated macrophages into the tumour with overall pro-tumorigenic effect[145]. It can be inferred that TNF inhibition would then restrain the function of immunosuppressive MDSCs and MSCs.

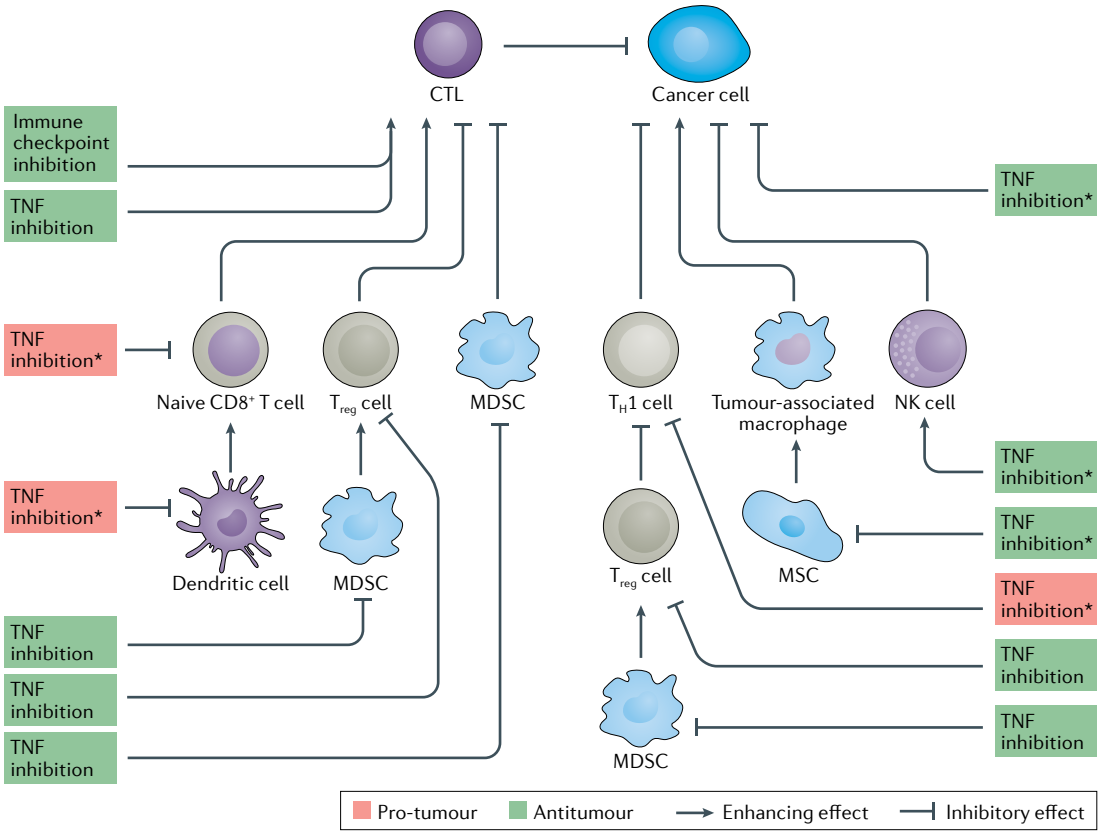


Fig. 1 | **Pro-tumour and antitumour effects of TNF inhibition and immune checkpoint inhibition.** A model of immune interactions in the tumour microenvironment. $CD8^+$ cytotoxic T lymphocytes (CTLs) have direct cytotoxic effects on cancer cells; moreover, they serve as a hub to integrate the indirect effects of other immune cell types, TNF inhibition and immune checkpoint inhibition. Naive $CD8^+$ T cell differentiation replenishes the CTL pool. Natural killer (NK) cells, $CD4^+$ T helper ($T_H1$) cells and tumour-associated macrophages have direct effects on cancer cell proliferation, and integrate the indirect effects of other immune cell types and TNF inhibition. TNF inhibition has a direct inhibitory effect on cancer cell proliferation. This model maps the different paths by which TNF inhibition exerts pro-tumour or antitumour effects. Each path starts with TNF inhibition or immune checkpoint inhibition exerting a direct effect on a cell type. *Denotes that the effect of TNF inhibition is inferred from experimental data on TNF[68,128,129,137,145,146,148–150,152]. MDSC, myeloid-derived suppressor cell; MSC, mesenchymal stromal cell; $T_{reg}$ cell, regulatory T cell.

***Effect of TNF on cancer cells.*** Finally, TNF signalling in cancer cells helps them to evade immune surveillance while promoting their survival and proliferation[137], with an overall pro-tumorigenic effect. In several cancer cell lines, TNF signalling increases the surface expression of PDL1, an immune checkpoint ligand that helps cancer cells to evade T cell immune surveillance[146]. In human melanoma lesions, PDL1 gene expression correlates positively with TNF gene expression[147]. TNF signalling also promotes de-differentiation of melanoma cells accompanied by loss of immunogenicity, which helps cancer cells to evade T cell immune surveillance[148]. Hence, TNF inhibition is expected to exert a direct inhibitory effect on cancer cells.

Overall, the model of interactions depicted in FIG. 1 predicts that TNF inhibitors augment ICI antitumour activity by promoting CTL activity and that TNF inhibitors promote additional antitumour activity through ICI-independent pathways.

### *Prolonged use of TNF inhibitors*

In contrast to short-term TNF inhibition, prolonged use of a TNF inhibitor might deplete the antitumour CTL pool via inhibition of naive $CD8^+$ T cell differentiation into CTLs, leading to decreased numbers of tumour antigen-specific CTLs relative to that of tumour antigen-specific naive $CD8^+$ T cells. This depletion would occur because TNF signals through TNFR2 on naive $CD8^+$ T cells to provide a co-stimulatory signal that promotes TCR-mediated proliferation, activation and differentiation into CTLs[149,150]. Moreover, TNF signalling supports the dendritic cells that promote naive $CD8^+$ T cell differentiation[151]. TNF signalling through TNFR1 in immature dendritic cells induces their maturation, whereas TNF signalling through both TNFR1 and TNFR2 in dendritic cells promotes their survival[152].

Although a CTL depletion effect has not been investigated in preclinical models or in patients, this effect is of particular concern with regard to chronic use of TNF inhibitors and has relevance to the management of ICI-induced inflammatory arthritis, which is often persistent. It would be worthwhile studying whether CTL depletion occurs in patients receiving ICI therapy plus prolonged TNF inhibitor therapy compared with patients receiving ICI therapy alone or ICI therapy plus prolonged therapy with corticosteroids. If CTL depletion is observed, another important question would be whether this effect correlates with clinical outcomes such as progression-free survival.

### *Data from preclinical models*

Predictions of our model (FIG. 1) have been tested in mice engrafted with B16K1 melanoma and treated with an anti-PD1 therapy[147]. In this mouse model, treatment with a TNF inhibitor augmented the antitumour activity of ICI therapy, as assessed by the proportion of tumours that completely regress and by overall survival. Moreover, this improved ICI efficacy was associated with an increased proportion of CTLs out of the total number of cells in the tumours, and a decreased amount of cell death of these CTLs, suggesting that the improved ICI efficacy was attributable to a TNF inhibitor-mediated increase in CTL activity. The researchers also found that TNF inhibitor treatment alone did not have antitumour activity in this tumour model, contradicting our prediction that TNF inhibitors have ICI-independent antitumour activity. Thus, additional factors might be present that affect the tumour response to TNF inhibition that are not accounted for in our model.

In another preclinical study, researchers created a mouse model of ICI-induced colitis, in which the mice were given a combination of anti-CTLA4 and anti-PD1 therapy to treat engrafted MC38 tumours[153] and were concomitantly given dextran sulfate sodium to induce colitis, which is exacerbated by the combination ICI treatment. In this model, TNF inhibitor treatment both ameliorated colitis and augmented ICI antitumour activity. The improved ICI efficacy was associated with an increased proportion of tumour antigen-specific CTLs out of the total number of cells in the tumours and decreased cell death of these CTLs. A limitation of both of the studies described in this section is that the duration of TNF inhibitor treatment was at most 10 days; thus, the studies did not address the effect of chronic TNF inhibitor treatment on CTL activity or ICI efficacy. Preclinical and clinical studies that look at an extended duration of TNF inhibitor treatment would be valuable.

## Conclusion

TNF is a pleiotropic cytokine with pro-inflammatory and immunosuppressive effects in inflammatory disease and cancer. TNF inhibitors are an effective treatment for a number of inflammatory diseases, including RA, IBD and ICI-induced inflammatory arthritis. Multiple clinical studies of TNF inhibitors in patients with inflammatory disease support the hypothesis that TNF inhibition poses a relatively low risk of cancer, but limited clinical data are available regarding its risk profile in patients with cancer undergoing ICI therapy. TNF can promote or inhibit the activities of the immune cells and cancer cells within tumours. The net effect of TNF inhibition on tumorigenesis might be positive or negative depending on qualitative (that is, the presence of specific cell types) and quantitative (that is, the local concentration of TNF) factors. Moreover, acute versus chronic TNF inhibition might have opposing effects on tumour growth. Preclinical models can be extrapolated to the clinic only to the extent that the qualitative and quantitative details of the experimental model match those found in patients. Despite these caveats, most of the current data support two conclusions: TNF inhibitor treatment of rheumatic diseases does not seem to increase the risk of cancer, except for non-melanoma skin cancer and possibly lymphoma, and preclinical data suggest that short-term TNF inhibitor treatment of irAEs should not diminish the anticancer efficacy of ICI therapy. Thus, short courses of TNF inhibitors should be safe to use in the treatment of ICI-associated irAEs. Further studies in preclinical models are required to directly assess the safety of long-term TNF inhibitor use in the context of ICI cancer treatment. Clinical studies that directly assess the effect of TNF inhibitor treatment on ICI efficacy are required to draw conclusions regarding the short-term and long-term safety of

TNF inhibitor treatment for irAEs. Preclinical studies provide evidence that TNF inhibitors, despite their efficacy in ameliorating irAEs, do not also restrain anticancer immune activity. The data from these studies suggest that different arms of the immune response are important for anti-self versus anticancer activities, with TNF inhibition restraining some arms of the immune response while promoting or having a neutral effect on others. The cellular and molecular details of how the pleiotropic effects of TNF signalling interact with different arms of the immune response remain to be fully delineated. A more complete map of these interactions might reveal novel drug targets for the treatment of inflammatory disease and cancer.

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# The gut–joint axis in rheumatoid arthritis

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Abstract | Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder that primarily affects the joints. One hypothesis for the pathogenesis of RA is that disease begins at mucosal sites as a consequence of interactions between the mucosal immune system and an aberrant local microbiota, and then transitions to involve the synovial joints. Alterations in the composition of the microbial flora in the lungs, mouth and gut in individuals with preclinical and established RA suggest a role for mucosal dysbiosis in the development and perpetuation of RA, although establishing whether these alterations are the specific consequence of intestinal involvement in the setting of a systemic inflammatory process, or whether they represent a specific localization of disease, is an ongoing challenge. Data from mouse models of RA and investigations into the preclinical stages of disease also support the hypothesis that these alterations to the microbiota predate the onset of disease. In addition, several therapeutic options widely used for the treatment of RA are associated with alterations in intestinal microbiota, suggesting that modulation of intestinal microbiota and/or intestinal barrier function might be useful in preventing or treating RA.

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder that primarily affects the joints[1], yet several pieces of evidence from epidemiological and translational research suggest that interactions between mucosal sites and dysbiotic microbiota might have a causal role in the development of RA[2–5]. Such evidence supports the idea that the pathogenesis of RA might begin at mucosal sites and then transition to the synovial joints[5]. However, the specific mucosal processes that influence arthritis development and disease evolution are not well understood.

Substantial data have been published in the past few years that demonstrate the presence of alterations in the composition of the microbial flora in individuals in the preclinical stages of RA[6], suggesting a role for intestinal dysbiosis in the development of RA and in maintaining the chronicity of systemic inflammation[7–14]. The models proposed account for the fact that the intestine is populated by the largest number of innate and adaptive immune cells in the body, and therefore is often considered to be the body's largest immunological organ[15]. The complex interactions that might occur between an altered intestinal bacterial flora and an immune system genetically predisposed to autoimmunity could potentially provide the basis for the development of systemic inflammation that also involves the joints. At present, it is impossible to establish whether these alterations are the result of interactions between the environment and the innate immune system in patients who are genetically susceptible to RA, or whether they are a consequence of a systemic inflammatory process that specifically involves the intestine. However, data derived from mouse models of arthritis and studies conducted in patients with early-stage RA strongly suggest that these alterations could precede the onset of disease and in some way represent a hidden trigger of systemic inflammation.

In this Review, we discuss the evidence supporting intestinal dysbiosis in RA with a focus on mucosal immunology. In particular, we offer an overview of the mechanisms that could link intestinal dysbiosis to the development and perpetuation of RA and speculate that, at least in a subset of patients with RA, dysbiosis might contribute to subclinical gut inflammation and promote the activation of specific innate and adaptive immune responses (FIG. 1). To this end, we outline the evidence for subclinical gut inflammation and derangement of the gut barrier in patients with RA and discuss the re-circulation of aberrantly activated immune cells from intestinal sites to secondary lymphoid organs and arthritic joints as a possible mechanistic link between mucosal alterations and arthritis development. Finally, we offer some thoughts on modulation of the intestinal microbiota and intestinal barrier function as possible new treatment approaches in RA.

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**Key points**

- Alterations in the composition of the microbial flora occurs in individuals in the preclinical stages of rheumatoid arthritis (RA) and in those with established RA.
- DMARDs modify the intestinal microbial composition in patients with RA.
- Subclinical gut inflammation occurs in some patients with RA and is associated with altered intestinal permeability.
- Zonulin family peptides are mediators of altered intestinal permeability in RA and their inhibition ameliorates the severity of arthritis in mouse models of disease.
- Dysbiosis and altered intestinal permeability could induce chronic activation of innate immune cells.
- Recirculation of innate immune cells from the gut to the peripheral joints has the potential to support the chronic inflammatory process in at least some patients with RA.

## Microbial dysbiosis in RA

Dysbiosis refers to an alteration in the composition and function of the microbiota that is promoted by a set of environmental and host-related factors[16]. The presence of dysbiosis has been demonstrated in the lungs, oral cavity and intestines of patients with RA, providing evidence in support of the hypothesis of a mucosal basis to the pathogenesis of this disease[5]. Because the mucous membranes are colonized by a specific microbial flora, dysbiosis at these sites could alter local, and possibly systemic, immune responses, thereby contributing to the pathogenesis of RA. However, it is important to underline that care is needed when judging the primary mechanisms involved in the generation and persistence of dysbiosis in RA; it is essential to distinguish the alterations to the microbiota that are associated with early stages of the disease (preclinical or very early disease) from the modifications that occur in established disease, when systemic inflammatory processes and pharmacological treatments might influence the composition of the microbial flora.

### *Lungs and oral cavity*

Although interesting, current data on changes to the composition of the lung microbiota in RA are limited, being confined to a single published study[3]. However, epidemiological data have shown an association between RA and lower airway conditions such as bronchiectasis and chronic obstructive pulmonary disease[17,18]. The hierarchy in this link is still not established, but in both bronchiectasis and chronic obstructive pulmonary disease, patients' lungs are prone to microbial colonization and become a site of peptide citrullination[17,19]. Bronchiectasis has also been proposed as a model for a chronic bacterial infection that induces autoimmunity; in one observational study, individuals affected by bronchiectasis had a higher frequency of positivity for rheumatoid factor or anti-citrullinated protein antibodies (ACPAs) than healthy individuals[17].

By contrast, several studies have documented the presence of an association between periodontal disease and RA[20]. This association has also been justified from a functional point of view by the ability of the periodontal pathogen *Porphyromonas gingivalis* to induce protein citrullination[20]. This post-translational modification generates a set of autoantigens that lead to the production of ACPAs. Although the existence of this association is adequately supported, conflicting results exist as to its extent and effect; in particular, a 2020 randomized nested study in the ESPOIR cohort of patients with established RA showed that good oral hygiene, together with regular dental scaling and polishing, can substantially reduce the load of periodontal pathogens without decreasing RA disease activity[21].

### *Intestines*

The intestine is the largest immune organ in the body, and includes a sophisticated integrated assembly of innate and adaptive immune cells that co-exist with specialized epithelial cells in a complex integrated system that maintains homeostasis between the microbiota and the host. Interesting data from studies conducted in different mouse models of RA support the presence of a specific intestinal dysbiosis that could potentially lead to arthritis, and the occurrence of intestinal dysbiosis has also been convincingly demonstrated in patients with RA and their relatives[7,8,11,22,23] (TABLE 1).

***Data from experimental models of arthritis.*** Collagen-induced arthritis (CIA) is an experimental model of RA, in which an inflammatory articular condition is induced in mice by injecting them with an emulsion of complete Freund's adjuvant and type II collagen. Substantial changes in the gut microbial community are present in mice during the preclinical phase of CIA, mainly characterized by the reduced representation of the Bacteroidetes phylum and an increased representation of Firmicutes and Proteobacteria, such as Ruminococcaceae, Lachnospiraceae and Desulfovibrionaceae[24,25]. CIA also causes an imbalance among 14 types of intestinal bacteria at the family level and a considerable perturbation of metabolites involved in energy production and the metabolism of tryptophan, fatty acids and secondary bile acid[26]. The reduction in *Bacteroides* spp. seen in mice with CIA could potentially promote a local pro-inflammatory environment by reducing $CD4^+$ T cell differentiation into regulatory T ($T_{reg}$) cells. Colonization of germ-free mice with *Bacteroides fragilis* is followed by an expansion of $T_{reg}$ cells and the induction of anti-inflammatory cytokine production in the intestine that seems to be caused by polysaccharide A, one of the immunomodulatory molecules of *B. fragilis*, which induces the conversion of $CD4^+$ T cells into IL-10-producing $FOXP3^+$ $T_{reg}$ cells during commensal colonization[27].

In the initial stages of CIA, dysbiosis and intestinal inflammation develop before overt signs of arthritis and persist throughout the course of the disease[25]. Actively modifying the microbiota of the mice with antibiotic treatment before the induction of CIA led to a substantial reduction in the severity of disease and in serum concentrations of pro-inflammatory cytokines and antibodies that recognize type II collagen compared with mice without antibiotic treatment[25]. Conversely, SKG mice, which spontaneously develop autoimmune arthritis, colonized with faecal microbiota from patients with RA (dominated by *Prevotella* spp.) showed an increased susceptibility to arthritis[10]. Interestingly, a 2019 study showed that ES-62, a phosphorylcholine-containing glycoprotein secreted by the parasitic filarial nematode

*Acanthocheilonema viteae*[28], is capable of normalizing the intestinal dysbiosis associated with CIA, reducing mucosal inflammation, normalizing intestinal permeability and blunting IL-17 responses in the mesenteric lymph nodes, leading to a reduction in the severity of joint inflammation[29]. These data suggest that reducing chronic gut inflammation could contribute to the amelioration of CIA[25,29] and are in line with the idea that intestinal microbiota could modulate the immune response by exerting both pro-inflammatory and anti-inflammatory functions.

In K/B×N mice with serum transfer-induced arthritis, the severity of arthritis is greatly reduced in the absence of intestinal bacteria and is accompanied by sharp reductions in serum concentrations of autoantibodies, the number of autoantibody-secreting splenic cells, the number of splenic T helper 17 ($T_H17$) cells and germinal centre formation[30]. In these mice, neutralization of IL-17 prevented arthritis in germ-free conditions, possibly as a result of the direct effect of IL-17 in inhibiting the formation of germinal centres. The introduction of segmented filamentous bacteria (SFB) into germ-free K/B×N mice restored the $T_H17$ cell population of the lamina propria and the production of autoantibodies, thus causing arthritis[30]. In this inflammatory process, SFB increase the ileal production of amyloid A serum isoforms, which in turn stimulate intestinal dendritic cells to induce the differentiation of $T_H17$ cells[31]. Commensal bacteria are also able to produce ATP, which activates a specific subset of $CD70^{hi}CD11c^{lo}$ intestinal dendritic cells that express molecules capable of supporting a $T_H17$ cell response, such as IL-6, the p19 subunit of IL-23 and transforming growth factor-β[32].

Another meaningful observation is the need for gut microbial colonization in the development of arthritis in IL-1 receptor antagonist (IL-1RA)-deficient mice, which spontaneously develop a T cell-mediated arthritis that is dependent on IL-1β[33]. Interestingly, the onset of arthritis is abrogated in these mice in the absence of microbial flora[34]. IL-1RA seems to regulate the composition of the gut microbiome in mice[35]; specifically, a lack of IL-1RA reduces intestinal diversity and microbial richness and induces specific taxonomic alterations characterized by increased representation of *Helicobacter* spp. and reduced representation of *Ruminococcus* and *Prevotella* spp. The presence of this aberrant intestinal microbiome specifically induces the expansion of $T_H17$ cells in the lamina propria, an effect that can be transferred to wild-type mice through faecal microbiota transplantation[35]. Therefore, the gut microbiota has the potential to support the differentiation of $T_H17$ cells, which are expanded in the blood of patients with RA[36]. Via the production of IL-17, IL-21, IL-22, granulocyte–macrophage colony-stimulating factor and TNF, $T_H17$ cells have been implicated in different aspects of the pathogenesis of RA including pannus growth, osteoclastogenesis and synovial neo-angiogenesis[37]. Whether the expanded circulating $T_H17$ cell population is entirely of intestinal origin, and, if not, what percentage of the population is of intestinal origin, remains to be clarified.

### *Data from patients with RA*

A cohort study of the faecal microbiome of first-degree relatives of patients with RA showed that, compared with asymptomatic individuals without autoantibodies, individuals with RA in the preclinical stages (positive for ACPAs or rheumatoid factor and/or displaying symptoms of RA) had a markedly modified microbiome that was characterized by a statistically significant over-representation of Prevotellaceae, particularly *Prevotella* spp.[11]. In a separate study, the amount of *Prevotella copri* was expanded in stool samples from patients with untreated new-onset RA (between 6 weeks and 6 months after diagnosis) and correlated with a reduction in *Bacteroides* spp. and a loss of reportedly beneficial microorganisms[4]. An HLA-DR-presented peptide was subsequently identified from the 27-kDa protein of *P. copri* (*Pc*-p27) that was capable of stimulating a $T_H1$ cell response in 42% of patients with new-onset RA[38]. Patients with RA (both new-onset and chronic) either

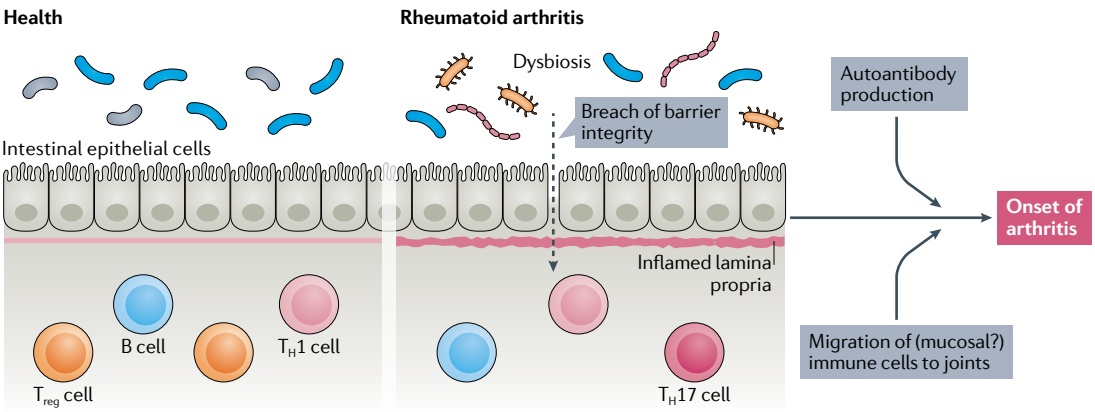


Fig. 1 | **The gut–joint axis in rheumatoid arthritis.** In healthy gut tissue, intestinal epithelial cells form a barrier, mediated by a layer of mucus and by tight junctions between cells, which limits the translocation of microorganisms and their products. Microbial dysbiosis and subclinical inflammation of the underlying lamina propria can be detected in individuals at risk of rheumatoid arthritis (RA) before the first clinical signs of arthritis occur, causing a breach in gut barrier integrity. The translocation of microbial products has the potential to trigger clinical arthritis via a number of possible mechanisms, but the exact contribution of the gut to RA development is unknown. $T_H$ cell, T helper cell; $T_{reg}$ cell, regulatory T cell.

Table 1 | **Statistically significant changes in microbiota composition in patients with RA**

| Cohort | Methodology | Increased | Decreased | Ref. |
|---|---|---|---|---|
| At-risk FDRs (seropositive or arthralgia) versus asymptomatic seronegative FDRs | 16S rRNA sequencing of faecal DNA | Prevotellaceae | None | 11 |
| Early RA versus fibromyalgia | 16S rRNA hybridization and flow cytometry | None | Bifidobacteria and the *Bacteroides–Porphyromonas–Prevotella* group | 174 |
| New-onset RA versus chronic RA | 16S rRNA sequencing of faecal DNA | *Prevotella copri* | *Bacteroides* | 4 |
| RA versus FDRs | 16S rRNA sequencing of faecal DNA | *Collinsella* and *Eggerthella* | *Faecalibacterium* | 8 |
| RA versus healthy individuals | Shotgun sequencing on faecal DNA | *Lactobacillus salivarius*, enterococci and *Bacteroides* | *Haemophilus*, *Klebsiella* and Bifidobacteria | 7 |
| RA and SpA versus healthy individuals | 16S rRNA sequencing of faecal DNA | Proteobacteria, Tenericutes and Synergistetes | Prevotellaceae and Bifidobacteriaceae | 175 |
| RA versus healthy controls | Shotgun sequencing of faecal DNA | *Prevotella* | None | 176 |

The sequencing and analytic methods are different between each study, making the comparison of findings difficult. FDR, first-degree relative of a patient with RA; RA, rheumatoid arthritis; SpA, spondyloarthritis.

had IgA-like antibody responses to *Pc*-p27 or *P. copri* that correlated with the production of $T_H17$ cell cytokines and the occurrence of ACPAs, or presented anti-*Prevotella* IgG antibodies that were associated with the presence of *Prevotella* DNA in the synovial fluid, *Prevotella*-specific $T_H1$ cell responses and a lower concentration of ACPAs. Unlike in RA, antibody responses to *Prevotella* have rarely been found in patients with other rheumatic diseases or in healthy individuals[38]. Although Prevotellaceae seem to be the main bacterial family associated with dysbiosis in patients with RA, an expansion of rare intestinal microorganisms could also be characteristic of RA. In a 2016 study, patients with RA showed a reduction in intestinal microbial diversity compared with healthy individuals and first-degree relatives characterized by an increased abundance of *Collinsella* and *Eggerthella*[8]. The abundance of *Collinsella* also strongly correlated with serum concentrations of the metabolites α-aminoadipic acid and asparagine, and with the production of IL-17A[8]. In addition, in line with the existing links between ACPAs and microbial flora, a Chinese study demonstrated that dysbiosis, expressed in terms of reduced microbial diversity, was stronger in ACPA-positive patients with RA than in ACPA-negative patients[39].

Although it is currently challenging to disentangle whether intestinal dysbiosis is causally linked to the development of RA or is the consequence of systemic inflammation in treatment-naive patients, all of these studies at least demonstrate that dysbiosis is present in all stages of RA. However, the role of dysbiosis in the development of RA is somewhat supported by evidence of early changes in the microbiota in preclinical disease. Notably, none of the studies discussed above was able to definitively show that the initial production of RA-associated antibodies occurs directly in the gut. Other studies have, however, demonstrated an abundance of secretory immunoglobulins (IgA and IgM) in RA that are normally produced at mucosal sites, and secretory rheumatoid factor, anti-carbamylated protein antibodies and ACPAs are frequently found in patients with RA[40]. In addition to the well-studied production of autoantibodies that occurs in the periodontium and the lung, the intestinal mucosa should be considered as a potential site for autoantibody production given that Peyer's patches are a major site for IgA plasmablast maturation, during which plasmablasts become resident or recirculate to other mucosal sites[41]. Indeed, some preliminary data have shown the presence of IgA ACPAs in the stool of patients with RA, which, when investigated in mice with CIA, was mediated by intestinal colonization by bacteria[42].

In contrast to the data from animal models that are providing new insights into the involvement of the intestines in the development of arthritis, data from humans are still unsatisfactory. Most of the studies performed in humans fail to offer a universal mechanistic explanation linking intestinal dysbiosis to autoantibody production and arthritis onset; however, they could be considered as pieces of a puzzle that is still in progress. Specifically designed prospective studies are still required to fully evaluate the role of intestinal dysbiosis in patients with RA.

### *Dysbiosis and sex bias in RA*

The links that exist between dysbiosis and the sex bias that occurs in RA have so far been under-investigated, but data from experimental models of autoimmunity have paved the way for some speculations. A link between sex-related microbiome alterations and autoimmunity has been demonstrated in different mouse models of autoimmunity. For example, in the non-obese diabetic model of type 1 diabetes, sex-related microbiota changes can promote hormone-dependent regulation of autoimmunity[43]. A 2020 study demonstrated that gut microbiota contribute to intestinal immune phenotype and systemic autoimmunity progression differently in female and male lupus-prone mice[44]. Notably, the composition of the gut microbiota in male and female littermates significantly differed only in adulthood, and the

absence of gut microorganisms reduced the progression of autoimmune disease only in female mice by reducing the pro-inflammatory cytokine response of the intestinal mucosa. In male mice, orchidectomy changed the composition of the gut microbiota but also caused a modest acceleration of the progression of autoimmune disease[44]. Looking specifically at mouse models of RA, conflicting data seem to have emerged around the possibility of sex-related alterations in the composition of the microbiome[45,46]. In the serum-transfer model of RA, male and female K/B×N mice similarly develop destructive arthritis and mechanical allodynia[47]. Conversely, transgenic mice carrying human HLA-DRB1*04:01, which is associated with susceptibility to RA, show a sex bias in the onset of arthritis (female to male ratio of 3:1) and in the production of rheumatoid factor and ACPAs[48,49].

Although some of the evidence from mouse models might suggest a sex-related alteration of the microbiome in RA, differences in the composition of the gut microbiome between men and women with RA have not been conclusively shown to influence the development of the disease. Given that RA occurs more frequently in women than in men, women with RA outnumber men with RA in studies evaluating intestinal dysbiosis, thus representing a potential bias in the interpretation of the results. However, an analysis of the gut microbiome compositions of a cohort of Chinese men and women with RA showed that Rikenellaceae, Porphyromonadaceae and Coriobacteriaceae were more abundant in women, whereas Pasteurellaceae, *Butyricicoccus* spp., Clostridiaceae 1, *Clostridium sensu stricto 1* spp. and *Allisonella* spp. were more abundant in men[50]. Taken together, these observations in mice and humans seem rather weak. Therefore, the contribution, if any, of dysbiosis to the sex bias that occurs in most autoimmune disease deserves a place in future research agendas.

## Intestinal inflammation in RA

Despite the evidence that gut dysbiosis occurs both in individuals at risk of RA and in patients with RA[4,11], it is unclear whether and how gut dysbiosis specifically promotes synovial inflammation. However, it is known that gut dysbiosis can initiate and perpetuate intestinal inflammation[51,52], and transplantations of stool or faecal microbiota are able to control intestinal inflammation in recipient mice[53–56]. Similar experiments have been performed in the context of inflammatory arthritis. In DBA/1 mice, CIA can be stably induced in 80–100% of animals; however, in this model, up to 20% of DBA/1 mice do not develop symptoms of arthritis[57]. Germ-free mice experience a greater increase in incidence and severity of arthritis when receiving faecal microbiota transplants from CIA-susceptible DBA/1 donor mice than when receiving transplants from CIA-resistant DBA/1 donor mice[57]. Taken together, these data seem to suggest a possible underlying mechanism linking the occurrence of intestinal inflammation with the development of arthritis; however, this theory is still speculative as potential inflammation in the intestines of germ-free recipient mice has not been investigated[57]. Therefore, it will be critical to search for evidence of intestinal inflammation in patients with RA.

If one considers the considerable spatial separation of synovial joints and the intestines, it is remarkable that in the early 1990s physicians had already detected microscopic gut inflammations in patients with arthritis, thereby discovering the well-known link between gastrointestinal inflammation and spondyloarthritis (SpA)[58–60]. The consolidated demonstration of a gut–joint axis in patients with SpA presents an excellent cognitive model to consider when studying RA. In SpA, the existence of subclinical gut inflammation has been confirmed by multiple studies to occur in more than 50% of patients regardless of their use of NSAIDs[61–64]. This gut inflammation was linked to the expansion of aberrantly activated intestinal innate immune cells that have the potential to reach extra-intestinal sites and promote inflammation[64,65]. The demonstration that the presence of intestinal dysbiosis can contribute to the onset of intestinal inflammation in SpA seems to suggest that similar mechanisms might also be operative in RA. Reinforcing the idea that, in some individuals, an altered composition of the microbial flora can contribute to intestinal and joint inflammation, the authors of one 16S rRNA study identified an expansion of pathogenic commensals such as *Escherichia coli* 2A in patients with SpA-associated Crohn's disease, but not in patients with Crohn's disease alone[66]. Moreover, isolates of *E. coli* 2A from patients with SpA-associated Crohn's disease induced strong $T_H17$ cell-mediated mucosal immunity that promoted disease in mouse models of inflammatory bowel disease (IBD) and autoimmune arthritis[66].

In comparison to SpA, evidence supporting the presence of subclinical inflammation in RA is limited. Early reports demonstrated increased amounts of *IL10*, *CCR5* and *CCR4* mRNA in duodenal tissue from patients with RA, suggesting immune cell activation[67]. However, histological analysis of intestinal tissue from patients with established RA demonstrated the presence of clear pathological findings, such as partial or complete loss of superficial epithelium, an increased number of plasma cells and granulocytes and the presence of vasculitic lesions, in only around 15% of patients[68,69]. By contrast, a subsequent study in a small cohort of patients with early RA demonstrated the occurrence of subclinical gut inflammation in virtually all patients[56]. Gut inflammation in these patients was characterized by an increased number of infiltrating mononuclear cells, T cells, B cells and $CD68^+$ macrophages, and by the presence of lymphoid follicles[56]. These histological findings suggest that a chronic inflammatory process occurs in the intestine of patients with early RA, in line with the concept that RA is a systemic disease. In support of this notion, a population-based study from South Korea highlighted a significant association between IBD and RA (OR 3.31)[70]. Interestingly, no associations were found between IBD and other inflammatory rheumatic diseases with the exception of the known link with ankylosing spondylitis (a type of SpA; OR 3.73)[70]. These data reinforce the idea of a potential common pathogenic mechanism between IBD and RA and underline the prospective role of the gut in the initiation of RA.

To date, clear histological alterations have not been observed in all patients with RA, but signs of altered

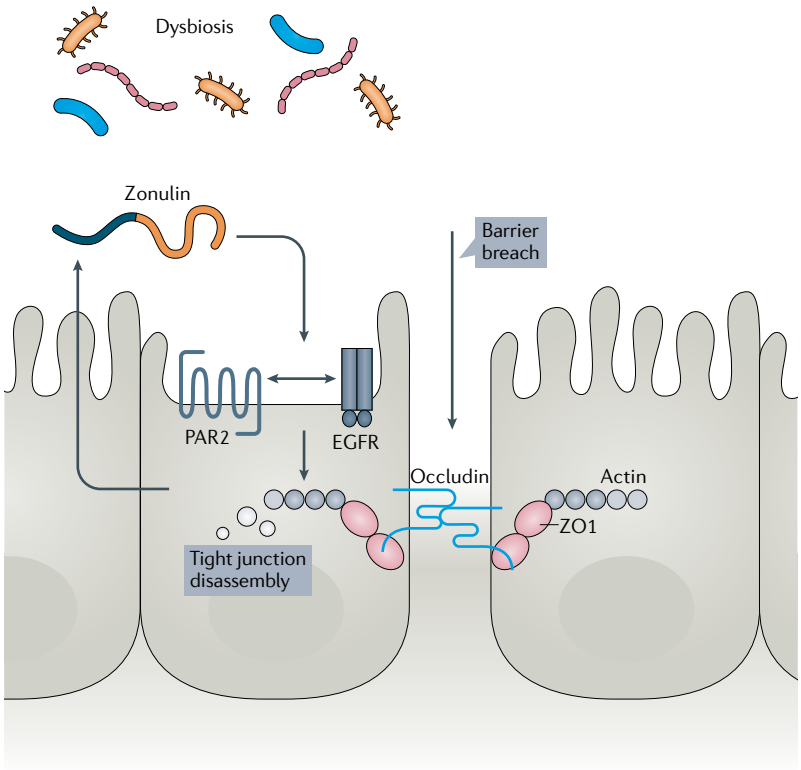


Fig. 2 | **Alteration of intestinal permeability in rheumatoid arthritis.** Following the development of intestinal dysbiosis, gut barrier integrity can be breached via zonulin production, which causes the disassembly of tight junction proteins. Zonulin contains an epidermal growth factor-like motif and a proteinase-activated receptor 2 (PAR2)-activating peptide. Zonulin triggers PAR2-dependent transactivation of epidermal growth factor receptor (EGFR), leading to the disassembly of the tight junction by the displacement of ZO1 and occludin from the junction complex.

intestinal permeability do seem to occur in patients with established RA and in patients during the pre-RA phase[56]. In a large cohort of French women, a history of chronic diarrhoea in the absence of defined gastrointestinal diseases was associated with an increased risk of developing RA (HR 1.70), suggesting that a perturbation in intestinal homeostasis might exist for many years before the onset of RA[71]. A critical analysis of these data could suggest that smouldering chronic alteration might precede the onset of arthritis and be under-diagnosed in clinical settings. However, the limited data currently available do not fully support the presence of gut inflammation and intestinal barrier disruption in patients with RA, and further studies are required specifically to define the exact prevalence of subclinical gut inflammation in RA and to establish whether patients with RA can be stratified into groups for personalized gut-targeting therapies. Further research is also warranted to define the disease phenotype of the patients who show signs of gut inflammation and the pathogenic factors that determine intestinal involvement, such as environment, genetic background, sex and age.

## Altered gut barrier function in RA

The primary function of the small intestine is the absorption of nutrients and minerals from food. Although representing a considerable surface area of the body that is exposed to the outside world, the epithelial cells of the intestine form a dynamic physical barrier to tightly control antigen trafficking via paracellular pathways. In the presence of inflammation, alterations to the intestinal barrier function and accompanying increases in gut permeability and bacterial translocation can promote IBD and autoimmunity in genetically predisposed hosts[72–74]. Similar to changes seen in patients with IBD, abnormal intestinal barrier permeability also occurs in patients with ankylosing spondylitis or RA[75]. The primary mechanism by which barrier disruption in the gut seems to occur is via an increased production of zonulin, the primary regulator of the integrity of the tight junctions in the intestinal epithelium[76] (FIG. 2). Epithelial intercellular tight junctions are critical structures in the regulation of paracellular trafficking at the intestinal barrier. Increased concentrations of zonulin have been linked to a mechanism that could lead to immune-mediated diseases[77]. Zonulin secretion, which is dependent on the adaptor protein MYD88 (REF.[78]), decreases intestinal barrier function by causing the disassembly of the proteins ZO1 and occludin from the tight junction complex[79,80]. The triggers for zonulin release from intestinal epithelial cells have mainly been described for gluten[81] (the protein that causes coeliac disease) and for dysbiotic microbiota[82,83].

In mice with CIA, intestinal barrier impairment has been observed during the preclinical autoimmune phase, as have increased concentrations of cytokines such as IL-17A, IL-22 and IL-23 (REF.[25]). Small intestine immune activation characterized by an increase in pro-inflammatory cytokine-secreting lamina propria $CD4^+$ T cells has also been proved in preclinical CIA[84]. Bacterial composition seems to influence gut permeability during arthritis. The transfer of human gut-derived *Prevotella histicola* to mice with CIA resulted in decreased arthritis severity, reduced intestinal permeability and increased expression of ZO1 in the jejunum, ileum and colon[9]. Occludin was also upregulated in the intestines of mice with CIA following *P. histicola* transfer, as well as in CACO2 cell cultures in vitro[9]. In line with these findings, arthritis-susceptible *HLADRB1*04:01* and arthritis-resistant *HLADRB1*04:02* transgenic mice differ in their microbiome composition, with the arthritis-susceptible strain also having a higher degree of intestinal permeability[46]. In another study, orally administered *P. gingivalis* worsened CIA, changed the gut microbiome, increased serum endotoxin concentrations and impaired gut barrier function[85]. Consistently, *P. gingivalis* decreased the expression of tight junction molecules in the intestines of mice with CIA[86–88]. Similar effects on tight junction proteins have also been reported for *Collinsella* spp.[8].

Peptides from the zonulin family could be good candidates to link gut dysbiosis with intestinal inflammation and reduced barrier function in patients with RA. Interestingly, two studies investigating the effects of a gluten-free vegan diet (which is expected to reduce zonulin concentrations) compared with a vegan diet on RA found substantial improvements in markers of inflammation in those who received the gluten-free diet[89,90]. Indeed, previous reports showed that gliadin, a constituent of gluten, induces the release of zonulin,

which increases intestinal permeability by binding to the chemokine receptor CXCR3 and amplifying damage in the small intestine[81,91,92]. Furthermore, the observation that the expression of zonulin family peptides is increased before the onset of RA and in the gut of patients with new-onset RA (who also have an accompanying downregulation of tight junction proteins)[56], together with evidence that bacteria might influence zonulin expression[83], supports a potential link between gut dysbiosis and an alteration of intestinal permeability in RA.

Abnormal intestinal permeability has been shown in patients with RA who have active disease compared with those who do not have active joint disease[75,93–96]. However, in some of these studies, the presence of altered intestinal permeability in patients with active RA was confounded by the intake of NSAIDs, which also influence intestinal permeability[97]. For example, in one study, altered gut permeability was described in patients with IBD, RA or SpA; however, whereas altered gut permeability was not linked to NSAID use in individuals with SpA or IBD, all of the patients with RA who participated in the study took NSAIDs, and therefore the effect of RA on gut permeability was difficult to judge[98]. Notably, the role of prostaglandin $E_2$ in modulating intestinal permeability, and therefore of its inhibitors (NSAIDs), is still controversial, as some studies have found that prostaglandin $E_2$ promotes the destruction of the epithelial barrier[99]. Overall, current data, especially data from individuals with preclinical and new-onset RA who did not take any NSAIDs, suggest that alterations in intestinal permeability might be intrinsic to RA and potentially modulated by intestinal dysbiosis then worsened by the use of NSAIDs.

## Mechanisms of the gut–joint axis

Taken together, the data presented above suggest that intestinal inflammation, along with reduced barrier function — both of which are observed in patients with RA — promotes the onset of clinical RA. The connections among disturbed barrier function, intestinal inflammation and arthritis could be mediated by two non-mutually exclusive pathways that are briefly discussed in this section. First, autoantibodies could be generated within the inflamed intestine[5] and second, pro-inflammatory immune cells primed in intestinal tissues could traffic to systemic sites and to the joints[9,56,100].

### *Autoantibody production*

In keeping with the mucosal origins hypothesis[5], increased amounts of autoreactive IgA antibodies have been found in the serum years before the onset of clinical RA[101–103]. Given that mucosal surfaces are the primary site for the development of IgA antibodies, these results suggest that autoantibodies associated with RA could initially be generated at mucosal surfaces such as the intestine[5]. Interestingly, increased serum concentrations of rheumatoid factor are associated with mucosal inflammation in the lungs and periodontal disease even before the onset of clinical RA[20,104–106]. This finding further highlights the early link between inflammation at mucosal sites and the generation of autoantibodies, which subsequently leads to autoimmunity and clinical signs of RA[107–109]. A 2020 cohort study revealed that IgA rheumatoid factor concentrations substantially differed from those in matched controls as early as 14 years before diagnosis of RA and several years prior to a detected increase in IgG rheumatoid factor[110]. Furthermore, the appearance of IgA ACPAs occurred 6 years before the onset of clinical RA, suggesting mucosal processes in the preclinical phase of the disease that facilitate the transition to the onset of clinical RA[110]. Strikingly, intestinal tissue samples from patients with RA also show increased concentrations of IgA and IgM antibodies that recognize food antigens[111]. Overall, these data point towards mucosal surfaces, specifically the gut, as sites of antibody generation in response to external stimuli, including food-derived antigens and, in predisposed individuals, autoantigens.

### *Mucosa-derived immune cells*

The presence of the alterations in gut barrier function and increased intestinal permeability has been linked with the possibility that inflammatory immune cells primed in intestinal tissues could traffic to the joints. Some studies in humans support the general idea of cell trafficking from the intestine to the joints. For example, gut-activated B cells adhere efficiently to both gut and synovial high endothelial venules but not to high endothelial venules in peripheral lymph nodes, suggesting that immune cells from the gut might enter the joints[112]. In addition, identical T cell clones have been identified in the joints and intestines of patients with SpA, and the synovium of patients with RA contains T cells that express the gut homing receptor αEβ7 integrin[113,114]. Several mucosa-derived innate immune cells are expanded and activated in patients with RA, supporting the idea of a gut–joint cellular axis (FIG. 3), and are discussed below.

***Group 3 innate lymphoid cells.*** Group 3 innate lymphoid cells (ILC3s) are mainly tissue-resident cells that belong to a heterogeneous family of cells with a preferential tropism for the epithelial barrier surfaces and have been well studied in the context of SpA and IBD (reviewed elsewhere[115]). The term ILC3 itself refers to a group of RORγt-dependent cells: lymphoid tissue inducer (LTi) cells, natural cytotoxicity receptor (NRC)-positive ILC3s and NRC-negative ILC3s[116]. Through strict interactions with the intestinal environment, ILC3s are a potential immunological bridge between intestinal microbiota and local systemic immune responses, acting mainly via the secretion of IL-17 and IL-22 (REF.[115]). In addition, LTi cells are fundamental to the formation of lymphoid structures[117]. The response of ILC3s to intestinal microbiota composition has been previously demonstrated in IBD and seems to partially depend on microorganism-sensing $CX3CR1^{hi}CD14^{+}$ macrophages[118]. Bacterial bioproducts such as aryl hydrocarbon receptor ligands and retinoids can also directly modulate ILC3 activity by stimulating LTi and ILC3 maturation[119,120]. Similarly, the bacteria-derived short-chain fatty acid (SCFA) butyrate suppresses ILC3 activation[121], and conversely the agonism of free fatty

acid receptor 2 (FFAR2), a microbial metabolite-sensing receptor, induces ILC3 activation and IL-22 secretion in colonic mucosa[122].

The recirculation programme of ILC3s to extra-intestinal sites is not well known; however, ILC3s have been found circulating in peripheral blood and in human spinal entheses[123,124]. In the context of RA, ILC populations have been investigated in the inguinal lymph nodes of healthy individuals, individuals at risk of RA (identified as rheumatoid factor-positive or ACPA-positive) and individuals with early RA[125]. Despite the fact that the overall number of ILCs did not differ among the groups, differences in the relative composition were observed. The percentage of ILC3s was increased in patients with early RA but not in healthy individuals or those at risk of RA. The LTi population was substantially decreased in patients with RA and declined progressively in parallel with disease

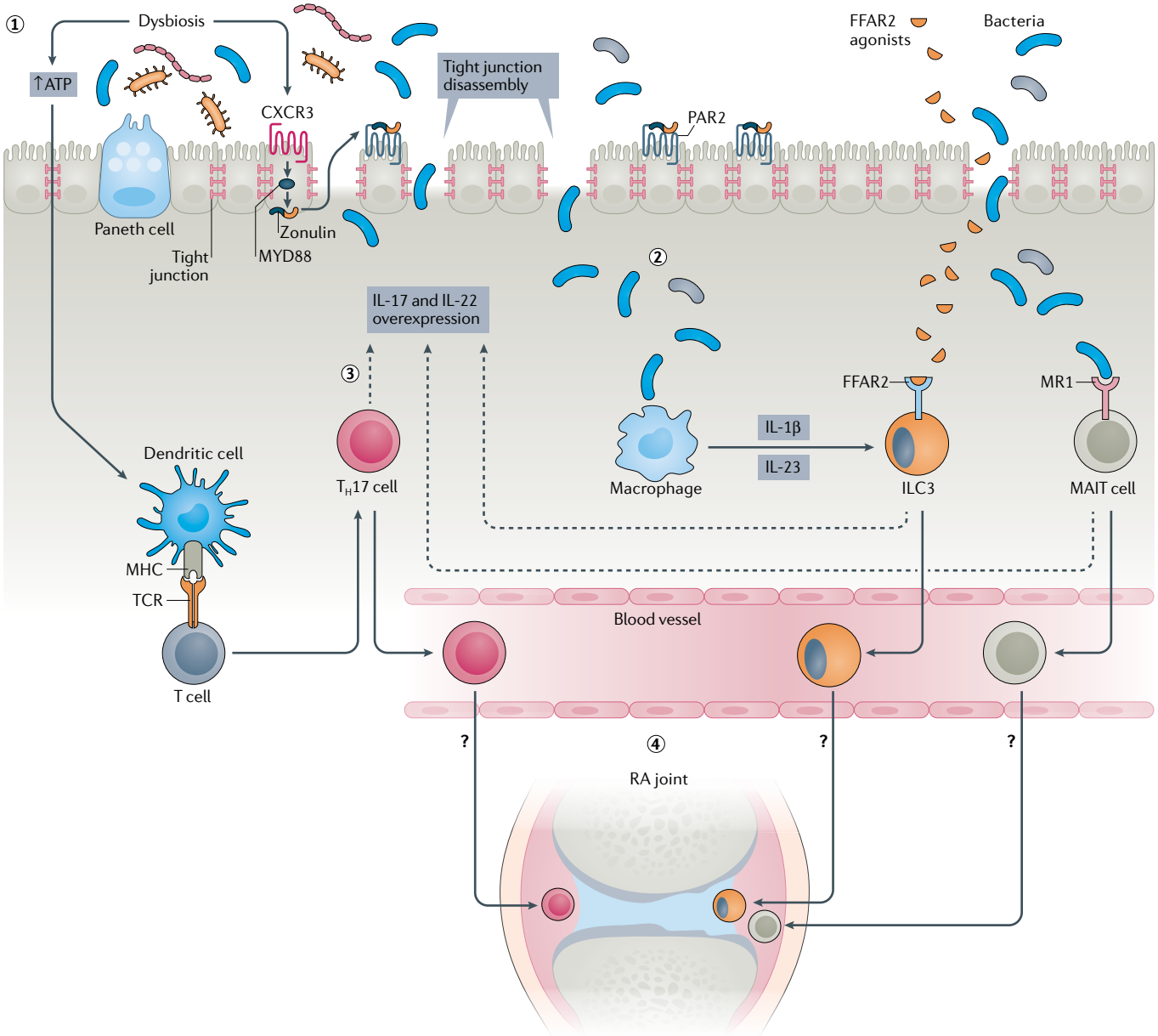


Fig. 3 | **Interactions between microbiota, intestinal epithelium and immune cells in RA.** Pathobionts contribute to the inflammatory profile of epithelial cells via the chemokine receptor CXCR3 and myeloid differentiation primary response 88 (MYD88)-mediated signalling, which increases the production of zonulin (1). Zonulin causes the derangement of epithelial tight junctions, thereby increasing the penetrance of microorganisms and microbial products such as ATP and free fatty acid receptor 2 (FFAR2) agonists in the submucosae (2). Dendritic cells respond to increased ATP production and prime T cells to become T helper 17 ($T_H17$) cells, which produce IL-17. Bacteria-derived FFAR2 agonists and other bacterial products directly activate group 3 innate lymphoid cells (ILC3s), thereby inducing IL-22 secretion. Microorganisms also directly activate innate immune cells, such as macrophages, and innate-like cells, such as mucosa-associated invariant T (MAIT) cells, triggering an amplificatory cascade that leads to intestinal inflammation and local activation and differentiation of T cells, macrophages and ILC3s (3). $T_H17$ cells, ILC3s and MAIT cells can migrate into the blood, raising the possibility that inflammation could be transferred to the joints by these cells (4). MR1, MHC class I-related gene protein; PAR2, proteinase-activated receptor 2; RA, rheumatoid arthritis; TCR, T cell receptor.

duration[125]. Overall, these data could suggest a shift in the ILC profile in RA from a homeostatic phenotype to a pro-inflammatory phenotype[126]. ILC3s contribute to type 3 immune responses by secreting IL-17 and IL-22 and have high expression of the activation marker NKp44, further supporting the idea that ILC3s could participate in the pathogenesis of RA[125]. In mice with CIA, the number of $CCR6^+$ ILC3s was significantly increased in arthritic joints compared with non-arthritic joints and produced IL-22 and IL-17A[127]. In patients with RA, although an intestinal origin has not yet been established, $CCR6^+$ ILC3s were enriched in synovial fluid in two cohorts of individuals, and the frequency of these cells correlated with disease activity and the concentration of CCL20 in synovial fluid[127,128]. The relevance and importance of the contribution of ILC3s to RA is still unknown, as is how ILC3 blockade might affect the development and severity of RA. In the future, it will be worth investigating the presence and phenotype of ILC3s directly in RA synovial tissue.

***Mucosa-associated invariant T cells.*** Mucosa-associated invariant T (MAIT) cells are a population of innate-like $ROR\gamma t^+CD3^+CD4^-CD8^-$ T lymphocytes that typically reside at mucosal and epithelial barriers and in the liver. MAIT cells express an invariant T cell receptor that is restricted to recognizing antigens presented by the MHC class I-like molecule MR1. In line with the principal localization of MAIT cells at mucosal surfaces, they respond to products of bacterial origin, thereby acting as a potential immunological bridge between the intestinal microbiota and the immune system[129]. MAIT cell involvement has been observed in IBD and ankylosing spondylitis, in which they represent one of the principal sources of IL-17 (REFS[130–132]); however, this production of IL-17 has not been observed in RA, suggesting a disease-specific profile[133]. In treatment-naive individuals with early RA, the frequency of total circulating MAIT cells did not differ from that seen in individuals with SpA or healthy individuals[133]. However, compared with those from patients with SpA, MAIT cells from patients with RA were predominantly $CD4^+$, had reduced expression of CD161 and were hyporesponsive to *E. coli* stimulation[133]. Notably, the chronic exposure of MAIT cells to bacterial products could lead to exhaustion[134], offering a possible interpretation of their hyporesponsiveness and the downregulation of CD161 that occurs in RA. By contrast to early RA, data from patients with established RA show a reduction in circulating MAIT cells[135]; high concentrations of TNF and IL-1β in the synovial fluid promote MAIT cell migration to the joints[136]. The inflammatory milieu in the joints also leads to the upregulation of the cell adhesion molecules E selectin, ICAM1 and VCAM on endothelial cells, which facilitate the migration of MAIT cells (potentially of mucosal origin) into the joint[136].

To date, it has not yet been established if the MAIT cells found in the joints have an intestinal origin. However, it is known that MAIT cells are rare in lymphoid tissues as they lack lymph node homing receptors; instead, MAIT cells express the gut-homing molecule α4β7 integrin[137]. Multiple studies characterizing the distribution of MAIT cells in humans have demonstrated that these cells are preferentially enriched in the gastrointestinal system, where they represent a substantial proportion of the total $CD4^-$ T cells: 20–50% in the liver, 10% in the colon, 1.5% in the ileum, 2% in the rectum and around 60% in the jejunum[137]. Therefore, it is likely that MAIT cells, once activated in the mucosal environment, traffic to the inflamed joint as part of the hypothetical gut–joint axis.

***Intestinal T follicular helper cells.*** The migration of T follicular helper ($T_{FH}$) cells from the gut to the joints has been studied using photoconvertible transgenic mice that ubiquitously express the green-to-red photoconvertible fluorescent protein KikGR in their cells[138,139]. By performing a surgical procedure to specifically photoconvert Peyer's patch cells with violet laser light, the migration of the photoconverted cells to other organs can be monitored using a variety of methods. In a study that used KikGR in mice with the K/B×N autoimmune arthritis background, SFB were shown to mediate the egress of $T_{FH}$ cells from Peyer's patches into the spleen[100]. This egress is essential for SFB-induced arthritis because the production of autoantibodies predominantly occurs in the spleen and lymph nodes and not in the Peyer's patches; the deletion of Peyer's patches considerably reduced splenic $T_{FH}$ cell numbers and autoantibody titres in these mice[100]. The underlying pathogenesis of this disease is mediated by the gut-residing SFB, which induce the differentiation and egress of $T_{FH}$ cells to systemic sites and distally boost the systemic $T_{FH}$ cell and autoantibody responses that exacerbate arthritis. SFB induce $T_{FH}$ cell differentiation by enhancing the main $T_{FH}$ cell regulator, BCL-6, in a dendritic cell-dependent manner[100].

Molecularly, it is interesting to understand the factors that regulate gut-derived $T_{FH}$ cell responses that trigger arthritis. The purinergic receptor P2RX7 has been identified as a potential therapeutic target for inflammatory arthritis[140,141]. Although initially identified for its essential role in innate immune responses[142], P2RX7 also has important roles in the regulation of T cell-mediated adaptive immune responses. P2RX7 activation negatively controls the number of intestinal $T_{FH}$ cells in C57BL/6 mice to support host–microbiota homeostasis[140]. Furthermore, P2RX7 deficiency enhances autoimmune arthritis in K/B×N mice by reducing apoptosis in $T_{FH}$ cells, thereby leading to an increase in autoantibody production[141]. Interestingly, some patients with systemic-onset juvenile idiopathic arthritis have loss-of-function mutations in *P2RX7* (REF.[143]). This condition is an example of a 'human knock-out', further confirming the involvement P2RX7 in modulating $T_{FH}$ cell function and preventing autoimmunity. $T_{FH}$ cells promote B cell differentiation and autoantibody production by producing IL-21; therefore, it is not surprising that the expansion of $T_{FH}$ cells occurs in RA, both in secondary lymphoid organs and in the periphery, including in synovial tissue and blood[144]. Although some clues exist as to the role of $T_{FH}$ cells in the pathogenesis of RA, their exact role has not been defined; however, data from 2020 suggest that the altered ratio between $T_{FH}$ cells and T follicular regulatory cells, which suppresses excessive $T_{FH}$ cell proliferation, might

be crucial in determining the autoantibody production associated with RA[145]. The intestinal microbiota also seems to be involved in maintaining this balance. In K/B×N mice, SFB-induced arthritis was linked to a reduction in CTLA4, an important regulatory molecule, on T follicular regulatory cells. SFB could also contribute to arthritis onset by perturbating the balance between $T_{FH}$ cells and T follicular regulatory cells, favouring autoimmunity[146]. This delicate balance deserves further study in humans and could be one of the targets of abatacept (which affects CTLA4 signalling) and of potential novel therapies.

## Modulating the gut–joint axis

### *Anti-rheumatic drugs and the gut microbiota*

The quantitative and qualitative alterations in the intestinal microbiome that are associated with RA raise questions about the effects of current anti-rheumatic treatments on the gut microbiota of patients with RA. Chemical anti-rheumatic drugs can perturb microbial composition by modulating immune function and by acting directly as xenobiotics in the metabolism of microbial cells. Minocycline, a tetracycline-class antibiotic, has historically been used as a DMARD for the treatment of RA and in some countries is still used in a small number of patients[147]. The use of antibiotic therapy in the treatment of RA stems from a theory about a potential infectious trigger for RA and was aimed at the eradication of mycoplasma[147]. However, the efficacy of minocycline in RA was later explained by the inherent anti-inflammatory and immunomodulatory effects exerted by tetracyclines[147,148]. Currently, in light of advances in understanding the relevance of intestinal and extra-intestinal microbiota in inflammatory arthritis, questions are being raised about the potential of tetracycline to influence RA manifestation by perturbing the gut microbiota. Although there is a lack of direct data on the effect of minocycline on gut microbiota, a single exposure to minocycline is known to cause a robust shift in faecal microbiota in healthy individuals[149]. Interestingly, minocycline treatment is associated with a reduction in the abundance of intestinal RA-associated microbial taxa[8,13], such as Actinobacteria, including *Collinsella* spp., and some Firmicutes[149]. Conversely, *Bacteroides* spp., which have been preliminarily reported to be reduced in RA[150], were increased in healthy individuals after minocycline administration[149].

In a metagenome-wide association study of faecal, dental and salivary samples from a cohort of individuals with RA and healthy individuals, the RA-associated microbiome differed from that of healthy individuals, as has been shown in other studies, but after 3 months of methotrexate therapy, these differences were partially lost[7]. Interestingly, a predictive model based on the expression of microbiome genes that were clustered into metagenomic linkage groups could be used to distinguish between patients with RA who responded well to DMARD therapy and those who did not[7]. Similarly, in a small cohort case–control 16S rRNA sequencing-based study of the faecal microbiome that included 42 patients with RA and 10 healthy individuals, methotrexate therapy was associated with a decrease in the relative abundance of the order Enterobacteriales[151]. One potential explanation for these findings is that methotrexate can affect the highly conserved enzyme dihydrofolate reductase, which is expressed by bacteria, so could have an off-target effect on bacterial survival and proliferation[152,153]. The link between methotrexate and gut microbiota is now more relevant than ever as evidence is emerging that points to the gut microbiota as an important determinant of methotrexate metabolism and pharmacokinetics, thereby contributing to the rate of treatment response in RA[154].

Sulfasalazine has both antibacterial and anti-inflammatory properties and requires activation by the gut microbiota to be effective[155]; the inactive drug sulfasalazine is converted by the microbial-encoded enzyme azuroreductase in the distal gut into active 5-aminosalicylic acid[156]. Nevertheless, the amount of data produced so far on the effects of sulfasalazine therapy on the gut microbiota in RA is limited. One study conducted before the advent of next-generation sequencing found a substantial fall in faecal *Clostridium perfringens* and *E. coli* during sulfasalazine therapy[157]. This reduction in *E. coli* was subsequently confirmed in another study, in which it was associated with a concomitant reduction in *Bacteroides* spp. and an increase in *Bacillus* spp.[158]. Treatment of patients with RA with the anti-rheumatic drug hydroxychloroquine was also associated with increases in intestinal bacterial richness and diversity in a 16S rRNA sequencing study, contributing to the restoration of a healthy-like microbiome[8]. Interestingly, hydroxychloroquine seemed to restore the abundance of *Faecalibacterium* spp., which are known producers of butyrate, a SCFA with anti-inflammatory and gut barrier regulatory functions[8,151].

Although TNF blockade is a cornerstone of the treatment of RA, the number of studies investigating the effect of TNF inhibitors on the microbiome is limited. Increases in Cyanobacteria and Nostocophycideae and decreases in Clostridiaceae and Deltaproteobacteria occurred in the faecal microbiomes of patients with RA who had been treated with etanercept[151]. By contrast, in mice with CIA, etanercept treatment led to a reduction in intestinal microbial richness and diversity characterized by an increase in *Escherichia* and *Shigella* spp. and a decrease in *Lactobacillus*, *Clostridium* cluster XIVa and *Tannerella* spp.[159]. Further studies are warranted to investigate the effect of cytokine inhibitors on the RA-associated intestinal microbiome. However, caution should be taken in translating data from other conditions, particularly IBD, to RA. Despite some commonalities suggestive of the existence of a common dysbiosis, distinct alterations in the microbiome have been observed among different immune-mediated inflammatory diseases[23,160].

### *Dietary interventions*

The possibility that current effective treatments for RA can additionally modify the intestinal bacterial flora raises the idea that modulation of gut microbiota could be used for therapeutic purposes in patients with RA. Going against this hypothesis are data from a French cohort showing that strict oral hygiene does not modify

disease activity in patients with established RA[21]; however, this approach has not yet been explored in patients with RA in the early phases. The interaction between dysbiosis and the intestinal innate immune system is likely to be important in the triggering phase of the inflammatory process which, once established, cannot be modified by interventions that target dysbiosis. Efforts to modify dysbiosis in the preclinical stages of diseases or in patients at high-risk of developing RA will be of great value to better understand how these changes can affect the onset of RA. Some randomized controlled trials have been conducted in RA to investigate the effects of probiotics on disease activity and the cytokine profile. A meta-analysis of four such trials did not demonstrate any efficacy of probiotics as adjunct therapy for RA[161]. However, considering the sample sizes and the design of the available studies, this result cannot yet be considered conclusive. Among the dietary interventions tested in RA to date, only the Mediterranean diet and a vegetarian diet have been shown to contribute to a reduction in disease activity[162,163].

SCFAs such as butyrate, which are produced by bacterial metabolism of dietary components, have a direct immunomodulatory function. The effect of SCFAs on immune cells seems to be largely mediated by direct binding to FFAR2 on colonic T cells and ILC3s[122,164]. In mice, a lack of FFAR2 is associated with a pro-inflammatory intestinal phenotype and increased susceptibility to colonic inflammation and infection[122]. SCFAs and FFAR2 agonists induce the expansion of ILC3s and the production of IL-22 in mice, which has a local protective effect on colonic inflammation[122]. In T cells, SCFAs and FFAR2 activation lead to the local and systemic expansion of $T_{reg}$ cells[164]. SCFA administration has also been tested therapeutically in mice with CIA, in which it reduced the severity of arthritis via the modulation of IL-10 (REF.[164]).

The main substrate for the production of SCFAs by gut-resident microbiota is fibre. In light of the aforementioned data on the biological activity of bacterial metabolites such as SCFAs in modulating mucosal integrity, it seems reasonable to suggest that a diet rich in fibre could have a protective effect on the gut and reduce systemic inflammation in RA. Moreover, fibre could represent one of the main contributors to the benefit induced by vegetarian or Mediterranean diets, as both are high in fibre. Building on this theory, a feasibility study was performed using a high-fibre diet supplement in 36 patients with RA for 28 days[165]. After the intervention, blood samples showed an increase in circulating $T_{reg}$ cells, amelioration of the $T_H1$ cell to $T_H17$ cell ratio and a decrease in markers of bone erosion, and the intervention also led to improvement in patient-reported outcomes[165].

Another fascinating hypothesis is that the modulatory effect of the natural flavonoid resveratrol on arthritis might function via a beneficial influence on intestinal microbiota. Resveratrol has demonstrated an anti-inflammatory effect and some signs of efficacy in reducing RA symptoms in rats and humans[166,167]. The results of a 2019 study suggest that resveratrol might also influence intestinal microbiota[168], but whether this is one of the mechanisms of action in RA has yet to be demonstrated. Taken together, although many interventions are at an early stage of investigation, these data could pave the way for large double-blind randomized controlled trials investigating microbiota-targeted dietary interventions in RA.

### *Inhibiting immune cell trafficking*

On the basis of the discussions on potential immune cell trafficking from the intestines to the joints, it is conceivable that interventions that affect the migration of T effector cells between systemic sites and the gut might be used for treating inflammatory arthritis. For example, the retinoic acid analogue AM80 increased the expression of the gut-homing molecule α4β7 integrin on $T_{FH}$ cells, diverting these cells away from inflamed sites in the intestine and reducing the severity of arthritis in K/B×N mice[169]. Conversely, blockade of β7 integrins worsened arthritis severity in these mice, but only when SFB were present[169]. The combination of β7 integrin blockade and SFB colonization negatively affected disease development because the presence of SFB causes the expansion of $α4β7^+$ $T_{FH}$ cells and $α4β7^+$ $T_H17$ cells[30,100], which then become concentrated at sites of inflammation because β7 integrin blockade prevents these cells from re-entering the intestine, thereby aggravating arthritis. Keeping in mind that altered intestinal barrier function is just one of the steps along the way to mucosal immune cell activation and recirculation, it is worth noting that restoration of the intestinal barrier in the pre-clinical phase of arthritis using butyrate or a CB1 agonist attenuates the development of arthritis[56]. Moreover, it is intriguing to understand whether and how zonulin-mediated increased intestinal permeability could contribute to the transfer of inflammation to the joint. Short-term blockade of zonulin with larazotide acetate before the onset of arthritis in mice with CIA effectively reduced arthritis by 50%[56]. Treatment improved intestinal barrier function in these mice and prevented the trafficking of primed intestinal cells to systemic organs, as well as to the joints[56]. In line with these findings, $CD11c^+CD103^+$ dendritic cells, which are normally located within the intestinal lamina propria[170], are present in the spleen of mice with CIA, suggesting that dendritic cells could have migrated from the gut to the spleen in these mice[9]. These experimental strategies suggest not only that a gut–joint axis exists in RA but also that it contributes to arthritis and that it can be a source of multiple novel therapeutic targets.

## Conclusions

Although better understood in SpA, it is becoming clear that gut–joint interactions also constitute an important aspect in the pathogenesis of RA, which could open up new therapeutic opportunities. Considering the data coming from experimental models of arthritis and studies in humans with RA, it seems likely that dysbiosis already occurs before the clinical onset of disease and influences the development of arthritis. Studies from the past few years have clearly shown dysbiosis of the gut microbiota in patients with RA. The direct immunological consequences of that dysbiosis are now the focus of ongoing research, which has already provided some new treatment opportunities, such as maintaining

and restoring a functional gut barrier or preventing the migration of gut-primed immune cells out of the intestines. These treatment avenues also present a potential window for interfering at an early point in the disease course to possibly reduce RA symptoms and the necessity of lifelong pharmacological treatments. Future studies should go one step further and concentrate on the cause of the dysbiosis and how that is related to the known genetic risk factors for RA. Whatever might cause the dysbiosis and its immunological consequences, it will be interesting to know if this is simply a trigger that reduces the immunological threshold needed to induce clinical disease onset in the joints.

As illustrated in this Review, similar to other diseases, many microbiome studies in RA are association studies that attempt to correlate changes in the bacterial composition in the gastrointestinal tract with disease. Although these studies imply clinical relevance, mechanistic studies are still required to harness these findings for future diagnostic and therapeutic approaches. One possible avenue for such studies involves the use of a humanized microbiota mouse model, in which germ-free mice are colonized with human commensal bacteria[171,172]. Along this line, *Bifidobacterium adolescentis* and *E. coli* 2A isolated from patients with Crohn's disease-associated SpA have been successfully colonized in antibiotic-treated K/B×N mice to demonstrate a causative effect in initiating disease[66,172]. Moreover, laboratory mice (unlike adult humans) lack effector-differentiated and mucosal memory T cells[173]. By contrast, these cell populations are present in mice from pet stores, which have robust and diverse microbial exposure. Therefore, in addition to the standard laboratory mice housed in specific pathogen-free conditions, 'dirty' pet store mice could offer a complementary angle for studying microorganism–host interactions in RA by providing a normalizing microbial environment that recapitulates adult human immune traits.

Restoration of microbial homeostasis in the gut could potentially be reached via nutritional changes such as the ingestion of a fibre-rich diet, which influences the bacterial composition of the gut and skews the metabolome of intestinal microbiota towards an anti-inflammatory, SCFA-rich pattern. Dysbiosis seems to be an important step in influencing the epithelial barrier function of the gut by affecting tight junction turnover at the intestinal surface and thereby enabling immune cell activation as a result of increased bacterial migration. Tight junction restoration seems to be another important intervention step to consider for the prevention of RA, as the breakdown of barrier function occurs before arthritis onset in both mice and humans. The link between intestinal immune cell activation and arthritis is based on the possible migration of gut-derived immune cells to the joints. This process is the third important step in the onset of arthritis and constitutes another target for interventions that aim to prevent arthritis. All these steps might not only be important in the very early autoimmune phase of the disease, but might also have adjuvant functions in established disease, as data show that dysbiosis and impaired barrier function are also present in patients with established disease.

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CONSENSUS STATEMENT

OPEN



# Consensus terminology for preclinical phases of psoriatic arthritis for use in research studies: results from a Delphi consensus study

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Abstract | The concept of psoriatic arthritis (PsA) prevention is gaining increased interest owing to the physical limitation, poor quality of life and low remission rates that are achieved with current therapies for PsA. The psoriasis-to-PsA transition offers a unique opportunity to identify individuals at increased risk of developing PsA and to implement preventive strategies. However, identifying individuals at increased risk of developing PsA is challenging as there is no consensus on how this population should be defined. This Consensus Statement puts forward recommended terminology from the Psoriasis and Psoriatic Arthritis Clinics Multicenter Advancement Network (PPACMAN) for defining specific subgroups of individuals during the preclinical and early clinical phases of PsA to be used in research studies. Following a three-round Delphi process, consensus was reached for three terms and definitions: 'increased risk for PsA', 'psoriasis with asymptomatic synovio-entheseal imaging abnormalities' and 'psoriasis with musculoskeletal symptoms not explained by other diagnosis'. These terms and their definitions will enable improved identification and standardization of study populations in clinical research. In the future, as increasing evidence emerges regarding the molecular and clinical features of the psoriasis-to-PsA continuum, these terms and definitions will be further refined and updated.

Psoriatic arthritis (PsA) is a chronic, immune-mediated inflammatory disease, characterized by both skin and joint involvement. Synovio-entheseal involvement is present in up to 30% of those with psoriasis[1,2], and individuals with psoriasis progress to PsA at a rate of up to 3% per year[3]. PsA can lead to joint erosions and deformities[4], as well as to decreased quality of life[5], high levels of psychosocial stress[6] and increased rates of comorbidities, unemployment, absenteeism and productivity loss[7]. Despite this burden, PsA remains both underdiagnosed and undertreated, even within dermatology practices[8,9]. The current challenges in diagnosing and treating PsA produce a considerable gap in the care of patients with psoriatic disease, given that a delay in treatment of as little as 6 months can lead to worse disease outcomes[10,11].

Highly effective treatment strategies are a major unmet need in PsA, and various interventions have been envisioned, including innovative therapeutic targets, combination therapies or potentially preventive measures. These last have become a focus in the field of PsA research and will be aimed at defining, predicting and, ultimately, preventing synovio-entheseal inflammation. To this end, it will be necessary to identify individuals at increased risk of developing PsA and to characterize clinical and molecular features that are specific to preclinical stages of disease. Distinguishing these high-risk individuals will help to shape the development, design and implementation of PsA prevention trials. In addition, it will enable improved screening, earlier diagnosis, timely treatment initiation and, eventually, should improve overall disease outcomes.

The Psoriasis and Psoriatic Arthritis Clinics Multicenter Advancement Network (PPACMAN) is an international non-profit organization that aims to "optimize the clinical care of patients with psoriatic disease through multidisciplinary collaboration, education and innovative research"[12]. Within PPACMAN, the Preventing Arthritis in a Multicenter Psoriasis At-Risk Population (PAMPA) study group was established to

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understand the clinical, genetic, environmental and immune events that occur during the natural history of the psoriasis-to-PsA transition[13]. To facilitate research focused on the preclinical and early clinical phases of PsA, the PAMPA study group conducted a consensus-building exercise to agree on common terminology related to the preclinical phases of PsA for use in clinical trials and translational research. The development of standardized nomenclature and common definitions to be used exclusively for research in this area should help in the recruitment of well-defined, homogeneous cohorts of patients and enable comparison across future trials and experimental therapeutic studies. This exercise emulates the efforts taken to create the EULAR recommendations for terminology for those at risk of rheumatoid arthritis (RA)[14]. In this Consensus Statement, we describe the process and results of a consensus-building exercise to develop nomenclature for preclinical PsA that can be integrated into future research studies.

## Methods

### *Scientific committee*

The consensus exercise was led and designed by the PAMPA study group and the PPACMAN steering committee, composed of methodologists, as well as dermatologists and rheumatologists who are experts in psoriatic disease.

### *Overview of study design and methods*

In this study, an online Delphi process was used that included international experts in psoriatic disease to achieve consensus on the terminology related to the preclinical phases of PsA for research purposes. The Delphi method is an iterative series of structured rounds that surveys experts until group consensus is reached. This method lends itself well to an online format, which enables a larger number of international experts to participate in the survey and avoids any strong influences from a small number of individuals or from standards of practice in certain countries. The Delphi method is used widely in health-care-related research that relies on expert opinion[15,16]; however, some limitations must be acknowledged. The Delphi methodology has been criticized for a possible lack of sufficient details about the background information provided to participants, differing response rates for all rounds, lack of formal feedback between rounds and lack of ability to discuss disagreement directly[17,18]. This Delphi exercise was therefore carefully designed to provide sufficient background detail and feedback for each round and to provide intermittent opportunities for direct discussion. For this study, a pre-Delphi exercise and three rounds of Delphi surveys were used.

***Pre-Delphi exercise.*** Prior to the Delphi development, experts in psoriasis and PsA ($n = 28$) were queried via e-mail regarding terms and definitions of the phases that individuals with psoriasis might go through prior to PsA development. This group of experts was recruited from PPACMAN and also included members of the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA), an international non-profit organization, and the National Psoriasis Foundation (NPF), a non-profit organization from the USA. On the basis of these results, preliminary terms and definitions were drafted and presented at the PPACMAN 2018 Annual Meeting[19]. After an introductory session, four breakout workshop sessions took place in which attendees suggested changes and provided open-ended opinions about the terms and definitions proposed in the form of small group discussions. These workshop sessions were followed by a plenary session in which the outcomes of each breakout workshop were summarized, and culminated with a voting exercise via an anonymous automated response system. This meeting included dermatologists ($n = 8$), rheumatologists ($n = 13$), rheumatologist–dermatologists ($n = 2$) and industry representatives ($n = 17$). Notably, the industry representatives met in a separate breakout session and did not participate in the voting process to avoid the introduction of bias into the study findings. Further details on the pre-Delphi session can be found in the Supplementary Information. The collected input and results were used to inform the development of the subsequent Delphi survey.

***Delphi process.*** The Delphi survey was designed using feedback from the pre-Delphi exercise. The survey was created and distributed using the New York University REDCap software[20] to 45 international experts in psoriasis and PsA who were selected by the scientific committee. As with the pre-Delphi exercise, experts were recruited from PPACMAN and included members of GRAPPA and the NPF, and all members who participated in the pre-Delphi exercise were invited to participate in the Delphi exercise. These experts had an average of over 18 years of experience in their fields (TABLE 1). No members of industry were invited to participate. All answers were anonymous, and participants

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Table 1 | **Numbers and demographics of participants in each Delphi round**

| Demographic | First round (*n* = 29) | Second round (*n* = 33) | Third round (*n* = 35) |
|---|---|---|---|
| ***Type of participant*** | | | |
| Dermatologist | 5 (17.2%) | 6 (18.2%) | 10 (28.6%) |
| Rheumatologist | 20 (69.1%) | 22 (66.7%) | 22 (62.9%) |
| Rheumatologist–dermatologist | 3 (10.3%) | 3 (9.1%) | 3 (8.6%) |
| Non-clinician researcher | 1 (3.4%) | 2 (6.1%) | 0 (0.0%) |
| ***Location of participant*** | | | |
| USA | 18 (62.1%) | 20 (60.6%) | 24 (68.6%) |
| Europe | 7 (24.1%) | 9 (27.3%) | 7 (20.0%) |
| Canada | 4 (13.8%) | 4 (12.1%) | 4 (11.4%) |
| ***Gender of participant*** | | | |
| Female | 12 (41.4%) | 15 (45.5%) | 16 (45.7%) |
| Male | 17 (58.6%) | 18 (54.5%) | 19 (54.3%) |
| ***Amount of experience of participants*** | | | |
| Mean number of years (s.d.) | 18.70 (10.47) | 17.36 (10.42) | 18.86 (11.24) |
| ***Type of experience of participants*** | | | |
| Academic clinicians | 25 (86.2%) | 26 (78.8%) | 28 (80.0%) |
| Clinicians who work privately | 1 (3.4%) | 3 (9.1%) | 3 (8.6%) |
| Both | 3 (10.3%) | 4 (12.1%) | 4 (11.4%) |

were asked to vote on and rank their preferred terms and definitions for describing populations of individuals with preclinical PsA. Space for free-form comments was also provided for each question. In all rounds, participants were provided with the results and discussion points generated in the previous rounds, as well as links to published literature to provide background information related to the voting topics[3,13]. Results from the first Delphi round were discussed at the PPACMAN meeting adjacent to the 2019 Annual Meeting of GRAPPA by multiple stakeholders. Consensus for multiple choice and ranking questions was defined a priori as ≥70%. For questions that use a visual analogue scale (VAS) ranging from 0 mm (should not be considered) to 100 mm (should definitely be considered), items were retained if the median score was >70 mm. If consensus was not reached, the question was carried through to the next round; however, Delphi items rated on a VAS that had a median score of <60 mm were removed.

***Data analysis.*** Descriptive statistics were used to report the voting results. Continuous data are presented as medians and interquartile range unless otherwise noted.

## Results

### *Pre-Delphi exercise*

At the 2018 PPACMAN Annual Meeting, stakeholders were presented with preliminary terms and definitions that were informed by the input collected from the initial survey distributed by e-mail. Expanded discussions and voting followed, but no consensus was reached. Further details of the pre-Delphi exercise are provided in the Supplementary Information.

### *Delphi exercise*

Round 1 of the Delphi exercise received 29 responses (response rate 64.4%), round 2 received 33 responses (response rate 73.3%) and round 3 received 35 responses (response rate 77.7%). Although the invited participants varied with regard to demographics and experience, the respondents were rheumatologists, dermatologists and rheumatologist–dermatologists who were mostly academic clinicians and/or researchers (TABLE 1). The original terms and definitions presented for voting in the Delphi exercise are provided in the Supplementary Information. BOX 1 shows the final terms and definitions proposed after consensus was reached.

### *Terms and definitions*

***Individuals with psoriasis at increased risk for PsA.*** During round 1 of the Delphi exercise, 80% of the panellists agreed on the term 'increased risk for PsA', and 86% voted that this term defines a meaningful subgroup for future research studies. Other terms proposed included 'at risk', 'high risk', 'higher risk' and 'elevated risk'. The term 'at risk' for PsA was not favoured by participants, who noted that any patient with psoriasis has the potential to develop PsA. During round 1, consensus for the definition was not reached.

In round 2 of the Delphi exercise, the definition of 'any individual with psoriasis and one or more risk factors for progression to PsA' reached consensus with 84.4% agreement. The alternative definition proposed was 'any individual with psoriasis and one or more risk factors for progression to synovio-entheseal disease'. Participants noted that the term synovio-entheseal disease might not be commonly used, particularly by dermatologists.

Regarding which risk factors for progression from psoriasis to PsA should be considered, obesity, the presence of arthralgia, severe psoriasis, a history of uveitis, nail psoriasis, scalp psoriasis and having a first-degree relative with PsA reached consensus in round 1 of the Delphi exercise (median >70 mm on a 100-mm VAS), whereas any associated gene (such as *HLA-B*08*, *HLA-B*27*, *HLA-B*38* or *HLA-B*39*) reached consensus in round 2 of the Delphi exercise (Supplementary Figure 1).

***Individuals with psoriasis and asymptomatic synovio-entheseal imaging abnormalities.*** In round 1 of the Delphi exercise, the terms 'subclinical PsA', 'potential PsA', 'psoriasis with imaging findings', 'psoriasis with asymptomatic synovio-entheseal imaging findings' and 'psoriasis with imaging abnormalities' were proposed (Supplementary Table 1). Subclinical PsA was generally disliked because the term implies that patients will definitely go on to develop PsA. As consensus was not reached, the three terms with the highest number of votes were moved to round 2 of the Delphi exercise. In round 2, the term 'psoriasis with asymptomatic synovio-entheseal imaging abnormalities' outstripped the other terms, gaining almost 60% of the votes. Consensus was finally reached for this term by 85.7% of participants in round 3 of the Delphi exercise. Overall, 86.2% of the

Box 1 | **PAMPA consensus terminology for preclinical phases of PsA**

According to the proposed terminology, in prospective research studies, individuals would be described in the following ways:

**Individuals with psoriasis at increased risk for PsA**
Any individual with psoriasis and one or more risk factors for progression to psoriatic arthritis (PsA).

- Risk factors include obesity, the presence of arthralgia, severe psoriasis, a history of uveitis, nail psoriasis, scalp psoriasis, having a first-degree relative with PsA and any associated gene (such as *HLA-B*08*, *HLA-B*27*, *HLA-B*38* or *HLA-B*39*).
- Can be combined with either of the other two terms.

**Individuals with psoriasis and asymptomatic synovio-entheseal imaging abnormalities**
Any individual with psoriasis and imaging evidence of synovio-entheseal abnormalities that is not associated with clinical signs or symptoms.

- Imaging modalities include MRI for axial disease, MRI for peripheral arthritis, ultrasonography for peripheral arthritis, ultrasonography for enthesitis and plain radiography for peripheral arthritis. Specific MRI findings used to define imaging abnormalities include enthesitis, bone marrow oedema, synovitis, tendonitis, bone erosions and new bone formation. Specific ultrasonography findings used to define imaging abnormalities include enthesitis, synovitis, tendonitis and bone erosions.
- Can be combined with 'individuals with psoriasis at increased risk for PsA'; for example, an individual with psoriasis might have uveitis (a risk factor for PsA) and have asymptomatic enthesitis defined by ultrasonography.
- Cannot be combined with 'individuals with psoriasis and musculoskeletal symptoms not explained by other diagnosis'.

**Individuals with psoriasis and musculoskeletal symptoms not explained by other diagnosis**
Any individual with psoriasis and heel pain, stiffness and/or arthralgia not explained by another diagnosis.

- Can be combined with 'individuals with psoriasis at increased risk for PsA'; for example, an individual with psoriasis might have uveitis (a risk factor for PsA) and have heel pain that is not explained by another diagnosis.
- Cannot be combined with 'individuals with psoriasis and asymptomatic synovio-entheseal imaging abnormalities'.

participants agreed that this term defines a meaningful population for future research studies.

In round 1 of the Delphi exercise, two definitions were initially proposed for this term (Supplementary Table 2). Consensus was not reached, and panellists suggested two new definitions, which were included in rounds 2 and 3 of the Delphi exercise. Consensus was finally reached in round 3 for 'any individual with psoriasis and imaging evidence of synovio-entheseal abnormalities that is not associated with clinical signs or symptoms' (88.6% of the votes).

Imaging modalities that reached consensus for use to define 'imaging abnormalities' in round 1 of the Delphi exercise included MRI for axial disease (median on a 100-mm VAS = 97 mm), MRI for peripheral arthritis (86 mm), ultrasonography for peripheral arthritis (90 mm), ultrasonography for enthesitis (90 mm) and plain radiography for peripheral arthritis (70 mm) (Supplementary Figure 2). Participants also voted on which specific MRI and ultrasonography signs should be considered for use in defining imaging abnormalities. MRI signs that met consensus for inclusion were enthesitis (median on a 100-mm VAS = 90 mm), bone marrow oedema (90 mm), synovitis (95 mm), tendonitis (70 mm), bone erosions (81 mm) and new bone formation (80 mm) (Supplementary Figure 3). For ultrasonography, enthesitis (median on a 100-mm VAS = 89 mm), synovitis (97 mm), tendonitis (77 mm) and bone erosions (87 mm) all met consensus in round 1 of the Delphi exercise (Supplementary Figure 4).

***Individuals with psoriasis and musculoskeletal symptoms not explained by other diagnosis.*** During round 1 of the Delphi exercise, 93.1% of participants agreed on the term 'psoriasis with musculoskeletal symptoms not explained by other diagnosis' (BOX 1). In round 2 of the Delphi exercise, 87.9% voted that this term was meaningful for future research studies. Previous iterations of this term included 'prodromal PsA', 'psoriasis with arthralgia', 'psoriasis with musculoskeletal symptoms' and 'psoriasis with musculoskeletal symptoms without musculoskeletal signs'. Similar to the term 'subclinical PsA', participants argued that the term 'prodromal PsA' implied that all patients with psoriasis would progress to PsA and was therefore inappropriate.

To define which musculoskeletal symptoms should be considered, participants were provided with a list of symptoms that had previously been identified in the literature as predictors of PsA, along with their hazard ratios and 95% confidence intervals[21]. Participants then scored the factors on a VAS, and a median score of ≥70 mm was defined as reaching consensus. Of these factors, heel pain, stiffness and arthralgia all reached consensus (median scores of 84 mm, 80.5 mm and 75 mm, respectively), whereas fatigue and problems with physical function (median scores of 67.5 mm and 51 mm, respectively) did not.

***Terms and definitions that did not achieve consensus.*** Participants were also asked to comment on time points after the diagnosis of PsA, specifically looking at the 6-month and 24-month time points. Although consensus was reached for 6 months from diagnosis being a meaningful time point (90.9%), consensus was not reached on whether this population of individuals should be termed 'early PsA', 'very early PsA' or 'new onset PsA'. Similarly, consensus was not reached on whether diagnosis should be defined by satisfying Classification for Psoriatic Arthritis (CASPAR) criteria[22] and/or musculoskeletal symptom onset. There was a lack of consensus that 24 months was a meaningful time point.

## Future research agenda

During the face-to-face PPACMAN meeting adjacent to the 2019 Annual Meeting of GRAPPA, the results of the consensus exercise were presented, and several areas were identified as priorities for investigation.

### *Imaging*

Imaging studies (primarily ultrasonography or MRI modalities) have the potential to improve the definition of meaningful subclinical inflammatory states and their ability to predict PsA development. In particular, ultrasonography represents a feasible and adaptable modality that is already being applied in the clinical setting to identify patients with psoriasis who have subclinical enthesitis and/or synovitis[23–25]. High-resolution peripheral

quantitative computed tomography (HR-pQCT) also shows promise for use in assessing if the presence of structural entheseal lesions can predict future PsA among patients with psoriasis[26]. Whether sonographic findings (such as synovitis, enthesitis, tenosynovitis or peritonitis) or radiological findings (such as bone erosions, tenosynovitis or bone proliferations) represent abnormal inflammatory features or are simply physiological immune-mediated responses aimed at containing disease progression remains a subject of intense debate. However, reports that the treatment of psoriasis using IL-12–IL-23 blockade[27,28] or IL-17 blockade[29] in patients without overt joint symptoms resulted in suppression of these sonographic and radiological abnormalities are promising and will surely aid in the design of future preventive studies, particularly if paired with clinical and molecular risk factor enrichment strategies. These studies will likewise inform a future revision of the term 'psoriasis with musculoskeletal symptoms not explained by other diagnosis'.

### *Prodromal phase and non-specific pain*

In an attempt to characterize a 'preclinical' phase of PsA, the term 'psoriasis with musculoskeletal symptoms not explained by other diagnosis' was selected by stakeholders over 'prodromal' and 'preclinical', as these terms might imply that the progression from psoriasis to PsA is definite. Indeed, the terms 'preclinical' or 'prodromal' PsA can only be applied retrospectively at this time owing to a lack of ability to truly predict progression to PsA. Currently, limited data exist with which to conclusively define the non-specific musculoskeletal symptoms that should be considered as part of this phase for research purposes[21]. Further understanding of this stage is crucial to distinguish who might be at the highest risk of progression to PsA and, ultimately, to implement early treatment and prevention strategies for PsA. Information gathered from ongoing[30] and future longitudinal studies of musculoskeletal symptoms will be needed to further inform and revise this definition.

### *Preventive trial design*

A preventive medicine approach is not foreign to the field of rheumatology and chronic immune-mediated inflammatory diseases. Specifically, investigators have pioneered trials in preclinical or pre-damage stages of systemic lupus erythematosus and RA, resulting, in some cases, in improved outcomes and even prevention[31–35]. Several prevention trials supported by the US National Institutes of Health are currently underway, including the SMILE study[35] and the StopRA study[36], and many other studies are in progress in Europe[37].

Although the field of psoriatic disease is rapidly moving forward in the design of preventive trials, several questions inevitably remain unanswered and will require a retrospective analytical understanding of the psoriasis-to-PsA transition to be achieved before they can be answered and preventive trials can be initiated. Chief among those questions is how to ascertain the relative weight for each proposed risk factor (in other words, the risk enrichment). The clinical, demographic, genetic and molecular features currently associated with progression from psoriasis to PsA have been mostly derived from retrospective and cross-sectional studies. One strategy for identifying relevant risk factors for progression in prospective studies consists of studying individuals with psoriasis at increased risk of developing PsA who have at least moderate skin disease and imaging evidence of entheseal abnormalities, plus one or more of the following features: scalp involvement, psoriatic nail disease or genetic factors linked to progression (such as a first-degree relative with PsA)[38].

The specific therapeutic approach in prevention trials will also be challenging, as arguments exist for using medications with any of the available mechanisms of action (such as TNF inhibitors, IL-17 inhibitors, IL-23 inhibitors or phosphodiesterase 4 inhibitors). Importantly, the role of natural history registries (following patients on immunomodulatory therapies as well as those with psoriasis who elect not to be treated with systemic medications) will be of the utmost relevance, as the ultimate goal will be to create a risk-score that incorporates the relative predictive value of each of the proposed risk factors alongside rigorous cut-off thresholds for sensitivity and specificity.

## Conclusions

Given that psoriasis commonly precedes the development of PsA[39], a unique pre-disease window of opportunity exists in the psoriasis-to-PsA continuum for studies on the clinical and molecular features of transition. To capitalize on this window of opportunity, it is imperative that the preclinical stages of PsA are better understood. The terms and definitions developed by the PAMPA study group describe clinical and imaging features of pre-disease states that individuals might traverse prior to PsA development. The use of standardized nomenclature and common definitions for PsA research will help to facilitate communication and comparison across future studies, which will enable robust validation between efforts in this particularly complex and heterogeneous disease. Overall, it is hoped that the consensus definitions set out in this Consensus Statement will catalyse the development of preventive strategies and, ultimately, improve outcomes in PsA[13].

Importantly, the overarching output derived from this consensus exercise is to be used exclusively for research purposes at this time. Although necessarily an evolving process, this work represents a much-needed starting point. Furthermore, this terminology should not be viewed as restrictive or unchangeable, nor should it be used for clinical care, given the preliminary nature of these terms and definitions. Adoption of terminology in the clinical sphere will require a natural refinement process and an iterative validation approach as research in this area progresses. Together, these efforts will characterize novel clinical and molecular features associated with the transition of psoriasis to PsA, which in turn will oblige the field to revise the proposed definitions and risk factors.

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### Author contributions
L.M.P.-C., R.H.H., J.F.M. and J.U.S. researched data for the article. L.M.P.-C., R.H.H., S.R., A.O., J.F.M. and J.U.S. provided substantial contributions to discussions of the content and wrote the article. All authors reviewed and/or edited the manuscript before submission.

### Competing interests
R.H.H. declares they have received consultation honoraria from Janssen. V.C. declares they have received research grants and/or advisory board honoraria from AbbVie, Amgen, BMS, Celgene, Eli Lilly, Janssen, Novartis, Pfizer and UCB. V.C. also declares that their spouse is employed by Eli Lilly. C.F.R. declares they have received consultation and/or investigator honoraria from AbbVie, Eli Lilly and Novartis. L.E. declares they have received consultation honoraria and non-restricted research and educational grants from AbbVie, Amgen, Eli Lilly, Janssen, Novartis, Pfizer and UCB. P.M. declares they have received research grants, consultation and/or speaker honoraria from AbbVie, Amgen, BMS, Boehringer Ingelheim, Celgene, Eli Lilly, Galapagos, Gilead, GlaxoSmithKline, Janssen, Novartis, Pfizer, Sun Pharma and UCB. S.R. declares they have received consultation honoraria from Amgen, Janssen, Novartis and Pfizer and have been involved in clinical trials with Amgen and Celgene. A.O. declares they have received consultation honoraria from AbbVie, Amgen, BMS, Celgene, Corrona, Eli Lilly, Janssen, Novartis and Pfizer and have received grant support from Amgen (to Forward/National Databank for Rheumatic Disease) and from Novartis and Pfizer (to the University of Pennsylvania). J.F.M. declares they have received consultation and/or investigator honoraria from AbbVie, Arena, Avotres, Biogen, Celgene, Dermavant, Eli Lilly, EMD Sorono, Janssen, Leo Pharma, Merck, Novartis, Pfizer, Regeneron, Sanofi, Sun Pharma and UCB. J.U.S. declares they have received consultation and/or investigator honoraria from AbbVie, Janssen, Novartis, Pfizer, Sanofi and UCB. All other authors declare no competing interests.


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# Diagnostic role of anti-dsDNA antibodies: do not forget autoimmune hepatitis

*Alessandro Granito, Luigi Muratori, Francesco Tovoli and Paolo Muratori*

In their Review (Pisetsky, D. S. & Lipsky, P. E. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* **16**, 565–579 (2020))[1], Pisetsky and Lipsky highlight the clinical role of antinuclear antibody (ANA) testing in patients with systemic lupus erythematosus (SLE) and properly state that anti-double-stranded DNA (dsDNA) antibodies are highly specific for the diagnosis of SLE; however, they refer to two papers that do not take into account the widely and historically recognized presence of these autoantibodies in individuals with autoimmune hepatitis[2,3]. In this regard, we would like to point out that knowledge of anti-dsDNA antibody positivity in autoimmune hepatitis dates back to 1956 when, because of the similarities to SLE, Mackay et al. proposed that this chronic liver disease be named 'lupoid hepatitis'[4].

The term lupoid hepatitis was first used to describe patients with chronic active hepatitis and positivity for the lupus erythematosus cell test, suggesting that these individuals have a specific form of liver disease that is often associated with extrahepatic complaints that are typically seen in SLE, including arthralgia and a cutaneous rash. In our opinion, the link between the two autoimmune diseases is of considerable clinical and immunological relevance. Patients with autoimmune hepatitis often have anti-dsDNA antibodies and ANAs with a 'homogeneous pattern', a typical feature of SLE, whereas patients with SLE often have a mild hepatic involvement, known as 'lupic hepatitis' to distinguish it from lupoid hepatitis (renamed autoimmune hepatitis), which, in turn, includes ANAs in its diagnostic criteria[5]. In addition, from a genetic perspective, both conditions share a strong association with *HLA-DR3*, suggesting a further parallel between the two diseases[6].

Positivity for anti-dsDNA antibodies occurs in ~30% of patients with autoimmune hepatitis, and concomitant positivity of anti-dsDNA and anti-mitochondrial antibodies occurs in up to 60% of patients with an autoimmune hepatitis–primary biliary cholangitis overlap syndrome[7,8]. ANA detection in autoimmune hepatitis should always be performed by indirect immunofluorescence (IIF) using HEp-2 cells as a substrate, as this method is the only way to ensure the correct identification of ANA IIF-patterns of diagnostic relevance[9]; for example, the homogeneous pattern can be distinguished with high confidence from the 'speckled pattern' on the basis of positive staining of chromatin in mitotic cells (FIG. 1a).

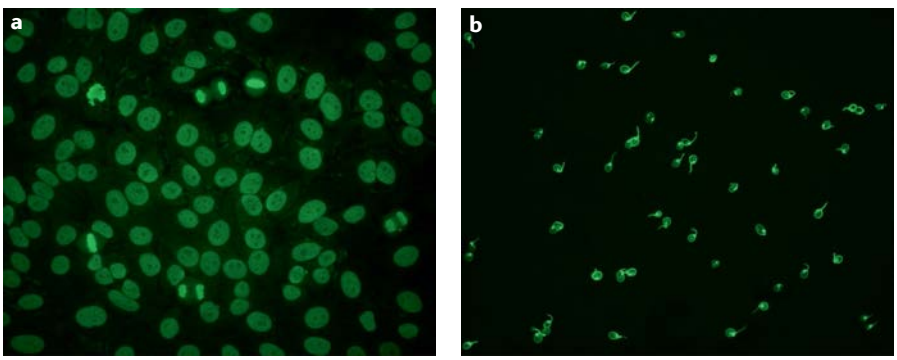


Fig. 1 | **Antinuclear and anti-dsDNA antibody immunofluorescence patterns in autoimmune hepatitis. a** | A typical autoimmune hepatitis antinuclear antibody staining pattern by indirect immunofluorescence in HEp-2 cells. A homogeneous fluorescence staining of the cell nuclei using a 1:40 dilution of serum from a patient with type 1 autoimmune hepatitis (serum titre 1:1,280). The condensed chromosomes of mitotic cells are positive, and the area surrounding the chromosomes is dark. **b** | Anti-double stranded DNA (dsDNA) antibody staining with serum from a patient with type 1 autoimmune hepatitis (titre 1:1,280). The pattern of positivity on *Crithidia luciliae* shows a strong kinetoplast staining (magnification 40x).

For the detection of anti-dsDNA antibodies, solid-phase immunoassays that use a mixture of multiple nuclear antigenic sources have lower specificity than immunofluorescence tests with *Crithidia luciliae*[10], which contain high amounts of dsDNA in the kinetoplast and enable easy and simple interpretation of positive staining (FIG. 1b).

We agree with Pisetsky and Lipsky that whoever orders an ANA test must be aware of which test will be used and, ideally, of its specificity and sensitivity; however, we would like to emphasize that in view of the potential clinical and immunological similarities between SLE and autoimmune hepatitis, a diagnosis of autoimmune hepatitis should always be considered in patients with ANA and anti-dsDNA antibody positivity.

There is a reply to this letter by Pisetsky, D. S. & Lipsky, P. E. *Nat. Rev. Rheumatol.* https://doi.org/10.1038/s41584-021-00574-6 (2020).

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The authors declare no competing interests.

# Reply to: Diagnostic role of anti-dsDNA antibodies: do not forget autoimmune hepatitis

*David S. Pisetsky and Peter E. Lipsky*

As Granito and colleagues correctly highlight in their correspondence (Granito, A., Muratori, L., Tovoli, F. & Muratori, P. Diagnostic role of anti-dsDNA antibodies: do not forget autoimmune hepatitis. *Nat. Rev. Rheumatol.* https://doi.org/10.1038/s41584-021-00573-7 (2020))[1] on our Review (Pisetsky, D. S. & Lipsky, P. E. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nat. Rev. Rheumatol.* **16**, 565–579 (2020))[2], anti-DNA antibodies, among other antinuclear antibodies (ANAs), occur prominently in autoimmune hepatitis. ANAs and other serological markers enable the division of autoimmune hepatitis into two types that differ in clinical course[3–7]. ANAs and anti-SMA antibodies are markers for type 1 autoimmune hepatitis, whereas anti-LKM1 antibodies are markers for type 2 autoimmune hepatitis[8].

Patients with type 1 autoimmune hepatitis produce ANAs that recognize many nuclear antigens (including histones, centromeres and ribonucleoproteins), but the expression of anti-DNA antibodies is perhaps the most surprising. Anti-DNA antibodies are a serological criterion for classification for systemic lupus erythematosus (SLE) as well as a marker of disease activity, particularly renal disease[9]. In SLE, anti-DNA antibodies can form immune complexes that are deposited in the kidneys and induce nephritis; these complexes can also stimulate the production of cytokines, including type I interferons, that promote widespread immune abnormalities[2]. In autoimmune hepatitis, anti-DNA antibodies do not seem to have the same consequences[8].

The expression of anti-DNA antibodies in both SLE and autoimmune hepatitis raises important questions about the putative role of anti-DNA antibodies in disease manifestations. Although characterizing the fine specificity of anti-DNA antibodies is complicated because of the size and structural complexity of DNA[9], the anti-DNA antibodies in autoimmune hepatitis can be detected using the same assays as for those in SLE[3–5]. The behaviour of anti-DNA antibodies from patients with autoimmune hepatitis in these assays suggests that these antibodies are bona fide anti-DNA antibodies and have binding properties similar to those in SLE.

The expression of anti-DNA antibodies in autoimmune hepatitis and SLE provides an opportunity to consider the role of these antibodies in pathogenesis and the reasons for differing pathologies in these conditions. Unlike in SLE, the role of anti-DNA antibodies, or other ANAs, in autoimmune hepatitis is unclear. Nevertheless, it is possible that these antibodies bind to hepatocytes in some way and cause cell death or injury: a mechanism that does not seem to pertain to SLE.

The absence of certain clinical and laboratory features in autoimmune hepatitis is also notable. Activation of complement does not seem to be common in autoimmune hepatitis[10], although disturbances in complement often occur in SLE concomitant with increased anti-DNA antibody levels. Glomerulonephritis is also not a manifestation of autoimmune hepatitis, raising the question of why anti-DNA antibodies can lead to nephritis in SLE but not in autoimmune hepatitis; perhaps a relevant source of DNA to form immune complexes is lacking in autoimmune hepatitis. On the basis of these interesting considerations, we appreciate the comments of Granito and colleagues[1] because they suggest that comparative studies of anti-DNA antibodies in SLE and autoimmune hepatitis might provide novel insights into the origin of these antibodies and their role in pathogenesis.

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The authors declare no competing interests.

# Author Correction: Raynaud phenomenon and digital ulcers in systemic sclerosis

*Michael Hughes, Yannick Allanore, Lorinda Chung, John D. Pauling, Christopher P. Denton and Marco Matucci-Cerinic*

Correction to: *Nature Reviews Rheumatology* (2020) https://doi.org/10.1038/s41584-020-0386-4, published online 25 February 2020.

In the originally published version of this article there was an error in the text. The sentence "For example, if the ulcer is 'wet' then appropriate dressings that contain hydrogels and hydrocolloids should be selected with the aims of reducing moisture and drying the wound" has been corrected to "For example, if the ulcer is 'dry' then appropriate dressings that contain hydrogels and hydrocolloids should be selected with the aims of promoting moisture and avoiding drying the wound." This error has now been corrected in the HTML and PDF versions of the manuscript.

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# Author Correction: Location, location, location: how the tissue microenvironment affects inflammation in RA

*Christopher D. Buckley, Caroline Ospelt, Steffen Gay and Kim S. Midwood*

Correction to: *Nature Reviews Rheumatology* (2021) https://doi.org/10.1038/s41584-020-00570-2, published online 01 February 2021.

In the originally published version of this article there was an error in Table 1. The phenotypes for immunomodulatory sub-lining fibroblasts and perivascular sub-lining fibroblasts were mistakenly swapped. In the row entitled "Sub-lining layer (immunomodulatory)", the cell for "Marker genes (human)" has been corrected from "Negative (*CD34*); positive (*CD90* and *HLA-DRA*)" to "Positive (*CD90* and *CD34*)" and the cell for "Marker genes (mouse)" has been corrected from "Negative (*Cd34*); positive (*Cd90*)" to "Positive (*Cd90* and *Cd34*)". In the row entitled "Sub-lining layer (perivascular)", the cell for "Marker genes (human)" has been corrected from "Positive (*CD90* and *CD34*)" to "Negative (*CD34*); positive (*CD90* and *HLA-DRA*)" and the cell for "Marker genes (mouse)" has been corrected from "Positive (*Cd90* and *Cd34*)" to "Negative (*Cd34*); positive (*Cd90*)". This error has now been corrected in the HTML and PDF versions of the manuscript.

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