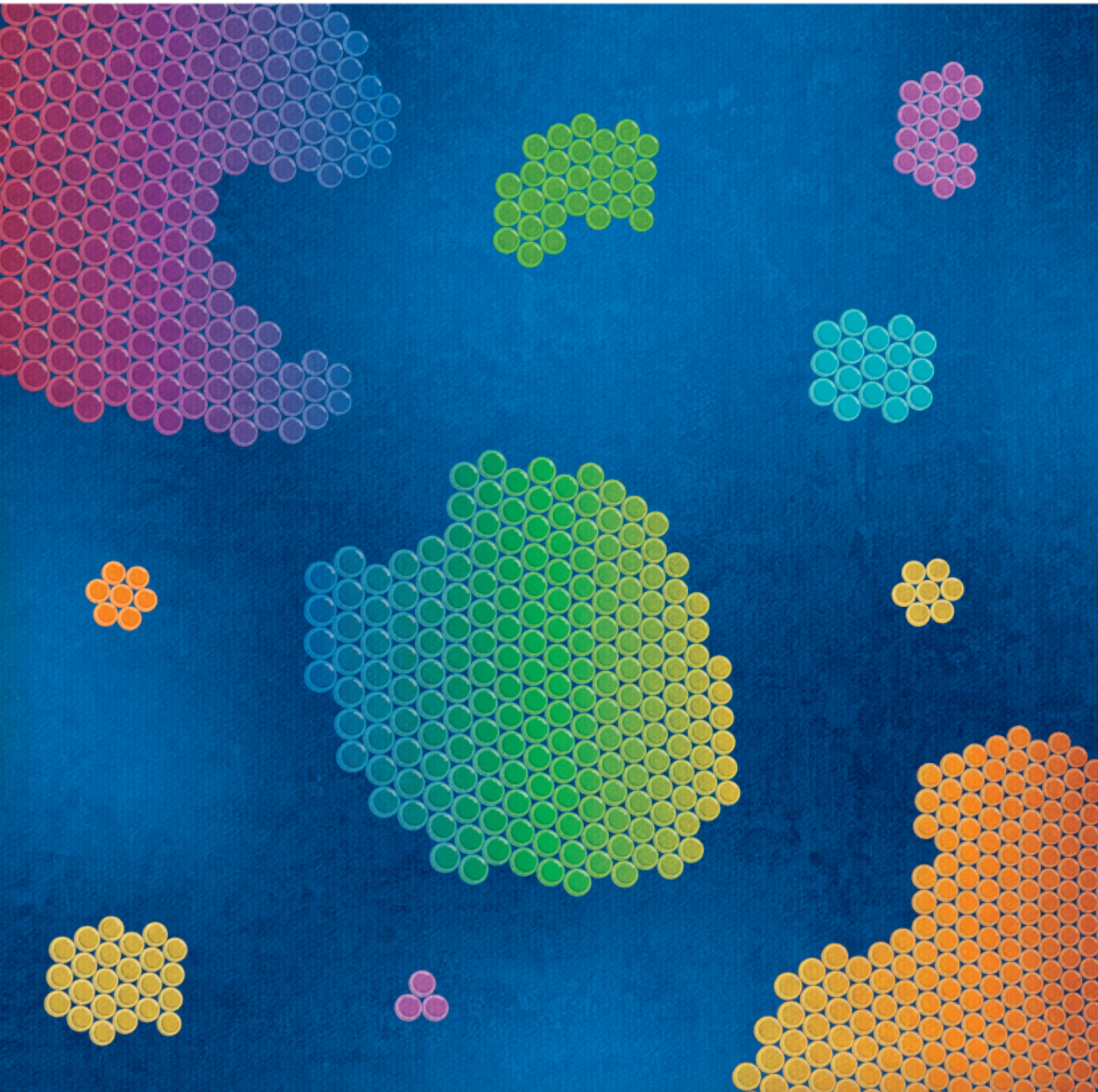
nature reviews
rheumatology

June 2025



https://doi.org/10.1038/s41584-025-01254-5

Understanding the causes of treatment failure is crucial for the management of axial spondyloarthritis

Denis Poddubnyy & Xenofon Baraliakos

Although numerous therapies are available for axial spondyloarthritis, more than half of patients do not achieve remission or respond to treatment. Understanding the reasons for non-response in axial spondyloarthritis is essential for effective management of this disease.

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that primarily affects the axial skeleton. Despite the availability of effective treatments, more than 50% of patients in clinical studies do not achieve a sufficient treatment response[1]. Addressing the causes of non-response is crucial for the optimization of management strategies. Biological non-response owing to uncontrolled inflammation despite adequate therapy is one challenge, but another major factor that contributes to treatment failure is incorrect diagnosis.

Despite correct diagnosis of axSpA and adherence to state-of-the-art anti-inflammatory treatment, satisfactory control of symptoms is not always achieved, and there are two major reasons for this lack of response. The first explanation, which probably applies to most patients with incomplete or non-response, is the presence of a condition that mimics the symptoms of axSpA (such as degenerative, mechanical changes in the spine or sacroiliac joints) or the presence of factors that can interfere with the assessment of axSpA symptoms (such as central or peripheral nervous system sensitization, anxiety, depression and sleep disturbances). The latter factors might contribute to the development and dominance of nociplastic pain mechanisms[2] (the extreme phenotype that is frequently diagnosed as fibromyalgia). The second explanation, which seems to apply to a smaller proportion of patients but is still clinically important, is the persistence of inflammation despite the appropriate use of advanced therapy; this outcome represents a major therapeutic challenge and highlights the need for further investigation into the immunological mechanisms of true non-response.

However, what if the diagnosis is not correct? The extent of the problem of misdiagnosis is unclear as current data are scarce, although this issue has been recognized in the latest update of the Assessment of SpondyloArthritis international Society (ASAS)–EULAR management recommendations for axSpA. According to these guidelines, ensuring the correct diagnosis in cases of non-response to biologic or targeted synthetic DMARDs is an essential step in the management approach before switching therapy[1]. This recommendation reflects the importance of ruling out alternative explanations for symptoms to avoid unnecessary changes in treatment strategy and improve patient outcomes.

The Improve-axSpA project, a telemedicine initiative conducted in Germany and Austria, has provided interim results suggesting that up to 35% of patients initially diagnosed with axSpA might have alternative explanations for their symptoms[3]. A key issue that contributes to misdiagnosis is the interpretation of MRI findings, particularly the attribution of unspecific bone marrow oedema to axSpA. Although bone marrow oedema is a hallmark feature of axSpA, studies demonstrate that a substantial proportion of the general population can present with this finding without inflammatory disease. Another population-based study[4] demonstrated a relatively high prevalence (17%) of active inflammatory lesions on MRI of sacroiliac joints in individuals under 45 years of age. In this study, a history of giving birth and high BMI were associated with the presence of bone marrow oedema, back pain and HLA-B27-positivity[4]. In the spine, bone marrow oedema was even more common than in sacroiliac joints (28%) and associated with older age and physically demanding work. In an analysis of the German National Cohort study, approximately one-third of healthy individuals exhibited active inflammatory (bone marrow oedema) or structural changes (mostly sclerosis) on MRI of sacroiliac joints[5].

Osteitis condensans ilii (OCI), a mechanical condition frequently associated with the presence of bone marrow oedema in the sacroiliac joints[6], is increasingly recognized as an important differential diagnosis for axSpA. According to current understanding, mechanical stress associated with pregnancy and giving birth (which can persist for many years), anatomic variations of sacroiliac joints or physically demanding jobs and other recurrent physical impacts can induce bone marrow oedema (predominantly in the anterior part of the joint, with both iliac and sacral sides possibly involved), which, over time, might evolve into fat lesions and sclerosis. Importantly, erosions, a hallmark of axSpA, are virtually non-existent in OCI.

In axSpA, inflammation primarily involves the middle of the cartilaginous compartment of the sacroiliac joint and leads to the relatively rapid (within months) development of axSpA-specific structural lesions — erosions and backfill (depicted as fat signal from repair tissue in the erosion cavity). Figure 1 represents examples of inflammatory (axSpA) and mechanical (OCI) patterns of changes in the sacroiliac joint. The differentiation of axSpA from mechanical or degenerative causes of back pain requires the use of an appropriate imaging protocol. The ASAS–Spondyloarthritis Research and Treatment Network (SPARTAN) imaging recommendations[7] advocate for a standardized four-sequence MRI protocol (detailed in Supplementary Box 1).

Clear communication between referring physicians and radiologists is an important factor contributing to the correct diagnosis. The 2024 ASAS recommendations for imaging requests emphasize the need

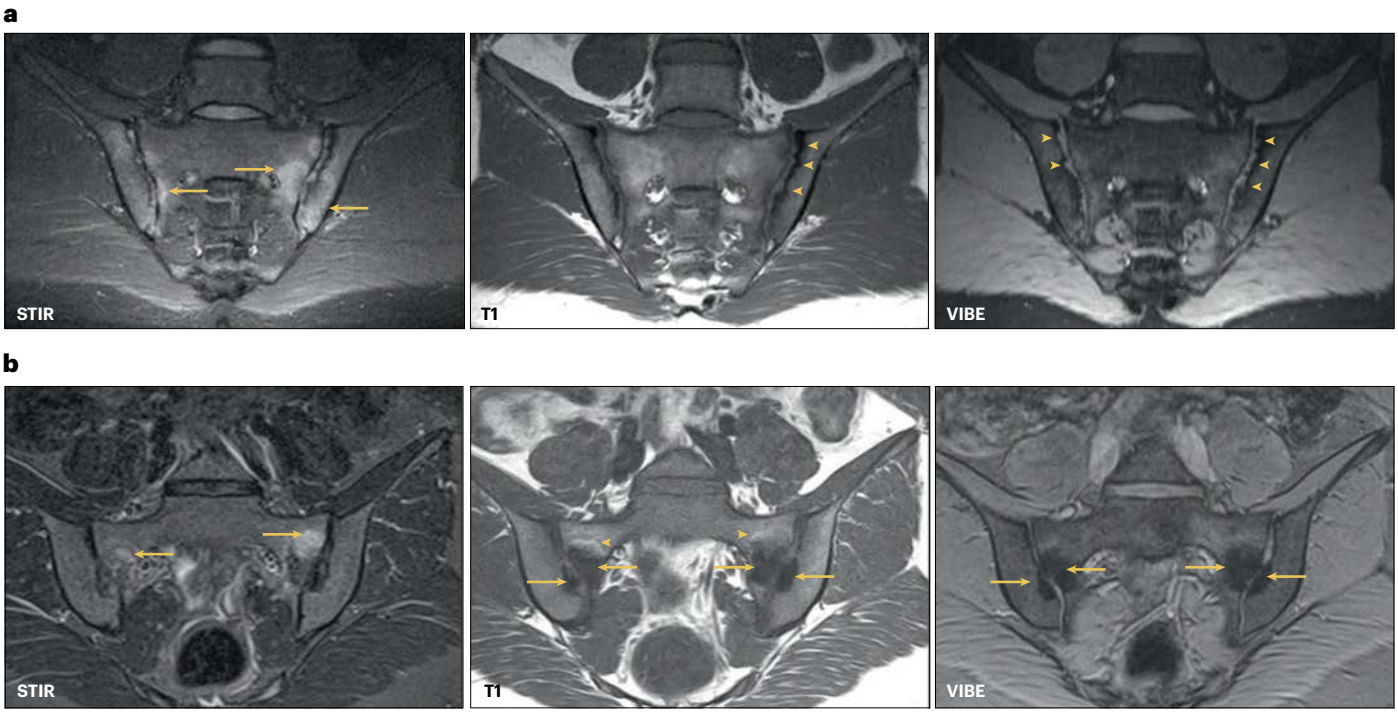


Fig. 1 | Typical patterns of MRI changes in the sacroiliac joints in axial spondyloarthritis and osteitis condensans ilii. a, Pattern of axial spondyloarthritis: subchondral bone marrow oedema (arrow on STIR (short tau inversion recovery)) in the middle part of the cartilaginous compartment of the left sacroiliac joint, accompanied by erosions (arrowheads on T1 and VIBE (volumetric interpolated breath-hold examination)). VIBE enables the depiction of smaller erosions with greater clarity. **b**, Pattern of mechanically induced changes in osteitis condensans ilii: bone marrow oedema in the ventral part of both sacroiliac joints (arrows on STIR), accompanied by massive sclerosis (arrows on T1 and VIBE) and some fat metaplasia (arrowheads on T1). There is no erosive damage.

to include detailed clinical information to guide radiologists in differentiating inflammatory from non-inflammatory changes[8]. Additionally, standardized reporting guidelines for sacroiliac joint imaging provide a structured approach to describe changes associated with axSpA that also allow alternative diagnoses to be considered[9].

Education has a crucial role in improving diagnostic accuracy and reducing misdiagnosis in axSpA. Rheumatologists need appropriate training to recognize the potential imaging pitfalls when diagnosing axSpA. Radiologists require specialized knowledge in interpreting MRI (as well as other imaging) findings in the sacroiliac joints and spine and guidance on how to differentiate inflammatory from mechanical or degenerative changes. Educational efforts should include in-person training sessions, workshops and the integration of these topics into specialist training curricula. The ASAS Case Library serves as an important resource for both specialties, providing reference cases that illustrate common diagnostic challenges and correct interpretations.

Obtaining an expert opinion seems to have value in reducing misdiagnosis and refining diagnostic pathways. The use of artificial intelligence (AI) in imaging interpretation is an emerging area that also holds promise for improving diagnostic accuracy. Several studies have successfully demonstrated AI-based detection of active inflammatory and structural changes on MRI of the sacroiliac joints[10]. AI-assisted tools not only help detect subtle patterns of inflammation and structural damage but also have the potential to contribute to the correct interpretation of axSpA-specific versus non-specific changes.

A possible future development that could improve diagnostic precision is the use of molecular imaging, such as positron emission tomography, to target cytokines and other molecules involved in the pathophysiology of inflammation in axSpA. Molecular imaging techniques might enable a more specific differentiation between inflammatory bone marrow oedema associated with axSpA and non-specific bone marrow oedema as a result of mechanical stress or degenerative changes.

In summary, non-response to treatment in axSpA is a multifactorial issue. Although true biological non-response remains a challenge that requires new therapeutic strategies, a substantial proportion of cases can be explained by incorrect diagnosis or incorrect interpretation of the symptom source. Ensuring diagnostic accuracy through standardized imaging protocols, improved communication between clinicians and radiologists, continuous education and appropriate training, as well as access to expert opinions, can help prevent misdiagnosis and optimize treatment decisions.

Denis Poddubnyy [1,2,3] ✉ **& Xenofon Baraliakos** [4]

[1]Division of Rheumatology, Temerty Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada. [2]Division of Rheumatology, Schroeder Arthritis Institute, University Health Network, Toronto, Ontario, Canada. [3]Department of Gastroenterology, Infectiology and Rheumatology (including Nutrition Medicine), Charité – Universitätsmedizin Berlin, Berlin, Germany. [4]Rheumazentrum Ruhrgebiet, Herne and Ruhr-University, Bochum, Germany.
✉e-mail: denis.poddubnyy@uhn.ca

Published online: 23 April 2025

References

1. Ramiro, S. et al. ASAS-EULAR recommendations for the management of axial spondyloarthritis: 2022 update. *Ann. Rheum. Dis.* **82**, 19–34 (2023).
2. Al Mohamad, F. et al. Association of nociplastic and neuropathic pain components with the presence of residual symptoms in patients with axial spondyloarthritis receiving biological disease-modifying antirheumatic drugs. *RMD Open* **10**, e004009 (2024).
3. Poddubnyy, D. et al. Enhancing the diagnostic accuracy and avoiding overdiagnosis in axial spondyloarthritis through central evaluation of imaging: IMPROVE-axSpA, a nationwide telemedicine project. *Ann. Rheum. Dis.* **83**, 476–477 (2024).
4. Baraliakos, X. et al. Which factors are associated with bone marrow oedema suspicious of axial spondyloarthritis as detected by MRI in the sacroiliac joints and the spine in the general population? *Ann. Rheum. Dis.* **80**, 469–474 (2021).
5. Torgutalp, M. et al. Frequency and factors associated with the presence of active inflammatory and structural changes in the MRI of sacroiliac joints: results from a population-based study. *Ann. Rheum. Dis.* **83**, 116–117 (2024).

6. Poddubnyy, D. et al. Clinical and imaging characteristics of osteitis condensans ilii as compared with axial spondyloarthritis. *Rheumatology* **59**, 3798–3806 (2020).
7. Lambert, R. G. W. et al. Development of international consensus on a standardised image acquisition protocol for diagnostic evaluation of the sacroiliac joints by MRI: an ASAS–SPARTAN collaboration. *Ann. Rheum. Dis.* **83**, 1628–1635 (2024).
8. Diekhoff, T. et al. Clinical information on imaging referrals for suspected or known axial spondyloarthritis: recommendations from the Assessment of Spondyloarthritis International Society (ASAS). *Ann. Rheum. Dis.* **83**, 1636–1643 (2024).
9. Diekhoff, T. et al. Reporting sacroiliac joint imaging performed for known or suspected axial spondyloarthritis: Assessment of SpondyloArthritis International Society recommendations. *Radiology* **311**, e231786 (2024).
10. Adams, L. C., Bressem, K. K., Ziegeler, K., Vahldiek, J. L. & Poddubnyy, D. Artificial intelligence to analyze magnetic resonance imaging in rheumatology. *Joint Bone Spine* **91**, 105651 (2023).

Competing interests

D.P. has received research support from AbbVie, Eli Lilly, Janssen, Novartis, Pfizer and UCB; consulting fees from AbbVie, Eli Lilly, Greywolf Therapeutics, Janssen, Merk, Moonlake, Novartis, Pfizer and UCB; and speaker fees from AbbVie, Canon, Eli Lilly, Janssen, Medscape, Novartis, Peervoice, Pfizer and UCB. D.P. is a member of the executive committee of ASAS. X.B. has received research support from Abbvie, Celltrion, Janssen, Moonlake and Novartis; and consulting fees from Abbvie, Advanz, Alexion, Alphasigma, Amgen, BMS, Cesas, Celltrion, Clarivate, Galapagos, J&J, Lilly, Moonlake, Novartis, Peervoice, Pfizer, Roche, Sandoz, Springer, Stada, Takeda, UCB and Zuellig. X.B. is past president of ASAS and president-elect of EULAR.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41584-025-01254-5.

Related links

The ASAS Case Library: https://cases.asas-group.org/

Crystal arthritis

https://doi.org/10.1038/s41584-025-01256-3

Current challenges in understanding the epidemiology of calcium pyrophosphate crystal deposition

Charlotte Jauffret & Tristan Pascart

Calcium pyrophosphate deposition (CPPD) disease is secondary to the pathological accumulation of calcium pyrophosphate (CPP) crystals inside joints and involves acute or chronic inflammatory arthritis. Epidemiological research on CPPD has been slow despite the suspected high prevalence of this condition. Here we highlight key challenges in CPPD imaging, diagnosis and nomenclature that need to be addressed for epidemiological research to progress at a faster pace.

The first challenge in understanding CPPD epidemiology relates to the suboptimal quality of imaging evidence used to estimate CPPD prevalence, whether symptomatic or not, and the associated underdiagnosis of CPPD. Second, it has been challenging to correctly identify and classify individuals with CPPD disease. Third, the non-consensual nomenclature used for CPPD disease phenotypes has hampered the systematic study of well-defined clinical cohorts. Although the nomenclature for CPPD clinical forms is still under discussion, in this Comment, we use the term 'CPPD' to refer to calcium pyrophosphate deposition, regardless of asymptomatic or symptomatic forms, and 'CPPD disease' to refer to symptomatic forms of CPPD.

CPPD underdiagnosis

Routinely used imaging methods, such as radiography, ultrasonography and computed tomography (CT), have the potential to detect crystal deposition[1], enabling estimations of CPPD prevalence. In conventional radiography, CPPD is often reported as 'chondrocalcinosis'[2], but this imaging modality has poor sensitivity — of approximately 50% in CPPD disease[3]. Thus, prevalence studies that rely on conventional radiography largely underestimate the numbers of individuals with CPPD. The use of ultrasonography is increasingly validated for the detection of CPPD. Ultrasonography has a sensitivity of above 90% for CPPD and provides more accurate assessments of CPPD prevalence than conventional radiography[3]. However, the lack of sonographers that have been specifically trained to detect CPPD is a limiting factor[4]. Similar to ultrasonography, CT seems to have an increased sensitivity for CPPD detection and is broadly applied for the assessment of axial CPPD involvement. However, a CT scan is not routinely used to assess peripheral CPPD involvement owing to limited availability, cost considerations and radiation exposure. Dual-energy computed tomography (DECT), which is now used in routine practice for gout, can be applied for CPPD detection[5]. DECT is not superior to CT for the detection of calcium-containing crystals, and hardly distinguishes between calcium-containing crystal types, such as CPP and basic calcium phosphate (BCP) crystals[5].

In addition to all the above challenges for identifying individuals with imaging evidence of CPPD, the estimation of CPPD prevalence is hampered by the fact that CPPD detection is often incidental — CPPD is often detected when imaging is performed for other purposes. CPPD of the symphysis pubis, which appears on abdominal CT scans performed for abdominal pain, and CPPD of the C1C2 transverse ligament, which appear on cranial CT scans performed for example for head trauma, are the most frequent incidental diagnoses of asymptomatic CPPD. By contrast, CPPD of peripheral joints, including knees and wrists — which are the most frequently involved locations — is usually detected on imaging of symptomatic individuals. In these instances, additional clinical information is still required to determine whether the identified CPPD underlies the patient's symptoms.

To overcome all the above limitations that are pertinent to CPPD imaging, the detection capabilities of imaging tools need to improve, and imaging protocols must be further combined with clinical and other paraclinical modalities to differentiate between CPPD and CPPD disease.

Misidentification of individuals with CPPD disease

CPPD disease is often misdiagnosed and under-recognized[6]. The main challenge for CPPD disease epidemiology is to identify symptomatic individuals with ascertained diagnosis, either in prospective general population-based cohorts that are not disease-specific, such as the UK Biobank, or, retrospectively, in large databases, such as healthcare data. However, coding systems are particularly imprecise when it comes to CPPD disease, as they do not distinguish between the symptomatic CPPD disease and the asymptomatic CPPD. The most common code is 'chondrocalcinosis' (codes M111*, M112* of the 10th revision of the International Classification of Diseases (ICD-10), and refers to the imaging evidence of CPPD, whether symptomatic or not. In some cases, the code 'pseudogout' captures the full phenotypic spectrum of symptomatic CPPD disease and not only the acute arthritis phenotype. Other apparently non-specific codes such as 'other crystal arthropathies' (M118* of ICD-10) are often used, but these encompass also other crystal deposition diseases, such as BCP deposition. Epidemiological studies have so far included a variety of ICD-10 codes to identify patients in heterogeneous cohorts, leading to non-comparable results. However, chart reviews that assess the performances of these codes showed that they performed better than expected, and some research

groups have tried to develop algorithms to overcome this issue[7]. The first ACR–EULAR classification criteria created in 2023 are a huge step forward in uniformizing patient profiles for clinical research, including epidemiological studies, but cannot be applied retrospectively to available databases[6].

Inconsistent nomenclature for CPPD disease phenotypes

The 2011 EULAR task force recognized the CPPD disease phenotypes 'asymptomatic CPPD (isolated chondrocalcinosis (CC), or osteoarthritis (OA) with CC)', 'OA with CPPD', 'acute CPP crystal arthritis' and 'chronic CPP crystal inflammatory arthritis' under the umbrella term 'calcium pyrophosphate deposition (CPPD)'. Since then, further terms have emerged in the literature, with some authors classifying the term 'recurrent CPPD disease' under 'acute CPPD disease' and others under 'chronic CPPD disease', whereas the term 'persistent CPPD disease' has been referred to as a 'chronic CPPD disease' form[8,9]. Less commonly described CPPD disease phenotypes include crowned dens syndrome, microcrystalline spondylodiscitis and other axial involvements, extra-articular tophaceous or pseudo-tumoral CPP depositions, pseudoneuropathic arthropathy, and mixed crystal depositions. Those imprecisely defined phenotypes reflect the heterogeneity of CPPD disease, which might in many cases mimic other inflammatory rheumatic diseases. A consensus on the nomenclature of CPPD disease phenotypes is required, so that epidemiological research can focus on homogeneous groups of patients and provide more accurate data.

Implementing the 2023 ACR–EULAR classification criteria was a necessary step to include individuals with CPPD disease in research albeit irrespectively of their specific phenotypes[6,10]. Ongoing efforts to characterize CPPD disease phenotypes are expected to help towards this direction[8]. The cohort that was enrolled to develop and validate the 2023 ACR–EULAR classification criteria was used to examine the distribution of the most common CPPD disease phenotypes ('unique' acute CPP crystal arthritis, 'recurrent' acute CPP crystal arthritis, and 'persistent' CPP crystal inflammatory arthritis) and how these phenotypes are associated with imaging evidence of CPPD and osteoarthritis, patient characteristics, and clinical symptoms. So far, no patient cohort has been sufficiently phenotyped to study the transition from one phenotype to another — for example, to study which patients with inaugural acute CPP crystal arthritis have a risk of developing chronic manifestations and in what form. The frequency and causes of the transition from asymptomatic CPPD to CPPD disease are still unknown. Large CPPD-focused cohorts need to be built to answer these various questions. An update with more precise codes for CPPD disease would be more than welcome and would be able to extract more informative and reliable data from large databases.

Conclusion

A research priority is to improve our understanding of the epidemiology of CPPD disease, as it is expected to improve general awareness of the disease and help to tackle CPPD underdiagnosis and, in turn, poor disease management. Overcoming challenges in CPPD imaging, diagnosis and classification will help to optimize the identification of individuals with CPPD disease through large databases and support further genetic studies in large population cohorts where the genetic data are already available.

Charlotte Jauffret [1,2] ✉ **& Tristan Pascart** [2]

[1]ULR 2694 – METRICS, CERIM, Public Health Department, Lille University, Lille University Hospital, Lille, France. [2]Department of Rheumatology, Saint-Philibert Hospital, Lille Catholic University, ETHICS Laboratory, Lille, France.

✉e-mail: jauffret.charlotte@ghicl.net

Published online: 17 April 2025

References

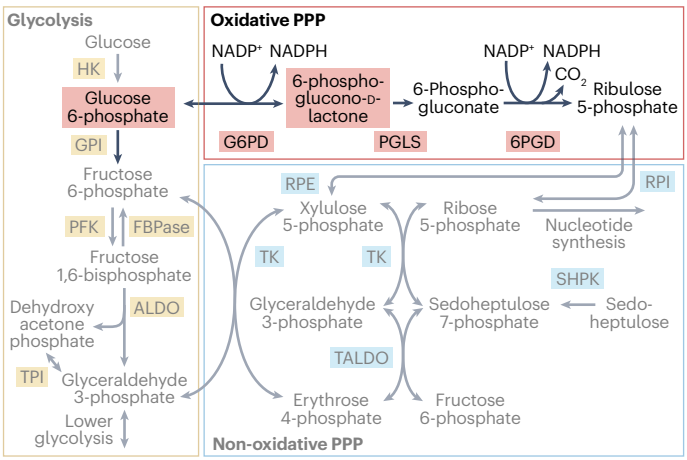
1. Pascart, T. et al. Calcium pyrophosphate deposition disease. *Lancet Rheumatol.* **6**, e791–e804 (2024).
2. Tedeschi, S. K. et al. Imaging features of calcium pyrophosphate deposition disease: consensus definitions from an international multidisciplinary working group. *Arthritis Care Res.* **75**, 825–834 (2023).
3. Cipolletta, E. et al. The diagnostic value of conventional radiography and musculoskeletal ultrasonography in calcium pyrophosphate deposition disease: a systematic literature review and meta-analysis. *Osteoarthritis Cartilage* **29**, 619–632 (2021).
4. Mandl, P. et al. 2023 EULAR recommendations on imaging in diagnosis and management of crystal-induced arthropathies in clinical practice. *Ann. Rheum. Dis.* **83**, 752–759 (2024).
5. Tedeschi, S. K. et al. A prospective study of dual-energy CT scanning, US and X-ray in acute calcium pyrophosphate crystal arthritis. *Rheumatology* **59**, 900–903 (2020).
6. Abhishek, A. et al. The 2023 ACR/EULAR classification criteria for calcium pyrophosphate deposition disease. *Ann. Rheum. Dis.* **75**, 1703–1713 (2023).
7. Bartels, C. M., Singh, J. A., Parperis, K., Huber, K. & Rosenthal, A. K. Validation of administrative codes for calcium pyrophosphate deposition: a Veterans Administration study. *J Clin. Rheumatol. Pract. Rep. Rheum. Musculoskelet. Dis.* **21**, 189–192 (2015).
8. Pascart, T. et al. Features associated with different inflammatory phenotypes of calcium pyrophosphate deposition (CPPD) disease: study using data from the international ACR/EULAR CPPD classification criteria cohort. *Arthritis Rheumatol.* **76**, 1780–1788 (2024).
9. Jauffret, C. et al. Systematic literature review on calcium pyrophosphate deposition (CPPD) nomenclature: condition elements and clinical states— A Gout, Hyperuricaemia and Crystal-Associated Disease Network (G-CAN) consensus project. *RMD Open.* **11**, e004847 (2025).
10. Tedeschi, S. K. A new era for calcium pyrophosphate deposition disease research: the first-ever calcium pyrophosphate deposition disease classification criteria and considerations for measuring outcomes in calcium pyrophosphate deposition disease. *Gout Urate Cryst. Depos. Dis.* **2**, 52–59 (2024).

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Systemic lupus erythematosus

Pentose phosphate pathway metabolite restores T cell balance in SLE



Autoimmune reactions, including inflammatory lesions in systemic lupus erythematosus (SLE), often involve a skewed balance between regulatory T cells (T_{reg} cells) and effector T helper 17 cells (T_H17 cells). A *Science Translational Medicine* study reports that the pentose phosphate pathway (PPP) metabolite gluconolactone (GDL) can potentially be used to increase T_{reg}–T_H17 cell ratio and treat inflammatory lesions in cutaneous lupus.

This translational finding was triggered by phosphoproteomic and metabolomic analyses of mouse T_{reg} cells that had or lacked expression of protein phosphatase 2A (PP2A), building upon the previous identification of the essential role of PP2A in T_{reg} cell differentiation and suppressive activity. The PPP enzyme G6PD produces 6-phospho-glucono-D-lactone as a by-product of glucose-6-phosphate oxidation into NADPH. G6PD levels and enzymatic activity, as well GDL and 6-phospho-glucono-D-lactone levels, were increased in PP2A-deficient T_{reg} cells, and G6PD was identified as a direct PP2A target. By contrast, the levels of PGLS, the downstream enzyme of the PPP that hydrolyses 6-phospho-glucono-D-lactone, were low in PP2A-deficient T_{reg} cells.

So, what is the effect of GDL on T_{reg} cells? Exogenous GDL improved T_{reg} cell differentiation and suppressive activity and suppressed the differentiation of T_H17 cells in vitro. This effect depended on the expression of PGLS, indicating that it is the yet unidentified downstream metabolites of GDL that promote T_{reg} cell differentiation and function.

To test the therapeutic potential of GDL — which is present in foods, as well as an approved compound for intramuscular, intravenous, oral and topical use — the authors applied a GDL-containing cream to skin lesions of imiquimod-treated or lupus-prone MRL/*lpr* mice. GDL improved skin histology and increased the T_{reg}–T_H17 cell ratio in the local lymph nodes of the treated mice. Oral administration of GDL to MRL/*lpr* mice also reduced the spleen and lymph node sizes and increased the T_{reg}–T_H17 cell ratio but failed to modulate autoantibody levels or kidney pathology.

Topical application of GDL-containing cream to skin lesions of three patients with cutaneous lupus improved clinical and histological components of these lesions compared with untreated lesions of the same patients. One of the three patients reported lesion clearance after 10 months of continuous GDL treatment.

"Although quite excited about this rapid conversion of basic findings to clinical practice, we still need to dissect each metabolic step that enables GDL to increase T_{reg} cell function," notes George Tsokos, corresponding author of the article. "At the clinical level we would love to pursue clinical trials [...] in patients with cutaneous lupus and other inflammatory skin diseases," Tsokos adds, suggesting that "GDL will be an adjuvant medication in treating systemic disease, but it may be sufficient for those with skin involvement."

Maria Papatriantafyllou

Original article: Li, W. et al. Gluconolactone restores immune regulation and alleviates skin inflammation in lupus-prone mice and in patients with cutaneous lupus. *Sci. Transl. Med.* **17**, eadp4447 (2025)

Inflammatory arthritis

Oestrogens implicated in progression to arthritis

Hormonal changes during menopause are thought to influence the development of rheumatoid arthritis (RA), but the nature of this link remains unclear. Findings from a prospective study of 433 women with clinically suspect arthralgia suggest that cumulative exposure to oestrogens increases the risk of progression to anti-citrullinated protein antibody (ACPA)-negative inflammatory arthritis (IA).

Compared with the risk for premenopausal women, postmenopausal women had an increased risk of ACPA-negative IA (HR 2.9; 95% CI 1.05–8.0) but not of ACPA-positive IA (HR 0.8; 95% CI 0.4–1.9). The risk for the development of RA followed similar trends.

Among postmenopausal women, several factors related to lifetime exposure to oestrogens were linked with arthritis development. A higher number of reproductive years was associated with a decreased risk of ACPA-negative IA, as was a higher number of ovulatory years (HR 0.88 for every 1-year increase; 95% CI 0.78–0.99). Conversely, onset of menopause before 45 years of age was associated with an increased risk of ACPA-negative IA (HR 6.03; 95% CI 1.34–27.10).

Thus, both the drop in oestrogens that occurs during menopause and the cumulative duration of exposure to oestrogens over a lifetime might have a role in the pathophysiology of ACPA-negative arthritis.

Sarah Onuora

Original article: Heutz, J. W. et al. Shorter reproductive time span and early menopause increase the risk of ACPA-negative inflammatory arthritis in postmenopausal women with clinically suspect arthralgia. *Rheumatology* https://doi.org/10.1093/rheumatology/keaf083 (2025)

Autoimmunity

Z-DNA as an inflammatory trigger in lupus

A study in *Science Immunology* delineates a molecular link between photosensitivity and exaggerated type I interferon (IFN) responses in skin diseases such as systemic lupus erythematosus (SLE), cutaneous lupus erythematosus (CLE) and dermatomyositis.

Type I IFN responses are upregulated in the skin, both lesional and non-lesional, of individuals with lupus or dermatomyositis, and are further enhanced by exposure to ultraviolet (UV) light, resulting in photosensitivity reactions. Thus, Benjamin Klein and Michelle Kahlenberg, co-corresponding authors of the study, initiated a project "to understand which factors are required for UV-mediated IFN activation and [...] how this is perturbed in an 'already' IFN-rich environment", as Klein notes.

This work associated UV-induced mitochondrial damage with the cytoplasmic accumulation of Z-DNA — a zigzag DNA conformation resulting, in this case, from the oxidation of mitochondrial DNA. The accumulation of Z-DNA in keratinocytes enhanced type I IFN responses.

Z-DNA binding protein 1 (ZBP1) is encoded by an IFN-inducible gene, and ZBP1 levels were increased in non-lesional skin tissues of individuals with SLE, CLE or dermatomyositis when compared with skin from healthy individuals. UV-induced Z-DNA was stabilized by ZBP1 in the skin of these individuals and further amplified type I IFN responses through the cytoplasmic DNA-sensing cGAS–STING signalling pathway.

As their findings underline how UV light might initiate chronic skin inflammation in autoimmune photosensitivity, the authors highlight Z-DNA and ZPB1 as potential therapeutic targets. "Understanding mitochondrial dysregulation in lupus skin will be important for getting at the root of the question of why type I IFNs are elevated in the first place", Kahlenberg concludes, adding that "it will also be interesting to determine whether other DNA sources, such as neutrophil extracellular traps, [contain] similar Z-DNA conformations".

Maria Papatriantafyllou

Original article: Klein, B. et al. Epidermal ZBP1 stabilizes mitochondrial Z-DNA to drive UV-induced IFN signaling in autoimmune photosensitivity. *Sci. Immunol.* **10**, eado1710 (2025)

Systemic sclerosis

Fumarate drives interferon release in systemic sclerosis monocytes

Fumarate hydratase (FH), a metabolite of the tricarboxylic acid (TCA) cycle that catalyses the conversion of fumarate to malate, is known to regulate innate immune responses. Research now implicates FH in the release of interferon in systemic sclerosis (SSc), through a mechanism involving the STING signalling pathway.

Previous studies have shown that FH suppression occurs in systemic lupus erythematosus (SLE); FH inhibition in SLE macrophages leads to fumarate accumulation, which causes mitochondrial stress and subsequent release of nucleic acids from SLE macrophage mitochondria, resulting in interferon production. In the present study, the researchers showed that levels of FH were significantly lower in $CD14^+$ monocytes isolated from 12 patients with early diffuse SSc in comparison with cells isolated from healthy individuals. The SSc monocytes also had increased expression of the interferon target gene *ISG15* and the mitochondrial stress markers *GDF15* and *ATF4*, and levels of mitochondrial DNA (mtDNA) were increased in the cytosolic fraction of SSc monocytes.

Depletion of mitochondria in SSc monocytes by incubation with the mitophagy-inducer urolithin A blunted lipopolysaccharide (LPS)-induced release of IFNβ. Treatment of cells with the voltage-dependent anion channel (VDAC) oligomerization inhibitor VBIT-4 also reduced IFNβ release, suggesting that the IFNβ response is at least partially mediated by the export of mtDNA from VDAC1 pores.

In cells from healthy donors, stimulation with the STING agonist cGAMP led to an increase in the release of IFNβ but this effect could be mitigated by pre-treatment with the STING inhibitor H-151 or with 4-octyl itaconate (4-OI), a cell-permeable derivative of the TCA cycle metabolite itaconate that has anti-inflammatory effects. Further experiments in SSc monocytes demonstrated that the inhibitory effects of 4-OI on IFNβ induction are downstream of mitochondrial nucleic acid release and probably mediated by its induction of the transcription factor NRF2, a negative regulator of STING signalling.

In skin samples from individuals with SSc, levels of fumarate, but not the TCA cycle components succinate or α-ketoglutarate, were increased in comparison with skin from healthy donors. In addition, levels of cGAMP and expression of *ISG15* and the STING target gene *CXCL10* were increased in SSc skin samples.

Together, the findings suggest a pathway by which altered fumarate metabolism in SSc monocytes leads to increased mitochondrial stress and the release of mtDNA into the cytosol that then triggers cGAS–STING signalling, resulting in interferon release. Targeting these altered metabolic pathways could offer a way to treat inflammatory diseases such as SSc.

Sarah Onuora

Original article: Steadman, T. & O'Reilly, S. Aberrant fumarate metabolism links interferon release in diffuse systemic sclerosis. *J. Dermatol. Sci.* **117**, 30–35 (2025)

Experimental arthritis

Lipid nanoparticles with PDL1-encoding mRNA spread tolerance

Tolerogenic antigen-presenting cells (APCs) downregulate the responses of effector T cells or promote the differentiation of regulatory T (T_{reg}) cells, and APC-based immunotherapies have been explored to target autoimmune diseases. Some tolerogenic APCs express the co-inhibitory cell surface molecule PDL1, which engages with PD1 on T cells to inhibit effector T cell activity. A study in *Nature Biomedical Engineering* now reports how a lipid nanoparticle (LNP)–mRNA formulation resembling that of the COVID-19 mRNA vaccines was harnessed to induce tolerogenic APCs in vivo and prevent disease progression in a mouse model of rheumatoid arthritis.

"The key innovation was optimizing these LNPs to be stealthy, minimizing immune activation while efficiently delivering their mRNA payload to APCs", notes Yucai Wang, co-corresponding author of the study. This was achieved through the in vivo screening of LNPs with varied chemical composition of the LNP components, with the aim to identify the least immunogenic formulation. Preferential targeting of APCs in vivo was attempted via subcutaneous administration of these LNPs, whereas tolerogenic potential was achieved via the integration of an mRNA that encoded PDL1 (*Cd274*).

The developed LNP–mRNA constructs were able to induce tolerogenic APCs that controlled activated T cells both in in vitro assays and in mice. Moreover, these LNP–mRNA constructs — when administered subcutaneously — inhibited disease progression in a mouse model of collagen-induced arthritis, with an efficacy that was comparable to that of the TNF inhibitor etanercept. T cell analyses of the spleen and lymph nodes of these mice indicated that *PDL1* mRNA-carrying LNPs reduced the numbers of pro-inflammatory effector T cells. T cell infiltration of the joints was also reduced.

"While our current method for generating tolerogenic APCs in the body works broadly against autoimmune inflammation (especially useful for diseases where the specific self-targets are unknown), the gold standard would be to develop therapies that specifically target only the problematic immune responses", Wang comments. "The good news is that our mRNA platform is like a modular toolkit — we can easily adapt it to include specific autoantigens relevant to different diseases, (...) [or] potentially substitute PDL1 mRNA with other [immunosuppressive] genes like IL-10. The proven success of LNP delivery in vaccines supports its clinical potential for autoimmune therapies", Wang concludes.

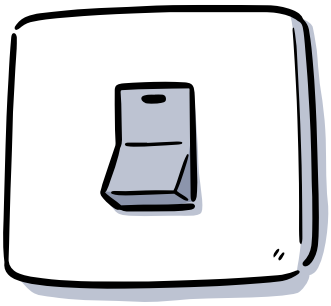
Maria Papatriantafyllou

Original article: Liu, Y. et al. Generation of tolerogenic antigen-presenting cells in vivo via the delivery of mRNA encoding PDL1 within lipid nanoparticles. *Nat. Biomed. Eng* https://doi.org/10.1038/s41551-025-01373-0 (2025)

Related article: Buckner, J. H. Antigen-specific immunotherapies for autoimmune disease. *Nat. Rev. Rheumatol.* **21**, 88–97 (2025)

Rheumatoid arthritis

Glycosylation switch in synovial fibroblasts promotes ECM degradation



Understanding the mechanisms that cause synovial fibroblast to degrade the extracellular matrix (ECM) in arthritic joints is key. In cancer, hyper O-glycosylation of cell-surface proteins leads to ECM degradation by cancer cells. This process is activated by the relocation of *N*-acetylgalactosaminyltransferases (GALNTs) from the Golgi to the endoplasmic reticulum (ER). This relocation is known as GALNT activation (GALA) and promotes high O-glycosylation and the synthesis of the Tn glycan. A study in *Nature Communications* investigates whether the GALA pathway is altered in synovial fibroblasts in arthritic joints.

Tran et al. report that joint tissues from individuals with rheumatoid arthritis (RA), psoriatic arthritis and osteoarthritis have higher levels of GALNT relocation and Tn glycan than those from healthy individuals.

Using the collagen antibody-induced arthritis (CAIA) mouse model of RA, the authors found that, similarly to humans, GALNT relocation and Tn glycan levels were increased in mice with CAIA compared with control animals. In both humans and mice, synovial-lining fibroblasts were identified as the cells that had high GALNT relocation and expression of Tn glycan. In synovial fibroblast from individuals with osteoarthritis and RA, exposure to ECM proteins activates the GALA pathway, whereas cells from healthy individuals had little or no response.

The authors generated a human synovial fibroblast cell line that expressed the GALNT inhibitor ER-2Lec, which inhibits GALNTs in the ER but not in the Golgi. When stimulated, the cells that expressed ER-2Lec had reduced ECM-degrading properties compared with control cells. Tran et al. also generated a mouse model in which synovial fibroblasts express ER-2Lec, and induced CAIA in these mice. ER-2Lec-expressing mice with CAIA had reduced disease activity compared with control mice with CAIA.

Further research revealed that GALNTs glycosylate calnexin (an ER-resident protein involved in ECM degradation) in the ER, and this results in translocation of calnexin to the cell surface where it contributes to ECM degradation. Blocking calnexin in vivo protected mice with CAIA from cartilage degradation and subsequent disease development.

Frederic Bard, the corresponding author of this article, concludes that "Our study sheds new light on the way synovial fibroblasts contribute to the ECM degradation process"; therapeutics that target calnexin are being developed for the treatment of arthritis.

Holly Webster

Original article: Tran, L. S. et al. ER O-glycosylation in synovial fibroblasts drives cartilage degradation. *Nat. Commun.* **16**, 2535 (2025)

Osteoarthritis

https://doi.org/10.1038/s41584-025-01257-2

Targeting osteoarthritis where it hurts: the osteochondral junction

Anne-Marie Malfait & Alia M. Obeidat

In joints with osteoarthritis, angiogenesis and neuronal growth occur at the osteochondral junction, a process that can contribute to structural joint damage and pain. Targeting this neurovascularization process represents a possible strategy for the development of disease-modifying drugs for osteoarthritis.

REFERS TO Qin, W. et al. Neurovascularization inhibiting dual responsive hydrogel for alleviating the progression of osteoarthritis. *Nat. Commun.* **16**, 1390 (2025).

In osteoarthritis (OA), neurovascular growth at the cartilage–bone interface might contribute to both disease progression and pain. Blood vessels grow into the normally avascular cartilage and can promote calcification and cartilage damage. In addition, pathological nerve growth into joint tissues that are exposed to high mechanical load can make these tissues very sensitive to stimuli, especially as the inflammatory milieu in the OA joint causes peripheral sensitization of nociceptors and subsequent joint pain[1]. Therefore, targeting neurovascularization at the osteochondral junction represents an attractive treatment strategy for OA. In a study in *Nature Communications*, Qin et al. report the development of a novel dual-responsive hydrogel that targets neurovascularization at the osteochondral junction of the temporomandibular joint (TMJ), with the aim of treating TMJ osteoarthritis (TMJ-OA)[2].

In diarthrodial joints, the osteochondral junction forms the cartilage–bone interface, where the deep layers of articular cartilage cover the subchondral bone to allow frictionless motion between bones. The osteochondral unit is formed by articular hyaline cartilage, beneath which a layer of calcified cartilage marks the transition from soft tissue to the underlying subchondral bone plate. The bone plate consists of dense cortical bone, which transitions into more porous and metabolically active trabecular bone[3] (Fig. 1). Subchondral bone is highly vascular, indicating the high nutritional needs of the bone tissue and the overlying avascular cartilage. In humans, arterial and venous vessels can send small branches into the calcified cartilage[4].

Functionally, the cartilage–bone interface at the osteochondral junction is a highly dynamic structure, uniquely adapted to transfer loads during joint motion and weight bearing[3], and permits the exchange of biochemical mediators between the two tissues. In OA joints, pathological changes at the osteochondral junction that start early in the disease have been described[5]. Gross pathological changes include thickening of the calcified layer, advancing endochondral ossification and duplication of the tidemark. Progressive changes in the subchondral bone ultimately lead to increased subchondral bone thickness and stiffness, which alters mechanical loading and renders the osteochondral unit vulnerable to microfractures. The formation of microcracks and channels between cartilage and subchondral bone compromises the integrity of the barrier between bone and cartilage, which promotes pathological processes. Thus, treatments that target the osteochondral unit could hold potential for disease modification in OA. Perhaps one of the most exciting observations is the intense neurovascularization that occurs at the osteochondral junction. Indeed, knees obtained from patients with OA at the time of total knee replacement show angiogenesis at the osteochondral junction, with increased vascular density extending through channels that breach the tidemark[6]. These osteochondral channels also contain peptidergic nerves and are associated with pain in knee OA[7]. Sprouting of nociceptors in subchondral bone channels occurs in all experimental models of OA, including primary age-associated OA, and is associated with cartilage degeneration scores[8] (Fig. 1). Furthermore, the angiogenic factor VEGF (which can also promote nerve growth), as well as the neurotrophic factor NGF (which can also promote vessel growth), are expressed at the osteochondral junction.

TMJ-OA is a debilitating chronic pain condition for which no treatments exist. To try to target neurovascularization in TMJ-OA, Qin et al.[2] used the unilateral anterior crossbite TMJ-OA model in female mice (which have a higher morbidity rate in this model). The authors have previously shown that augmented neurovascularization and overexpression of *Ngf*, *Vegf* and *Ntn1* (which encodes Netrin 1, an osteoclast-derived protein that can promote sensory nerve growth in the subchondral bone[9]) occur at the osteochondral junction in TMJ-OA mice. In this model, the authors proposed a role for extracellular RNA in regulating nerve and blood vessel ingrowth. Specifically, they report that RNA–VEGF complexes might promote the growth of trigeminal ganglion cells[10]. Now, the authors have developed a novel, self-healing, polycationic pH- and reactive oxygen species-responsive hydrogel loaded with bevacizumab (an anti-VEGF antibody). The polycationic nature of the hydrogel enables it to scavenge extracellular RNA, and the dual responsiveness allows controlled release of bevacizumab. The study provides in vitro evidence that the hydrogel effectively inhibits angiogenesis and neuronal growth. In TMJ-OA mice, a single injection of the hydrogel at day 0 had notable beneficial effects after 3 weeks, including fewer new blood vessels; healthier, proteoglycan-rich articular cartilage; fewer microcracks; increased bone mineral density; fewer calcitonin gene-related peptide-positive nerves at the osteochondral junction; and decreased expression of *Ngf*, *Vegf*, *Ntn1* and *Ntn3*. These beneficial effects on joint damage were accompanied by less maxillofacial pain, including decreased mechanical allodynia and decreased anxiety-driven behaviours.

This interesting study shows promise for targeting neurovascularization directly at the osteochondral junction and provides proof of concept that this approach can have a true disease-modifying effect in OA. The novel hydrogel has the potential to overcome the many barriers associated with intra-articular drug delivery that hamper

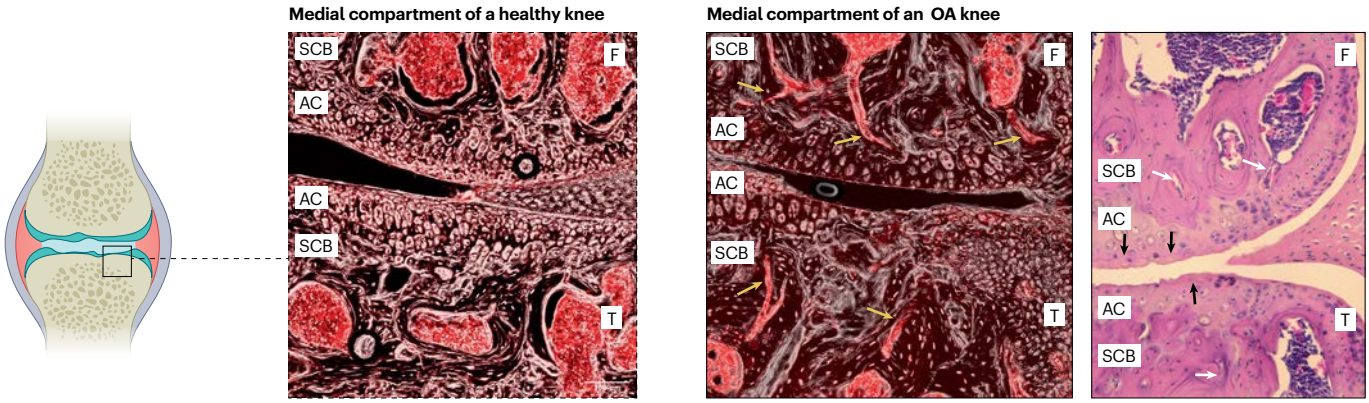


Fig. 1 | Neurovascularization at the osteochondral junction in OA. Images of the medial compartment of healthy age-matched knee (middle left) and a knee with osteoarthritis (OA), 12 weeks after partial meniscectomy (middle right and far right), showing neurovascularization at the osteochondral junction. Confocal images (middle left and middle right) and H&E image (far right) are from $Na_V1.8$-tdTomato mice, in which neurons that express the sodium channel $Na_V1.8$ express the tdTomato (red) fluorescent tag; these NaV1.8-positive neurons are nociceptors. White arrows show blood vessels within subchondral bone (SCB) channels. Black arrows indicate the tidemark. Yellow arrows indicate $Na_V1.8$-positive nociceptors within subchondral bone channels growing towards the tidemark. AC, articular cartilage; CC, calcified cartilage; F, femur; T, tibia. Scale bar, 100 μm.

biodistribution in the joint and interfere with pharmacokinetics; however, some key limitations warrant further exploration. Importantly, injecting the hydrogel at the time of model induction limits the observed effects to a prophylactic approach. An interventional approach to address the reversibility of neurovascularization would be very exciting. Also, although a supplemental figure suggests that the hydrogel overcame the 'joint barrier' and was detected both in cartilage and subchondral bone 3 weeks after intra-articular injection, careful assessment of the distribution of the gel over time is needed to understand the pharmacokinetics. Furthermore, in this TMJ-OA model, disease progresses for up to 6 weeks[10], whereas mice in this study were monitored for only 3 weeks. Including later time points and longitudinal monitoring of all relevant outcomes over a 6-week period would enable careful delineation of the observed effects and the relationships among blood vessels, neurons, structural changes and pain.

Overall, this stimulating study by Qin et al.[2] provides evidence that targeting neurovascular growth at the osteochondral junction can have beneficial effects on structural disease and pain, at least in a mouse model of TMJ-OA. The OA field is in dire need of innovative treatment approaches, and this hydrogel presents an exciting possible future approach; we look forward to seeing how this hydrogel performs in experimental models of knee OA and in human tissues.

Anne-Marie Malfait[1,2] ✉ **& Alia M. Obeidat**[1,2]
[1]Department of Internal Medicine, Division of Rheumatology, Rush University Medical Center, Chicago, IL, USA. [2]Chicago Center on Musculoskeletal Pain, Chicago, IL, USA.
✉e-mail: anne-marie_malfait@rush.edu

Published online: 22 April 2025

References

1. Malfait, A.-M. Mechanisms of joint pain: five short lessons from osteoarthritis. *Semin. Arthritis Rheum.* https://doi.org/10.1016/j.semarthrit.2025.152690 (2025).
2. Qin, W. et al. Neurovascularization inhibiting dual responsive hydrogel for alleviating the progression of osteoarthritis. *Nat. Commun.* **16**, 1390 (2025).
3. Goldring, S. R. & Goldring, M. B. Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage–bone crosstalk. *Nat. Rev. Rheumatol.* **12**, 632–644 (2016).
4. Imhof, H., Breitenseher, M., Kainberger, F. & Trattnig, S. Degenerative joint disease: cartilage or vascular disease? *Skeletal Radiol.* **26**, 398–403 (1997).
5. Suri, S. & Walsh, D. A. Osteochondral alterations in osteoarthritis. *Bone* **51**, 204–211 (2012).
6. Walsh, D. A. et al. Angiogenesis in the synovium and at the osteochondral junction in osteoarthritis. *Osteoarthritis Cartilage* **15**, 743–751 (2007).
7. Aso, K. et al. Contribution of nerves within osteochondral channels to osteoarthritis knee pain in humans and rats. *Osteoarthritis Cartilage* **28**, 1245–1254 (2020).
8. Obeidat, A. M. et al. Intra-articular sprouting of nociceptors accompanies progressive osteoarthritis: comparative evidence in four murine models. *Front. Neuroanat.* **18**, 1429124 (2024).
9. Zhu, S. et al. Subchondral bone osteoclasts induce sensory innervation and osteoarthritis pain. *J. Clin. Invest.* **129**, 1076–1093 (2019).
10. Qin, W. et al. Effect of extracellular ribonucleic acids on neurovascularization in osteoarthritis. *Adv. Sci.* **10**, 2301763 (2023).

Competing interests

A.M.M. receives funding from the National Institute for Arthritis and Musculoskeletal and Skin through grants R01AR060364, UC2AR082186, P30AR079206, R01AR064251 and R21AR085242-01, and has received consulting fees from Orion, Averitas and Novartis.

Molecular imaging

https://doi.org/10.1038/s41584-025-01239-4

Imaging inflammation with leukocyte-targeted PET tracers

Filippo Fagni

Molecular imaging techniques such as PET with the leukocyte-targeted probe ^{89}Zr-CD45 are promising tools for rheumatology, providing a non-invasive whole-body assessment of the mechanisms that drive tissue inflammation. These techniques could improve diagnosis and disease monitoring, but further research is required before clinical implementation.

REFERS TO Salehi Farid, A. et al. CD45-PET is a robust, non-invasive tool for imaging inflammation. *Nature* **639**, 214–224 (2025).

Over the past decades, advances in imaging technologies have changed the way we visualize inflammation by moving beyond mere structural and anatomical assessments and delving deeper into the molecular mechanisms that underpin inflammatory diseases. Molecular imaging with PET utilizes radiolabelled tracers targeted at key molecules and cells involved in the inflammatory process, offering unprecedented insights into the biological processes sustaining inflammation across the entire body in a single scan[1]. In particular, radiotracers aimed at specific immune cell compartments that are central to the pathogenesis of immune-mediated inflammatory conditions, such as fibroblasts and macrophages, are rapidly gaining traction in rheumatological imaging[1,2]. Until now, 18-fluorine-fluorodeoxyglucose-PET (^{18}F-FDG-PET) has been the most widely used nuclear imaging technique in rheumatology, owing to its capacity to detect areas of increased glucose metabolism, which serves as a surrogate for inflammation. However, ^{18}F-FDG-PET lacks specificity in distinguishing between different types of inflammation or malignancy.

Salehi Farid et al. have now developed a novel 89-zirconium (^{89}Zr)-labelled radiotracer aimed at the human leukocyte common antigen CD45 (ref. 3). The authors tested an ^{89}Zr-CD45 probe on mouse models of acute respiratory distress syndrome (ARDS) and inflammatory bowel disease (IBD), showing that radionuclide uptake reliably correlated with both histological and clinical indicators of inflammation and that the probe demonstrated superior efficacy compared to ^{18}F-FDG-PET in stratifying disease severity thanks to superior tracer accumulation at inflammatory sites.

^{89}Zr-CD45-PET enabled the visualization of immune cells throughout the whole body in healthy mice, with maximal uptake in the spleen, bone marrow and lymph nodes. Probe uptake was significantly increased at sites of organ inflammation. In the ARDS mouse model, ^{89}Zr-CD45 uptake in the inflamed lungs correlated with clinical severity, inflammatory infiltrate in CT and histological lung injury scores. Notably, ^{89}Zr-CD45-PET outperformed ^{18}F-FDG-PET in a head-to-head comparison, in which ^{18}F-FDG uptake was less correlated to all severity parameters. Similarly, in the IBD mouse model, ^{89}Zr-CD45-PET signal in the intestines was highly correlative with histological scoring, disease activity score and weight loss, whereas ^{18}F-FDG-PET failed in detecting rectal inflammation and did not correlate with disease severity scores or weight loss. Mice with IBD that underwent a treatment regimen received a follow-up PET examination to test the sensitivity of ^{89}Zr-CD45-PET to changes in disease activity over time. Treated mice showed a notable reduction in intestinal probe uptake and this strongly correlated with clinical improvement, including body weight gain.

To evaluate whether the ^{89}Zr-CD45 tracer can detect human leukocytes, the authors tested an anti-human CD45 radiotracer in humanized NSG mice that had been transplanted with human peripheral blood mononuclear cells. Notably, ^{89}Zr-CD45-PET was able to detect higher tracer uptake in the spleen of humanized mice compared with non-humanized controls, as well as increased uptake in multiple target organs in mice that developed symptoms of graft-versus-host disease. Human leukocyte infiltration in these organs was confirmed by immunohistochemistry, showing human CD45$^+$ cells in areas of increased ^{89}Zr-CD45 uptake.

Despite its promising potential, the ^{89}Zr-CD45 tracer has some limitations. The high ^{89}Zr-CD45 uptake in lymphoid organs such as the spleen, bone marrow and lymph nodes[3] does not allow a low threshold for the specific detection of inflammation in these organs, which would be required in common autoinflammatory diseases such as autoimmune connective tissue diseases, sarcoidosis and Castleman disease. Second, CD45 expression varies with leukocyte differentiation and activation, and aberrant expression occurs in several conditions including autoimmune diseases, haematological malignancies and infection with HIV[4], potentially leading to off-target signals. Lastly, no human clinical data are available on ^{89}Zr-CD45, leaving its safety, pharmacokinetics and diagnostic accuracy unverified. Furthermore, ^{89}Zr-CD45 uptake in malignant diseases remains to be investigated and could represent an important confounding factor.

Nonetheless, this study represents an important advance in molecular imaging of inflammation. In rheumatology, ^{89}Zr-CD45-PET has potential for clinical applications, particularly in inflammatory diseases in which immune cell infiltration in peripheral tissues drives pathology. ^{89}Zr-CD45-PET can track treatment responses over time but does not seem to be burdened by the limitations of non-specific uptake in glucose-avid tissues, nor does it require restrictive dietary regimens before imaging. But the main advantage of ^{89}Zr-CD45 over ^{18}F-FDG and previously developed cell-targeted tracers lies in the ability to achieve high sensitivity for inflammation detection while maintaining broad immune cell specificity, effectively differentiating inflammatory activity without compromising signal strength or precision.

Table 1 | Brief overview of clinically relevant cell-targeted radiotracers in rheumatology

Category	Target molecule	Radionuclide	Immune-mediated diseases with application
Macrophages[2,5,6]	Macrophage marker translocator protein (TSPO)	^{124}I-DPA-713 ^{11}C-R-PK11195 ^{18}F-FEDAC	Rheumatoid arthritis Large vessel vasculitis
	β-folate receptor (β-FR)	^{18}F-fluoro-PEG- folate ^{99}Tc-EC20	Rheumatoid arthritis
Fibroblasts[5,7–10]	Fibroblast activation protein (FAP)	^{68}Ga-FAPI ^{68}Ga-DOTA-FAPI-04	Rheumatoid arthritis Axial spondyloarthritis Psoriatic arthritis Inflammatory myopathies Systemic sclerosis IgG4-related disease
T cells[5]	CD4 receptor	^{99m}Tc-MAX.16H5 ^{99m}Tc-EP1645	Rheumatoid arthritis
B cells[5]	CD20 receptor	^{124}I-rituximab ^{89}Zr-rituximab ^{99m}Tc-rituximab	Rheumatoid arthritis Systemic lupus erythematosus Sjögren syndrome Inflammatory myopathies Sarcoidosis Behçet disease Psoriatic arthritis
Myeloid cells[5]	Triggering receptor expressed on myeloid cells 1 (TREM1)	^{64}Cu-TREM1	Multiple sclerosis
Granulocytes[5]	Non-specific cross-reacting antigen 95 (NCA95)	^{99m}Tc-BW250/183	Inflammatory bowel diseases

I believe we will see an increasing use of nuclear medicine techniques in rheumatology aimed at targeting different cell compartments to dissect the mechanisms that drive tissue inflammation (Table 1). For instance, multiple studies have investigated macrophage-targeted tracers (including ^{124}I-DPA-713, ^{68}Ga-DOTA and ^{11}C-R-PK11195) to depict synovial inflammation in rheumatoid arthritis (RA)[5], in which resident synovial macrophages are crucial in sustaining inflammation, and in large-vessel vasculitis[6], in which vessel macrophage infiltration is a key pathogenic step. Similarly, targeting activated fibroblasts with ^{68}Ga-fibroblast activation protein inhibitor-04-PET (^{68}Ga-FAPI-PET) has been successfully implemented to depict fibrotic processes, for example in systemic sclerosis[7] and IgG4-releated disease (IgG4-RD)[8], as well as in inflammatory arthritis[9]. In RA, fibroblasts contribute to synovial remodelling, whereas in axial spondyloarthritides, including psoriatic arthritis, entheseal fibroblasts are activated already in the early disease stages, with fibroblast activation resulting in increased synovio-entheseal ^{68}Ga-FAPI uptake[10]. Notably, ^{68}Ga-FAPI-PET outperformed ^{18}F-FDG-PET in correlating with clinical and serological disease activity parameters in RA[9] and in identifying organ involvement in IgG4-RD[8]. ^{89}Zr-CD45-PET has potential to fit into this broader framework by offering a pan-leukocyte imaging approach that captures the full spectrum of immune cell activity, enabling a comprehensive assessment of immune infiltration across various tissues and providing a more global view of systemic inflammation.

A radiotracer-based approach for diagnosing inflammatory diseases holds great promise by providing in vivo, non-invasive information on disease activity while simultaneously indicating viable therapeutic targets. However, to date, no tracers have been validated as diagnostic or prognostic imaging biomarkers for rheumatological diseases. Therefore, further research is needed for validation in human studies, assessment of their predictive value for disease progression, and evaluation of their responsiveness to therapeutic interventions. Standardization of imaging protocols and comparative studies against existing diagnostic tools will also be essential to determine their role in future practice.

Filippo Fagni [1,2]
[1]Department of Internal Medicine 3, Friedrich-Alexander University Erlangen-Nuremberg and Universitätsklinikum Erlangen, Erlangen, Germany. [2]Deutsches Zentrum für Immuntherapie, Friedrich-Alexander University Erlangen-Nuremberg and Universitätsklinikum Erlangen, Erlangen, Germany.
e-mail: filippo.fagni@uk-erlangen.de

Published online: 26 March 2025

References

1. Minopoulou, I. et al. Imaging in inflammatory arthritis: progress towards precision medicine. *Nat. Rev. Rheumatol.* **19**, 650–665 (2023).
2. Patel, S. K. & Janjic, J. M. Macrophage targeted theranostics as personalized nanomedicine strategies for inflammatory diseases. *Theranostics* **5**, 150–172 (2015).
3. Salehi Farid, A. et al. CD45-PET is a robust, non-invasive tool for imaging inflammation. *Nature* **639**, 214–224 (2025).
4. Tchilian, E. Z. & Beverley, P. C. L. Altered CD45 expression and disease. *Trends Immun.* **27**, 146–153 (2006).
5. Lee, H. J., Ehlerding, E. B. & Cai, W. Antibody-based tracers for PET/SPECT imaging of chronic inflammatory diseases. *ChemBioChem* **20**, 422–436 (2019).
6. Jiemy, W. F. et al. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging of macrophages in large vessel vasculitis: current status and future prospects. *Autoimmun. Rev.* **17**, 715–726 (2018).
7. Bergmann, C. et al. ^{68}Ga-FAPI-04 PET-CT for molecular assessment of fibroblast activation and risk evaluation in systemic sclerosis-associated interstitial lung disease: a single-centre, pilot study. *Lancet Rheumatol.* **3**, e185–e194 (2021).
8. Luo, Y. et al. Fibroblast activation protein–targeted PET/CT with 68Ga-FAPI for imaging IgG4-related disease: comparison to 18F-FDG PET/CT. *J. Nucl. Med.* **62**, 266–271 (2021).
9. Luo, Y. et al. 68Ga-FAPI PET/CT for rheumatoid arthritis: a prospective study. *Radiology* **307**, e222052 (2023).
10. Corte, G. et al. Anatomical pattern of entheseal and synovial fibroblast activation in patients with psoriasis and its risk of developing psoriatic arthritis. *RMD Open* **10**, e004294 (2024).

Acknowledgements

F.F. is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) — 493624887 (Clinician Scientist Program NOTICE).

Competing interests

The author declares no competing interests.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01253-6

Review article

Check for updates

Understanding rheumatic disease through continuous cell state analysis

Lysette Marshall [1], Soumya Raychaudhuri [1,2,3,4] & Sebastien Viatte [1,5,6]

Abstract

Autoimmune rheumatic diseases are a heterogeneous group of conditions, including rheumatoid arthritis (RA) and systemic lupus erythematosus. With the increasing availability of large single-cell datasets, novel disease-associated cell types continue to be identified and characterized at multiple omics layers, for example, 'T peripheral helper' (T_{PH}) ($CXCR5^{-}$ $PD\text{-}1^{hi}$) cells in RA and systemic lupus erythematosus and $MerTK^{+}$ myeloid cells in RA. Despite efforts to define disease-relevant cell atlases, the very definition of a 'cell type' or 'lineage' has proven controversial as higher resolution assays emerge. This Review explores the cell types and states involved in disease pathogenesis, with a focus on the shifting perspectives on immune and stromal cell taxonomy. These understandings of cell identity are closely related to the computational methods adopted for analysis, with implications for the interpretation of single-cell data. Understanding the underlying cellular architecture of disease is also crucial for therapeutic research as ambiguity hinders translation to the clinical setting. We discuss the implications of different frameworks for cell identity for disease treatment and the discovery of predictive biomarkers for stratified medicine — an unmet clinical need for autoimmune rheumatic diseases.

Sections

[1]Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, The University of Manchester, Manchester, UK. [2]Center for Data Sciences, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [3]Divisions of Rheumatology, Inflammation and Immunity and Genetics, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [4]Broad Institute, Cambridge, MA, USA. [5]NIHR Manchester Musculoskeletal Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK. [6]Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK. e-mail: sebastien.viatte@manchester.ac.uk

Key points

- The definition of cell types and cell states remains controversial and is driven by the advent of new technologies and analytical frameworks.
- Examples from rheumatology emphasize the inadequacies of traditional cell classification systems.
- Advances in single-cell technologies have revealed considerable heterogeneity within the cellular architecture of rheumatic diseases.
- Adopting a conceptual and computational approach that considers continuous cell states reveals additional insights beyond that of discrete clustering methods.
- The shift towards understanding cell identity as a dynamic continuum provokes important discussions regarding the future directions for the treatment of rheumatic diseases.

Introduction

The dysregulation of immune and stromal cell subsets is a key feature of autoimmune rheumatic diseases (ARDs), in which immune cells infiltrate target tissues and orchestrate inflammation and tissue damage. For example, in rheumatoid arthritis (RA), immune cells infiltrate the synovium that lines diarthrodial joints[1]. With the development of single-cell, high-dimensional technologies, such as single-cell RNA sequencing (scRNA-seq), we have gained unprecedented insight into the cellular and molecular heterogeneity of these diseases[2–14]. Identifying disease-relevant cell populations is essential for understanding disease mechanisms; certain cell populations might be causal in disease propagation. These insights are important for the identification of new treatment targets or drugs, as well as for the identification of biomarkers for disease monitoring and stratified medicine[15,16], a research priority.

However, what defines a cell 'type' or cell 'state' has long been debated across various fields[17,18] and our interpretation continues to evolve in tandem with advances in single-cell technology and computation[19]. As more data become available, there has been a shift towards the view of cell identity as a dynamic continuum rather than as a rigid hierarchical classification system of mutually exclusive categories. This debate extends beyond academic curiosity and has far-reaching implications; the perspective on cell identity influences all aspects of an analysis, including study design, technological and computational method selection and development, and interpretation and application of findings.

This Review explores the ongoing debate regarding the definition of cell identity, with a focus on the immune and stromal cell landscape within rheumatology. Moving from the past concept of discrete cell types, through the present concept of granular cell states and to the future model of a dynamic cellular continuum (Fig. 1), we examine how these different frameworks of cell identity have shaped our understanding of the pathogenesis of ARDs and consider how varying computational approaches to single-cell data analysis align with these concepts. For each concept, we highlight relevant examples of cell types and states across ARDs. Finally, we explore the broader implications of these frameworks for other domains of rheumatology research, including biomarker selection and stratified medicine.

Discrete cell classification systems

The traditional approach to defining cell identity is to categorize cells into discrete cell types on the basis of a small set of markers, such as expression of pre-defined proteins as measured by flow cytometry[19] (Fig. 1). This definition assumes a threshold for the presence or absence of a marker, mutual exclusivity of expression profiles between cell types and that cells expressing the same markers share functionality. A classic example is the T helper (T_H) 1–T_H2 dichotomy, describing two distinct $CD4^+$ T cell subsets that can be characterized by cytokine and transcription factor expression profiles: T_H1 cells, regulated by T-bet, produce interferon (IFN)-γ, whereas T_H2 cells, regulated by GATA-3, produce IL-4 (refs. 20–22). Within ARD research, concepts of discrete cell classification systems, along with their challenges, can be illustrated by examples of cell subset dysregulation or imbalances in RA (Box 1) and systemic lupus erythematosus (SLE) (Box 2).

Limitations of traditional cell-profiling techniques

Owing to spectral overlap in fluorophore signals, conventional hypothesis-driven flow cytometry studies are limited by the number of parameters that can be assessed simultaneously, limiting their discriminatory capacity and the depth at which cell types can be phenotyped[23,24]. However, the capabilities of flow cytometry are increasing; for example, spectral flow cytometry can better distinguish fluorophores based on the measurement of unique 'spectral fingerprints', allowing for large panels of >30 parameters[25]. For analysis of cell-type abundances, cells of interest are manually selected on a series of two-dimensional scatter plots by biaxial gating; here, the positioning of rigid boundaries around cells imposes the assumption that the populations are discrete (Fig. 2). However, this manual process introduces subjectivity and thus issues of reproducibility[26]. Inconsistencies in subset definition and subsequent gating strategy can lead to opposing results. For example, a meta-analysis of studies investigating the abundance of regulatory T (T_{reg}) cells in RA compared with controls found that conclusions varied depending on the choice of T_{reg} cell definition[27]; studies using a more precise definition, such as $CD25^+$ $FOXP3^+$, resolved a significant reduction in peripheral blood T_{reg} cell frequency in RA relative to controls, whereas studies with less precise definitions, such as $CD25^+$ alone, showed no difference. Combined with this issue, manual biaxial gating is becoming unfeasible in the high-dimensional single-cell era; researchers are unable to assess all marker combinations when using larger panels, leading to substantial information loss and hampering novel discoveries[26].

Granular cell states

Despite the historical contribution of hypothesis-driven studies to our understanding of cell-type disturbances in ARDs, traditional cell classification systems are weakened by emerging evidence, as presented in Boxes 1 and 2, and by the inherent limitations of the associated methodology. Thus, perspectives are shifting beyond the assumption of rigid cell categories and towards the concept of 'cell states' to better reflect the heterogeneous and dynamic nature of cell identity (Fig. 1). 'Cell state' is often used to refer to a transitory condition characterized by a certain molecular and functional phenotype[28]. However, delineating cell type from state remains a challenging task, largely owing to the lack of a precise definition of both terms. One potential distinguishing factor is the stability of a cell type, in which cells of different types, such as T cells and B cells, are resistant to interconversion under normal physiological conditions. Within each coarse cell type, however, could exist a spectrum of fluid cell states between which cells

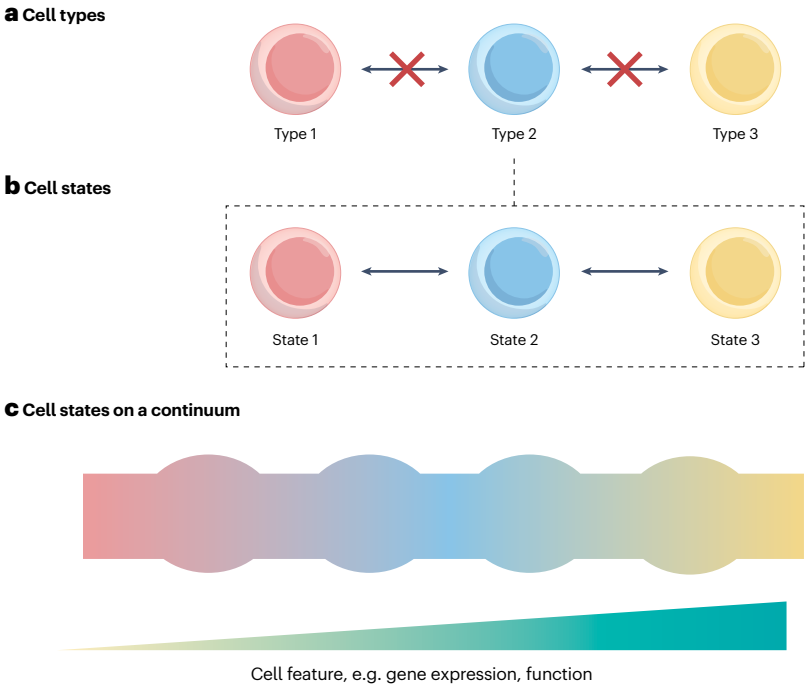


Fig. 1 | Frameworks for representing cell identity. a, Discrete cell types, indicating distinct and non-overlapping populations that do not interconvert. **b**, Granular cell states of a fluid nature. **c**, A continuum of cell states without the existence of discrete cell populations. This continuum might be reflected by a continuous feature of cell identity, for example gene or protein expression or the 'degree' of a particular function.

can interconvert. Testing this definition presents practical and technological challenges, although future advances in temporally resolved and single-cell multiomics technologies will help to clarify the distinction and enhance our understanding of cell identity (see elsewhere[29,30] for a detailed review of these technologies).

To better describe the heterogeneous landscape of cell states in ARDs, large consortia have been formed with the aim of 'disease deconstruction' at the single-cell level; for example, the Accelerating Medicines Partnership RA (AMP RA) and AMP SLE programs have generated comprehensive multi-modal reference maps of disease[3,4,6,31]. Here, we discuss examples of cell state heterogeneity from rheumatology.

T cell heterogeneity

The AMP RA phase 1 study generated multi-modal, single-cell data from synovial-tissue samples and was aimed at identifying disease-associated cell states at greater granularity than traditional studies[3]. Notable findings include the identification of three $CD8^+$ subsets that vary in expression of granzyme K (*GZMK*) and B (*GZMB*), which encode cytotoxic molecules[3]. Subsequent investigations have confirmed that the dominant $CD8^+$ populations in synovium express GZMK alone or alongside GZMB[32]. Interestingly, $CD8^+$ $GZMK^+$ $GZMB^+$ cells exhibit limited cytotoxic potential compared with those expressing GZMB alone[32]. Moreover, contrary to expectations, $CD8^+$ T cells, not $CD4^+$ T cells, were the main producers of IFNγ in RA synovium. Mechanistically, GZMK expression might contribute to disease pathogenesis by induction of pro-inflammatory cytokine production in synovial fibroblasts[32] and activation of the complement system, in which fibroblasts are a key source of complement proteins, amplified by IFNγ and TNF stimulation[33]. Accordingly, GZMK deficiency was associated with reduced complement activation and decreased joint swelling in murine models of RA[33]. Functional assays investigating the antigen-specificity of $CD8^+$ T cells have demonstrated that cytotoxic $GZMB^+$ $CD8^+$ T cells undergo clonal expansion in response to citrullinated antigens (a key feature of RA) to a greater degree than $GZMK^+$ $CD8^+$ T cells[34], although the latter might be responsive to alternative antigens[35,36].

An important discovery is the identification of a T peripheral helper (T_{PH}) cell population expanded in the synovium of patients with seropositive RA. Initially identified in 2008 (ref. 37), Rao and colleagues further characterized these cells in 2017 (ref. 38). Defined as $CD4^+$ $PD\text{-}1^{hi}$ $CXCR5^-$, T_{PH} cells produce high levels of IL-21 and CXCL13, promoting differentiation of B cells to a plasma cell lineage. T follicular helper (T_{FH}) cells are a similar $PD\text{-}1^+$ population whose frequency in the blood often correlates with that of T_{PH} cells; T_{FH} cells express CXCR5 and localize to lymph node follicles, where they carry out similar B cell helper functions[39]. T_{PH} cells have also been identified in other autoantibody-positive diseases, such as SLE, in which their abundance correlates positively with disease activity[40,41], as well as in Sjögren syndrome[42]. Given the phenotypic and functional overlap of T_{PH} and T_{FH}, work in SLE has explored their relationship[43]. T cell receptor (TCR) sequencing revealed dynamic clonal overlap between T_{PH} and T_{FH} cells in which TCR clones unique to either population can be shared over time, suggesting a shared developmental axis. Moreover, trajectory inference (see the section 'Trajectory inference') of scRNA-seq data indicates a transition from T_{FH} to T_{PH}, marked by decreasing *CXCR5* expression and increasing *CXCL13* and *IL21* expression. This finding might therefore represent an example of dynamic shifts between cell states along a phenotypic axis. By comparison, Law et al.[44] show an inverse relationship between blood T_{FH}–T_{PH} cells and T_H22 cells in SLE. Here, genetic and pharmacological perturbation studies showed that aryl hydrocarbon receptor is involved in repression of CXCL13 production and simultaneously promotes IL-22 expression; type I IFN antagonizes these effects to favour the T_{FH}–T_{PH}-dominant phenotype seen in SLE. These findings suggest that T_H22 and T_{FH}–T_{PH} are opposing, and potentially mutually exclusive, cell states[44]. However, it is unclear

Box 1 | Discrete cell subsets in rheumatoid arthritis

T cell subset dysregulation

Rheumatoid arthritis (RA) was historically designated as a T helper 1 (T_H1)-mediated disease; early studies identified the dominance of IFNγ-producing T_H1s over IL-4-producing T_H2s in the synovial membrane and fluid[138,139], although findings vary across studies and compartments (blood or synovium)[138,140]. The discovery of additional T cell subsets beyond T_H1 and T_H2 (ref. 141) shifted attention to IL-17-producing T_H17 cells as potential drivers of RA pathology[142]. T_H17 cytokines stimulate synovial fibroblasts to express IL-6, cartilage-degrading matrix metalloproteinases (MMPs)[143], and RANKL (receptor activator of nuclear factor kappa-B ligand), thus promoting osteoclastogenesis[144]. Other studies have identified imbalances in these newer subsets; for example, the balance of T_H17 cells to anti-inflammatory regulatory T cells (T_{reg} cells) might be altered in favour of T_H17s in the blood of patients with active RA[145,146]. However, observations of co-expression of markers associated with multiple T cell subsets complicates the standard T cell taxonomy[83,147–149], making it difficult to annotate cell identity and function under this framework. We now recognize that cells exhibit plasticity; they can alter their phenotype in response to external cues[150,151]. For example, under arthritic conditions $FoxP3^+$ $CD4^+$ T cells can adopt a pro-inflammatory T_H17 phenotype ('exFoxP3' T_H17 cells) with increased osteoclastogenic potential[152]. Overall, these findings support a revision of the canonical T cell classification system.

Innate immune cells

Monocytes are conventionally classified into classical ($CD14^{++}$ $CD16^-$), intermediate ($CD14^{++}$ $CD16^+$) and non-classical ($CD14^+$ $CD16^{++}$) monocytes, often appearing as a 'waterfall' pattern on plots of CD14 against CD16 expression[153,154]. In RA, intermediate monocytes seem to be expanded in the peripheral blood and are capable of inducing a T_H17 response[155]. For macrophages, one classification system describes their polarization towards an 'M1' or 'M2' phenotype, reminiscent of the T_H1–T_H2 paradigm[156]. In RA, pro-inflammatory M1 macrophages dominate in the synovial fluid[157]. This nomenclature is widely adopted, amongst other systems that describe additional M2 subtypes[158]. However, confusion arising from inconsistent terminology and defining markers has prompted calls for a refined system and conversations surrounding the applicability of the M1–M2 paradigm in vivo and in pathological conditions[158–160]. Moreover, most macrophages in the synovial sublining of patients with RA exhibit a hybrid M1–M2 phenotype, challenging the dichotomous model[161]. Similarly, single-cell studies have begun to uncover heterogeneity within the myeloid compartment in RA beyond these traditional classification systems[137,162] (see also the section 'Granular cell states'), further highlighting their oversimplification.

at what stage(s) in differentiation type I IFN exerts this influence or if T_H22 and T_{FH}–T_{PH} exhibit plasticity[45].

Of note, in RA and SLE, the frequency of T_{PH} cells correlates with that of age-associated B cells (ABCs)[40], a memory B cell population often reported as $CD11c^+$ T-bet^+, that expands with age and in autoimmune conditions[3,46]. However, the precise definition of ABCs, their relationship to other B cell populations and their role across different disease contexts remain unclear[46,47].

Finally, the regulatory T cell compartment has also been shown to exhibit heterogeneity in cell state in arthritic joints. One study identified several T_{reg} cell subsets in synovial fluid from patients with juvenile idiopathic arthritis (JIA), including resting T_{reg} cells that differentiate towards active T_{reg} cells, many of which express T_H1-associated markers[48]. Additionally, a differentiated, suppressive T_{reg} cell subset was defined as $GPR56^+$ $CD161^+$ $CXCL13^+$. Other T_{reg} phenotypes include cytotoxic T_{reg} cells[49] and T_{PH}-like T_{reg} cells (which are speculated to regulate T cell–B cell interactions facilitated by T_{PH} cells[50]). The mechanistic contribution of T_{reg} cell states to disease pathology remains largely unclear; a reduced suppressive capacity and an enhanced pathogenic potential might be contributing factors (reviewed elsewhere[51]).

Macrophage heterogeneity

Evidence has highlighted diverse disease-relevant myeloid cell states. For example, Alivernini et al.[52] identified that $MerTK^+$ $CD206^+$ synovial tissue macrophages are involved in the resolution of inflammation and might contribute to maintaining remission in RA. Conversely, Zhang et al.[53] identified an expanded inflammatory $CXCL10^+$ $CCL2^+$ macrophage state in RA synovium and affected tissue from other inflammatory conditions, such as inflammatory bowel disease (IBD), relative to non-inflamed tissue. This phenotype is suggested to be driven by IFNγ and TNF[53]. Supplementing this finding, IFNγ was found to drive the expression of SLAMF7, in which SLAMF7 engagement culminates in a downstream 'super-activated' macrophage state characterized by pro-inflammatory cytokine expression[54]. Notably, this macrophage state is not captured by the classically activated M1 macrophage gene signature, highlighting the oversimplification of the M1–M2 paradigm[54]. Similarly, Hanlon et al.[55] identified a $CD206^+$ $CD163^+$ $CD40^+$ macrophage population expanded in RA synovium that does not fully align with the M1–M2 paradigm. Although CD206 and CD163 are typically associated with M2, this subset also displayed M1-like features, such as secretion of pro-inflammatory cytokines (including IL-6 and IL-1β), suggesting that it lies on a spectrum between the two phenotypes[55]. Interestingly, CD40 expression was not seen in $CD206^+$ $CD163^+$ macrophages from healthy synovium, and inhibiting CD40 signalling in RA counterparts dampened their inflammatory response. Overall, these findings could point to a pathogenic transition of steady-state cells in disease that could represent a future therapeutic target[55].

Fibroblast cell states and spatial context

Stromal cells are crucial players in many ARDs; in concert with immune cells, they orchestrate the inflammatory response and can mediate tissue-destructive processes. Fibroblasts, the cells responsible for synthesis of the extracellular matrix (ECM), have garnered attention as major contributors to RA pathogenesis and are considered part of the innate immune system[56]. However, the study of fibroblast heterogeneity is still a relatively young field; functionally distinct fibroblast states in disease have only been described in the past 10 years[57–60]. This discussion leads us to an important layer of cell identity not yet discussed: physical location and interaction with the microenvironment. Beginning with

high-level spatial variation, synovial fibroblasts from different joint locations have variable transcriptional and functional phenotypes[61,62]. For example, processes associated with joint destruction are enriched in upper-extremity joints, such as those of the hand, compared with knee joints[61]. Positional identity of synovial fibroblasts is related to epigenetically regulated expression of homeobox (*HOX*) genes[61,62]; notably, the long non-coding RNA *HOTAIR* (HOX transcript antisense RNA), specifically expressed in lower limb joints, is an important regulator of RA-associated processes and fibroblast functionality and is itself regulated by inflammatory factors[62].

Moreover, fibroblasts exhibit heterogeneity in identity and function across spatial regions within the joint. In RA, fibroblasts in the synovial sublining ($THY1^+$ $FAP\alpha^+$) primarily produce cytokines to support inflammation, whereas lining fibroblasts ($THY1^-$ $FAP\alpha^+$) are attributed to tissue destruction[59]. A similar $THY1^+$ $HLA\text{-}DR^{hi}$ sublining population is strikingly expanded in leukocyte-rich RA samples relative to leukocyte-poor or osteoarthritis samples[3]. Synovial fibroblasts in RA exhibit continuous transcriptional variation across spatial gradients driven by NOTCH3 signalling from nearby endothelial cells[63]. Moreover, spatial heterogeneity in fibroblast phenotypes is influenced by differential cytokine responses; response to IL-1β is largely associated with lining fibroblasts, whereas TNF and/or IFNγ responses are distributed throughout the synovium[64].

Other fibroblast states relevant for ARD pathology include $CD200^+$ fibroblasts, which are associated with resolution of inflammation; high proportions of these cells are found in the synovium of patients with psoriatic arthritis (PsA) and RA in remission[65]. Functional analyses in mouse models of arthritis showed that inhibition of IL-17 promotes an expansion of these $CD200^+$ fibroblasts, and that numbers of these cells increase as arthritis resolves[65].

Overall, integration of the spatial layer illustrates a potential continuum of fibroblast cell states across spatial gradients, although further work is required to clarify the relationship of other phenotypes, such as $CD200^+$ fibroblasts, to other key states. Resolving the underlying dynamics might provide opportunities for intervention to skew the cell state landscape away from pathogenic states, such as destructive lining fibroblasts, and towards pro-resolution subsets such as $CD200^+$ fibroblasts. As proposed by Weinand et al.[31], in situations in which cell states are interconvertible, direct depletion of pathogenic states might be unproductive. Rather, a more successful approach might involve modulating the factors that drive cell state dynamics, such as cytokine signalling or NOTCH3 pathways[63], or promoting anti-inflammatory functions, for example, with CD200-Fc[65].

Similar findings have arisen in studies of other conditions involving autoimmune and inflammatory processes. In IBD, high expression of two gene modules was associated with treatment resistance and reflected activation of a pathogenic fibroblast subset that drives neutrophil recruitment through IL-1 signalling[66]. Distinct subsets have also been identified in cancer, including IL-6-producing inflammatory fibroblasts and a myofibroblast population[67]. These themes are reviewed further elsewhere[56]. Interestingly, cross-tissue analyses have revealed two fibroblast cell states that are expanded across tissue types from inflammatory conditions, including RA, Sjögren syndrome, IBD and interstitial lung disease, highlighting potential shared disease mechanisms[60]. One state, $CXCL10^+$ $CCL19^+$ fibroblasts, colocalizes with lymphoid cell niches and overlaps with synovial $THY1^+$ and $HLA\text{-}DR^{hi}$ sublining subsets, whereas the second state, $SPARC^+$ $COL3A1^+$, colocalizes with vascular and mural cell niches and might be associated with ECM modulation[60].

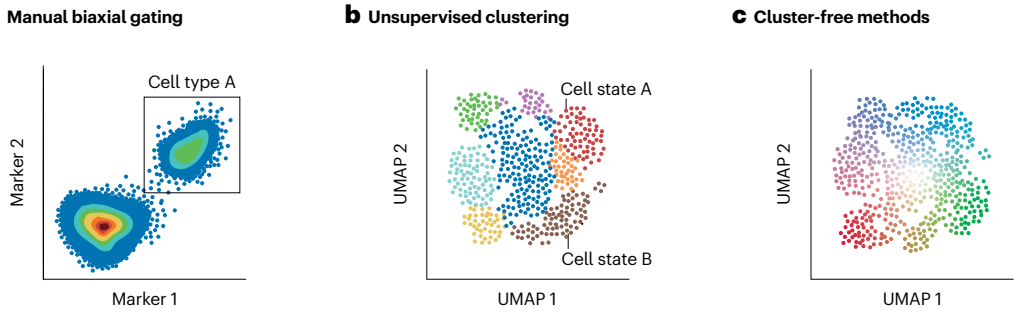
Notably, the resolution of spatial context as an additional layer of cell identity has important implications for conducting research at the site of disease. For example, synovial fibroblasts cluster based on the joint of origin, superseding even clinical diagnosis (RA or osteoarthritis) as the dominant signal in scRNA-seq data[61]; this finding highlights the requirement for consistency in joint sampling when studying disease mechanisms across patients to prevent potential confounding by joint location. Moreover, joint location will be important to consider for synovial biomarker discovery work; certain markers might represent location-specific processes and can thus only be considered in the context in which they were discovered.

Unsupervised methods of cell identification

These unprecedented insights into ARD pathology have been enabled by developments in single-cell sequencing and immunophenotyping technologies that better accommodate hidden complexities in cell identity. For example, cytometry by time-of-flight (CyTOF) uses metal isotopes in place of fluorophores as antibody labels, overcoming the restriction on the size of the panel that results from spectral overlap of fluorophore signals in flow cytometry[24,68]. The contributions of these technologies to defining cell identity is reviewed in detail elsewhere[19,69]. Single-cell technologies can address many of the limitations of traditional approaches by enabling comprehensive, more objective workflows. As well as revolutionizing our understanding of

Box 2 | B cell subsets in systemic lupus erythematosus

B cells are important in the pathogenesis of systemic lupus erythematous (SLE), an autoimmune disease with extensive multi-system manifestations, including arthritis and lupus nephritis[163]. B cell subtypes are traditionally defined by combinations of markers that reflect their developmental stage and/or function. Naïve B cells (canonical phenotype: $CD19^+$, IgD^+, $CD27^-$) are those that have not yet encountered their antigen, whereas plasma cells, characterized partly as $CD27^{++}$, are terminally differentiated and specialized for antibody production[47]. In SLE, the immunopathology involves a perturbation of the balance of these B cell subsets. Early studies highlighted a decline in naïve peripheral B cells ($CD27^-$), causing a relative increase in the proportion of memory B cells, defined as $CD27^+$, compared with controls[164]. Similarly, a population of $CD27^{high}$ plasma cells are expanded in SLE and correlate positively with disease activity[164,165]. However, a population of $CD27^-$ memory B cells known as double-negative (DN) cells (defined as IgD^- $CD27^-$) represent a large proportion of the peripheral blood B cell population in SLE, in some cases ≥40%, compared with typical proportions of <10% in healthy individuals and those with rheumatoid arthritis[166]. These cells do not neatly fit within the traditional B cell classification of memory and naïve cells based on CD27 expression[47]; they were identified as memory B cells based on additional features of cell identity, including functional observations[166]. These cells have been further characterized in other studies. This IgD^- $CD27^-$ subset is also elevated in bacterial infection relative to healthy controls; however, a subset expressing CD95 (IgD^- $CD27^-$ $CD95^+$) was elevated only in patients with SLE[167]. Here, the broad cell-type definitions conceal heterogeneity, requiring additional detail to uncover the populations specific to disease pathology.



	Concept 1	Concept 2	Concept 3
Biological insight	Pre-defined discrete cell types	Cell types and granular cell states	Continuous cell states
Technology	Conventional flow cytometry	High-dimensional omics, e.g. scRNA-seq, CyTOF	High-dimensional omics, e.g. scRNA-seq, CyTOF
Examples	FlowJo software	Louvain clustering, FlowSOM	PCA, NMF-based methods
Limitations	Subjective, time-consuming and unfeasible for large panels	Arbitrary choice of cluster number/resolution	Challenges for interpretation
Technical details	Manual selection of population of interest	Data-driven grouping of cells based on similarities in marker expression profiles	Matrix factorization for representation of continuous cell states

Fig. 2 | Computational approaches for assessing cell identity. a, Manual biaxial gating, illustrated as a two-dimensional dot plot upon which populations of interest are manually selected based on expression of predefined markers. **b**, Unsupervised clustering approaches, illustrated by a UMAP dimensionality reduction plot in which each individual cell is coloured by its cluster assignment. **c**, Cluster-free methods, illustrated by the same UMAP as **b**, but with cells coloured based on a continuous feature, capturing the continuum of cell states. The table below provides an overview of the technologies, examples, limitations and technical details associated with each approach. CyTOF, cytometry by time-of-flight; NMF, non-negative matrix factorization; PCA, principal component analysis; scRNA-seq, single-cell RNA sequencing.

complex ARDs, work on single-cell datasets has aided the development of reference resources, including large cell atlases across health and disease[70,71] (Table 1).

The generation of these high-dimensional datasets requires the development of computational methods to reduce their complexity whilst preserving the rich information contained within them. Indeed, the number of tools for scRNA-seq analysis, for example, has grown rapidly, with 1,059 tools catalogued between 2016 and 2021 (ref. 72). For problems of cell classification, a popular approach is unsupervised clustering methods that group cells based on similarities in their molecular profile[26] (Fig. 2). Common methods include the Louvain[73] or Leiden[74] community detection algorithms for scRNA-seq. For CyTOF data, benchmark studies highlight FlowSOM[75], an algorithm based on self-organizing maps and hierarchical clustering, as a top-performer[76,77]. For scRNA-seq data, these techniques are typically paired with prior dimensionality reduction, such as principal component analysis (PCA), in which the high-dimensional data is mapped to lower dimensions whilst preserving the overall structure in the data[78]. For downstream visualization, non-linear transformations such as t-distributed Stochastic Neighbour Embedding[79] and Uniform Manifold Approximation and Projection[80] are popular choices. If comparing across conditions, differential abundance testing, for example, with mixed-effects models, can be employed to test if cluster abundances are perturbed in the condition of interest[81,82].

Of note, comparisons of these approaches with biaxial gating highlight the information gain achieved with unsupervised methods[83]. Using CyTOF data, automated clustering revealed substantial imbalances in $CD4^+$ T cell populations in RA compared with healthy controls. For instance, an unconventional cluster positive for markers of both canonical T_H1 (T-bet) and T_H17 (IL-17) cells was enriched in RA, whereas $FoxP3^+$ $IL\text{-}2^+$ clusters were depleted in RA. However, using biaxial gating analysis limited to 'standard' definitions of T cell subsets, for example, '$CD4^+$ T-bet^{+}' for T_H1 cells, failed to reveal perturbations in $CD4^+$ T cell subsets besides a reduction in T_{reg} cells ($CD4^+$ $FoxP3^+$) in RA. Overall, these findings underscore the limitations of standard cell taxonomies in the single-cell era.

Limitations of unsupervised clustering. Although conceptually straightforward and intuitive, unsupervised clustering methods have several limitations. For example, choosing the final number of clusters or the clustering resolution is usually subjective. Automated methods for deciding the optimal cluster number exist but have performed poorly in benchmarking studies[76]. The clustering structure can be informative at multiple resolutions, for example, defining coarse cell types, or rarer cells with more granular clustering. However, it is unclear at what resolution cell types are represented over transient cell states, or if the cluster represents an artefact that lacks biological significance[78]. Related to this issue, with the growing number of single-cell datasets detailing diverse cell states (Table 1), a key challenge is mapping these states across studies and distinguishing technical variation from truly distinct cell states[84]. Several methods exist for the integration of single-cell datasets[85], although compiling unified reference resources for understanding cellular heterogeneity and disease pathogenesis is an ongoing pursuit[86].

Table 1 | Examples of single-cell atlases including human autoimmune rheumatic diseases

Disease(s)	Tissue(s)	Key technique(s)	Sample size[a]	Key outcomes	Ref.
Broad immune and stromal cell atlases					
RA	Synovium	scRNA-seq	5 RA	Developed a low-cost instrument for single-cell profiling; identified 13 cell populations	2
RA	Synovium	scRNA-seq, CyTOF	36 RA, 15 OA	Identified 18 cell populations; *THY1*$^+$*HLA-DRA*$^+$ fibroblasts and *IL1B*$^+$ monocytes were amongst those expanded in RA and were the main producers of *IL6* and *IL1B*, respectively	3
RA	Synovium	CITE-seq	70 RA, 9 OA	Six synovial subtypes were defined based on the frequencies of six main cell types; subtypes were further associated with granular-cell states and variation in treatment response	4
RA	Synovium	scATAC-seq, snATAC-seq, snRNA-seq	25 RA, 5 OA	Defined 24 'chromatin classes' associated with specific cell states and transcription factors	31
RA, spondylarthritis, PsA, UA	Synovium	scRNA-seq	19 RA, 6 spondylarthritis, 6 PsA, 9 UA	Optimized a dissociation protocol for fresh synovial biopsies; identified 42 cell populations	11
RA, SLE, CD, UC, ILD, COVID-19	Synovium, kidney, intestine, lung, BALF	scRNA-seq	125 (including healthy and disease-affected individuals)	A CXCL10$^+$ CCR2$^+$ inflammatory macrophage state was shared and expanded across several diseases	53
Broad immune cell atlases					
SLE	Kidney (lupus nephritis)	scRNA-seq	24 lupus nephritis, 10 controls	Identified 21 immune cell populations; a continuum of states was observed in B cells and monocytes	6
SLE	Peripheral blood	scRNA-seq	33 children with SLE with 11 controls, 8 adult SLE with 6 controls	IFN-stimulated genes enriched in cell populations expanded in children with SLE, including specific monocyte, DC and T cell subsets	5
SLE	Peripheral blood	scRNA-seq	162 SLE, 99 controls	Depletion of CD4$^+$ naïve T cells and expansion of cytotoxic GZMH$^+$ CD8$^+$ T cells in SLE; strong IFN-stimulated gene upregulation in classical monocytes	134
pSS	Peripheral blood	scRNA-seq	6 pSS, 6 controls	Naïve CD8$^+$ T cells and CD8$^+$ CD4$^+$ T cells were depleted in pSS; T_{reg} cells were expanded in pSS	135
JIA	SF and peripheral blood	Spectral flow cytometry	70 JIA (52 SF, 18 blood), 18 healthy controls	SF monocytes and NK cells lack CD16 expression; SF T cells are activated and exhibit altered expression of co-receptors	136
Macrophage atlases					
RA	Synovium	scRNA-seq	112 RA (45 treatment-naïve, 31 treatment-resistant, 36 remission), 10 healthy	MerTK$^+$ CD206$^+$ macrophage populations, including MerTK$^+$ TREM$^+$ and MerTK$^+$ LYVE1$^+$ cells, have regulatory roles and are associated with remission	52
RA	Synovium	scRNA-seq	10 RA, 2 OA	Identified four macrophage populations; inflammatory HBEGF$^+$ macrophages, enriched in RA, induce fibroblast migration via EGFR signalling	137
CD4$^+$ T cell atlases					
SLE	Peripheral blood	ATAC-seq, scRNA-seq	72 SLE, 30 controls	Exhausted CCR7^{low}CD74^{hi} T_{reg} phenotype in SLE; T_{reg} exhaustion was linked to type 1 IFN signalling	7
SLE, MG, MS	Peripheral blood	scRNA-seq	3 SLE, 3 MG, 4 MS, 3 controls	Identified 12 gene programs describing T cell features; their activity varied across diseases, e.g. an IFN program was enriched in SLE	128
Stromal cell atlases					
RA, pSS, IBD, ILD	Synovium, salivary gland, intestine, lung	scRNA-seq	Synovium: 15 RA, 6 OA Lung: 8 ILD, 11 IPF and RA-ILD, 4 controls Salivary gland: 7 pSS, 6 controls (non-Sjögren Sicca syndrome) Intestine: 7 UC, 5 controls	Two fibroblast states were shared and expanded across diseases: CXCL10$^+$ CCL19$^+$ and SPARC$^+$ COL3A1$^+$	60

BALF, bronchoalveolar lavage fluid; CD, Crohn's disease; CITE-seq, cellular indexing of transcriptomes and epitopes by sequencing; cSLE, children with SLE; CyTOF, cytometry by time-of-flight; DC, dendritic cell; EGFR, epidermal growth factor receptor; IBD, inflammatory bowel disease; IFN, interferon; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; JIA, juvenile idiopathic arthritis; MG, myasthenia gravis; MS, multiple sclerosis; NK, natural killer; OA, osteoarthritis; PsA, psoriatic arthritis; pSS, primary Sjögren syndrome; RA, rheumatoid arthritis; scATAC-seq, single-cell assay for transposase accessible chromatin using sequencing; scRNA-seq, single-cell RNA sequencing; SF, synovial fluid; SLE, systemic lupus erythematosus; snATAC-seq, single-nucleus ATAC-seq; snRNA-seq; single-nucleus RNA-seq; T_{reg}, regulatory T cell; UA, undifferentiated arthritis; UC, ulcerative colitis. [a]Sample size reflects the number recruited and may not reflect the sample size taken forward for all analyses.

A further consideration is the choice of markers used for clustering. Some workflows suggest clustering on 'lineage' markers only, then performing differential expression testing on 'functional' markers[87]. However, the distinction between lineage and functional markers can be controversial, comparable with the distinction between cell type and state. Here, the challenge is to prevent overfitting whilst ensuring that the markers are sufficient to capture relevant populations. Other general limitations of unsupervised clustering are reviewed by Kiselev et al.[78]. Most importantly, clustering methods are still discrete by nature: the underlying assumption is that cells can be partitioned into distinct groups. If this assumption is flawed, clusters might not be biologically meaningful at all resolutions.

Do discrete cell types really exist?

As alluded to in earlier discussions of granular cell states in ARDs, an emerging model extends the concept of cell states and plasticity to a model in which cells exist and transition within a continuum of cell states (Fig. 1). This concept of a cellular continuum has long been described in developmental biology and differentiation trajectories, such as within haematopoiesis[88], but can be applied to broader discussions of heterogeneity within cell types; for example, degree of activation[89], pathogenicity[90] or immune cell exhaustion[91].

Applied to $CD4^+$ T cell heterogeneity, Eizenberg-Magar et al.[92] demonstrated that, depending on the combination of several 'input' cytokines, differentiated T cells can be placed along a continuous landscape of phenotypes amongst the classical T cell subsets. Notably, these 'intermediate' phenotypes were stable over time, and therefore do not represent transitory states between the canonical phenotypes. Supporting this model, Kiner et al.[93] demonstrated the separation of gut $CD4^+$ T cells into high-level groups, including naïve, regulatory and effector T cells. However, effector T cells formed a continuum that could not be separated into the traditional subsets based neither on scRNA-seq nor on chromatin accessibility data, albeit some polarization towards specific cytokine expression was observed. Thus, a true discretized system for T cell classification might be limited to broad distinctions, such as $CD4^+$ versus $CD8^+$. Beyond this, an adequate discrete system for delineating T cell subsets might be unlikely, owing to the substantial heterogeneity, plasticity and contextual nature of T cell identity and function[94].

If cells exist along a continuum in which there is continuous intermediate expression of markers, attempts to partition cells into subsets will be an arbitrary decision and insufficient for describing cell identity. Therefore, additional insights are required, such as those from alternative computational approaches that can further leverage single-cell data, to uncover the position of a cell relative to others in the cellular landscape. Here, we discuss specific examples that illustrate how the approach to exploring cell identity is evolving in rheumatology research, followed by associated computational methods that relax the assumption of discrete cell populations.

Shifting perspectives on cell identity in rheumatology

The growing recognition of a dynamic continuum in some cell lineages has permeated rheumatology research, in which studies of disease-relevant cell states are moving beyond discrete classification. Much of this work has focussed on T cell states. For example, one study modelled continuous cell states from cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) data in memory T cells, defining continuous dimensions of T cell state through canonical correlation analysis, a dimensionality reduction technique that can be applied to multimodal datasets[95]. Here, the effects of many genetic variants on gene expression were dependent on the specific T cell state, such as the degree of cytotoxicity, an effect that was masked by simple dichotomization into $CD4^+$ and $CD8^+$ subsets. These state-dependent expression quantitative trait loci (eQTLs) often overlap with risk loci from genome-wide association studies of autoimmune traits. For example, an eQTL for *ORMDL3* exhibited the strongest effects in $GZMB^+$ cytotoxic $CD8^+$ T cells and is in linkage disequilibrium with an RA genome-wide association study variant. Another study took a similar approach using scRNA-seq data, focussing on eQTLs for *HLA* genes[96]. Here, *HLA* eQTLs were highly dynamic; in RA synovium, the strongest effects were for regulation of *HLA-DQ* genes that showed state dependence across broad cell types. For example, the eQTL for *HLA-DQA1* was strongest in T cell states defined by cytotoxicity. Together, this work highlights the advantage of considering a continuous cell state, as well as a coarse cell type, for studies of disease pathology. Of note, although fine-grained clustering might capture certain cell states, modelling cell states as continuous factors ensures that fine heterogeneity is captured and removes the ambiguity imparted by the choice of cluster resolution[95].

Alternative computational approaches to single-cell analysis

The shift towards a continuum model presents new challenges for data analysis, necessitating the development, or repurposing of, computational methods for accurate biological modelling. Alternative approaches for cell classification and annotation have emerged that do not necessarily assume homogenous, discrete cell populations as the underlying biology (Fig. 2). Notably, the choice of computational method is especially important as it can affect the conclusions drawn. For example, clustering performance is sensitive to the choice of cell–cell similarity metric, and these metrics consistently perform worse in real and synthetic scRNA-seq datasets with a continuous structure compared to those classed as discrete datasets[97]. Similarly, the utility of t-distributed Stochastic Neighbour Embedding and Uniform Manifold Approximation and Projection plots for accurately representing single-cell data is a divisive topic in the field[98–100]. However, a combination of approaches should be considered for maximal output. Discrete cluster-based approaches remain highly valuable, for example, for generating initial, intuitive representations of cellular heterogeneity and informing downstream analyses, although their blind spots should be recognized and addressed with the application of alternative approaches, as detailed below.

Non-cluster-based approaches include matrix factorization methods that generate low-dimensional representations of single-cell data[101]. These methods include PCA, in which principal components can capture continuous features of cell identity, such as T cell 'innateness'[102]. Other methods include non-negative matrix factorization (NMF), which might offer more biologically meaningful representations than PCA for transcriptomic data[101,103]. NMF-based methods can resolve continuous cell states, technical features such as doublets or batch effects, as well as discrete cell types[101,104]. Examples include scCoGAPS (Coordinated Gene Activity across Pattern Subsets)[104], and consensus NMF (cNMF), which implements a meta-analysis for robustness[103]. Moreover, NMF allows multiple cell state programs to be attributed to the same cell, whereas conventional clustering confines cells to a single cluster[103]. As such, NMF has been used for annotation of cells based on usage 'scores' for a set of gene expression programs (GEPs); for example, T-CellAnnoTator, an extension of cNMF, consists of 46 reference GEPs capturing features of T cell identity,

such as cytotoxicity or a 'T_H1-like' GEP[105]. This approach might more accurately reflect T cell identity, in which individual cells can exhibit multiple signatures corresponding to diverse states and functions. However, akin to the choice of cluster number, choosing the number of components in matrix factorization is also not trivial and can influence interpretation of the data[101,106]. Moreover, despite the potential for deeper insight, the output from matrix factorization methods can seem abstract and less immediately intuitive to interpret than the discrete groupings produced by clustering algorithms.

Of note, an alternative approach includes latent Dirichlet allocation, often used for textual analysis[107]. Applied to problems of cell profiling with scRNA-seq data, latent Dirichlet allocation can be used to probabilistically model cells as mixtures of latent functions or 'topics', in which each of these topics is associated with expression of certain genes[108,109].

Cluster-free differential abundance testing. Testing for differential abundance of discrete clusters is a common approach to case–control analysis for detecting shifts in cellular composition, as alluded to earlier. However, a disadvantage of these methods is the inability to resolve more complex signals in the data, such as within-cluster heterogeneity[110]. Alternatively, cluster-free methods have been designed for testing for differential abundance of cell states across groups of samples[110–112]. An example is covarying neighbourhood analysis (CNA)[110]. Here, instead of discrete clusters, CNA identifies overlapping, granular 'neighbourhoods' of cells within a graph-based representation of the data. CNA then leverages matrix factorization by PCA to identify groups of neighbourhoods that co-vary in their abundance across samples, followed by statistical testing for associations of an outcome measure with this structure. Milo, a cluster-free method like CNA, also defines cell neighbourhoods, yet tests the differential abundance of each neighbourhood without prior matrix factorization[112]. In ARD research, these methods have been used to identify cell states associated with clinical phenotypes[4,31,44] and experimental perturbations[65]; for example, specific T cell states were associated with measures of synovial inflammation at the level of chromatin accessibility data[31]. Furthermore, CNA was able to easily recover a dominant Notch signalling gradient previously identified in synovial fibroblasts (see the section 'Fibroblast cell states and spatial context')[63,110]. Notably, benchmarking studies show that these cluster-free methods perform similarly to cluster-based differential abundance methods when the signal within the dataset is a perturbation in a discrete cell cluster[82,110].

Trajectory inference. Single-cell datasets typically represent a snapshot of cell identity in time; although the data encompasses cells in various states and stages, their temporal dynamics are not explicitly visible[19]. However, 'pseudotime' analysis methods have been developed to infer continuous dynamic processes from single-cell snapshots assumed to encompass cell state continuums[113,114]. Pseudotime estimation provides an ordering of cells along some trajectory, though does not directly reflect real time[114]. Inferred cellular relationships might reflect developmental processes, cell activation, and axes of variation that are not necessarily temporal[88,114,115]. An abundance of methods now exist with various frameworks and inference capabilities, as reviewed by Saelens et al.[115]. In the disease context, inference of pseudotime trajectories might contribute to understanding cell state transitions associated with pathogenesis, perhaps representing candidate biomarkers or processes for intervention. Examples from ARD research include B cell activation dynamics and their correlation with ABC signatures in SLE[6]. Although trajectory inference might provide valuable hypothesis-generating insights, algorithms can infer spurious relationships or the 'incorrect' trajectory depending on the specific model's assumptions[116]. Ultimately, experimental validation, such as lineage tracing[117], is necessary to substantiate these relationships. Additionally, integrating further 'omics' layers, such as epigenetic information, might offer additional mechanistic insight and refine the understanding of pseudotime trajectories[118].

Of note, longitudinal sampling can reveal trends in cell state dynamics over time, for example, progression over the disease course[119], in response to intervention[120], or predicting events such as disease flares[14,121]. Illustrating this idea, longitudinal sampling of the blood of patients with recently diagnosed SLE over 1 year revealed an initial expansion of T_{FH} cells followed by their decline, whereas levels of T_{PH} cells remained elevated[119]. This finding might suggest differential involvement of T_{FH} and T_{PH} at different disease stages, and the potential importance of timing in therapeutic interventions[119]. However, practical challenges in obtaining repeat patient samples means that these studies are currently sparse and limited by sample size.

Cell identity frameworks and treatment

Despite advances in drug development for ARDs, drug response remains variable across patients; the heterogeneous nature of ARDs might be responsible, in which different underlying disease mechanisms might be amenable to treatment with mechanistically distinct drugs. Therefore, stratified medicine is a core research area in rheumatology, with the aim of allocating patients to the treatment most likely to be effective as early as possible in the disease course[16]. Considering their role in disease pathogenesis and as targets of biologic drugs, immune cells represent a sensible candidate for treatment response biomarkers. The success of these strategies could in part be influenced by the perspective on cell identity and taxonomy adopted by current studies.

Few prospective trials have implemented stratified medicine approaches based on cell biomarkers. Of these, the randomized trial R4RA assessed response to rituximab or tocilizumab in patients with RA classified as B cell poor or B cell rich based on histological assessment and RNA sequencing of synovial biopsy samples[122]. Here, patients with B cell-poor RA, classified based on expression of a B cell gene module, had significantly better outcomes with tocilizumab than rituximab (as measured by achievement of 50% improvement in clinical disease activity index). Of note, this effect was not seen when B cell status was determined based on immunohistochemical staining. As noted by the authors, the RNA-seq-based classification captures additional B cell states that might be relevant to treatment outcomes, and avoids elements of subjectivity associated with histological assessment[122]. However, in a subsequent trial, STRAP, synovial B cell status, again assessed by histological methods and RNA-seq, was not associated with a different response to rituximab compared with tocilizumab or etanercept, thus questioning the efficacy of this approach[123].

Other approaches to patient stratification delineate a disease into distinct 'pathotypes' or 'endotypes', on the basis of differences in immune and stromal cell composition, which correlate with clinical outcomes including disease activity and treatment response[124–127]. For example, in patients with RA a pauci-immune synovial pathotype, defined by findings of limited immune cell infiltration and abundance of stromal cells upon histological assessment, is associated with poor response to anti-TNF therapy[127]. Considering multiple cell

types might better reflect broad perturbations in the cellular architecture that drive disease processes. Conversely, the abundance of major cell types might not correlate with expected pathological consequences[51], although a consideration of granular cell state might provide a deeper understanding of pathogenesis by identifying specific pathogenic cell states. For example, a study as part of the AMP RA program highlighted six cell-type abundance phenotypes in RA synovium (delineated by the abundance of six major cell types and further refined by the results of association testing with fine cell states within the cell types using CNA[4]).

Importantly, the concept of cell states on a continuum complicates efforts towards stratified medicine: if creating cell boundaries is arbitrary, how do we approach identification of cellular predictors of response? Harnessing continuous measures of cell state could be a potential strategy to refine biomarker and target identification. Exemplifying the use of continuous representations of cell states, a cross-disease scRNA-seq analysis by Yasumizu et al. used NMF to identify 12 components that represent gene programs in peripheral CD4$^+$ T cell populations[128]. Importantly, these features can offer insights that might not be apparent from measures of associated cell-type abundance, such as indications of functional alteration, highlighting the added value of this approach[128]. This discussion can be combined with that of challenges for disease classification (nosology), which also has implications for stratified medicine. Despite the classification of ARDs as distinct entities, these disorders overlap in clinical presentation and genetic background and often co-occur in the same patient[129]. This has encouraged the exploration of alternative classification systems; for example, a multidimensional continuum along which all autoimmune and autoinflammatory diseases are placed, constructed on the basis of continuous variables representing characteristics such as degree of inflammation or cell type abundance[130,131]. At the cellular level, cross-disease analyses have identified multiple shared pathogenic cell states, as mentioned earlier. Similarly, Yasumizu et al. projected the 12 NMF features onto external datasets and highlighted disease-specific program usage, but also that the same gene program is often shared across multiple conditions[128]. Related to these considerations is the concept of basket trials, in which drugs are tested across multiple diseases with overlapping pathologies, rather than for one disease at a time[130]. These alternative trial designs are reviewed in detail elsewhere[132,133]. Here, patients across diseases might be grouped by multiple biomarkers for a stratified design[132]; this approach could be an opportunity for inclusion of continuous representations of cell state to identify shared and distinct signatures upon which patient stratification could be based. The potential strategies for using cell-based biomarkers for stratified medicine, as discussed in this section, are summarized in Fig. 3.

In summary, cellular biomarkers of response are a promising avenue for stratified medicine. Abundance of discrete clusters might not necessarily reflect the underlying mechanisms important for pathology and treatment response, although identification of granular pathogenic cell states might improve precision. Moreover, combining this information with continuous measures of cell state might provide a more nuanced understanding that could be harnessed for improving therapeutic pipelines.

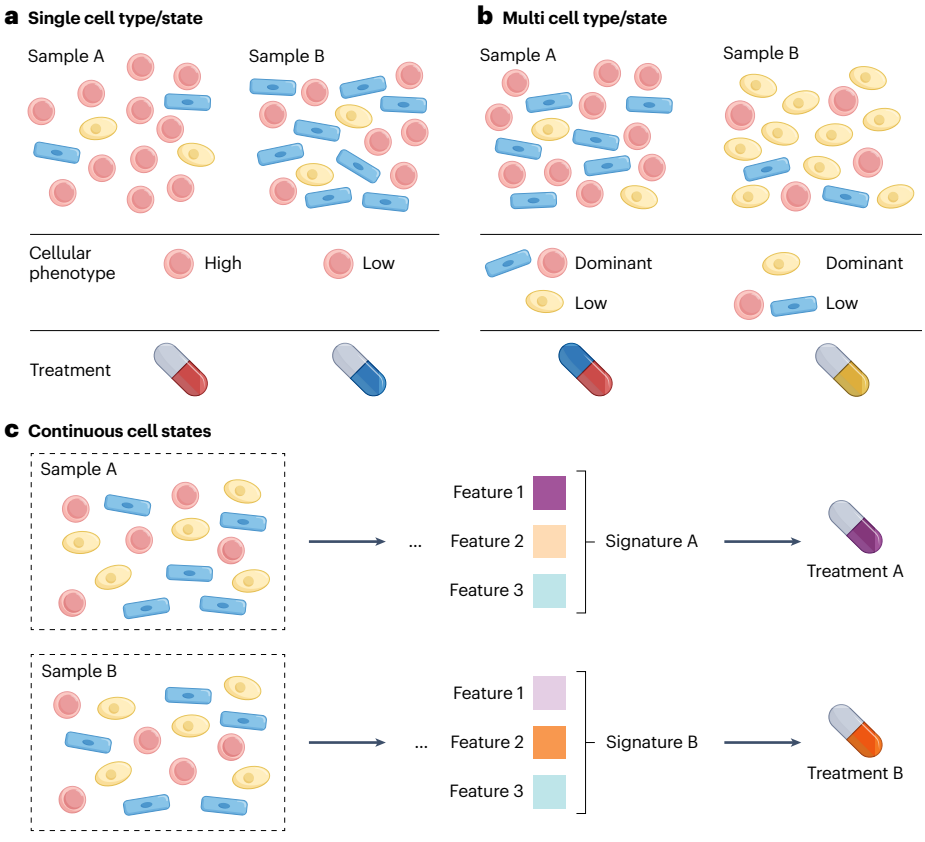


Fig. 3 | Potential approaches to using cellular signatures in stratified medicine pipelines. **a**, Stratification of patients to treatment regimens based on the abundance of a single cell type or state (for example, B cell-poor versus B cell-rich classification in R4RA[122]). **b**, Stratification based on proportions of multiple cell types/states within cell samples (as exemplified by studies of RA synovial pathotypes[125] or cell-type abundance phenotypes ('CTAPs')[4]). **c**, Incorporating continuous representations of cell state to identify signatures associated with response to different treatments. Each feature might reflect a continuous aspect of cell identity, such as degree of activation, and different features are represented here by different colours for which the intensity of the colour reflects how strongly each feature is represented in each patient cell sample.

Conclusions

The complexity of cell identity represents an example of how, despite our human tendency to categorize, many aspects of biology transcend discrete classification systems. Technological advances have intensified discussions surrounding how to best represent cell identity, introducing new perspectives to rheumatology research. As a result, emphasis is growing on the use of continuous representations of cell states that capture the heterogeneity within immune and stromal cell populations. Investigating cells on a continuum alongside more traditional approaches not only provides a more comprehensive understanding of disease pathology but also creates opportunities for future innovations in targeted treatments and discovery of predictive biomarkers for stratified medicine.

Published online: 7 May 2025

References

1. Smolen, J. S. et al. Rheumatoid arthritis. *Nat. Rev. Dis. Prim.* **4**, 18001 (2018).
2. Stephenson, W. et al. Single-cell RNA-seq of rheumatoid arthritis synovial tissue using low-cost microfluidic instrumentation. *Nat. Commun.* **9**, 791 (2018).
3. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
4. Zhang, F. et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. *Nature* **623**, 616–624 (2023).
5. Nehar-Belaid, D. et al. Mapping systemic lupus erythematosus heterogeneity at the single-cell level. *Nat. Immunol.* **21**, 1094–1106 (2020).
6. Arazi, A. et al. The immune cell landscape in kidneys of patients with lupus nephritis. *Nat. Immunol.* **20**, 902–914 (2019).
7. Guo, C. et al. Single-cell transcriptome profiling and chromatin accessibility reveal an exhausted regulatory CD4+ T cell subset in systemic lupus erythematosus. *Cell Rep.* **41**, 111606 (2022).
8. Hong, X. et al. Single-cell RNA sequencing reveals the expansion of cytotoxic $CD4^+$ T lymphocytes and a landscape of immune cells in primary Sjögren's syndrome. *Front. Immunol.* **11**, 594658 (2020).
9. Xu, T. et al. Single-cell profiling reveals pathogenic role and differentiation trajectory of granzyme K^+CD8^+ T cells in primary Sjögren's syndrome. *JCI Insight* **8**, e167490 (2023).
10. Penkava, F. et al. Single-cell sequencing reveals clonal expansions of pro-inflammatory synovial CD8 T cells expressing tissue-homing receptors in psoriatic arthritis. *Nat. Commun.* **11**, 4767 (2020).
11. Edalat, S. G. et al. Molecular maps of synovial cells in inflammatory arthritis using an optimized synovial tissue dissociation protocol. *iScience* **27**, 109707 (2024).
12. Povoleri, G. A. M. et al. Psoriatic and rheumatoid arthritis joints differ in the composition of CD8+ tissue-resident memory T cell subsets. *Cell Rep.* **42**, 112514 (2023).
13. Floudas, A. et al. Distinct stromal and immune cell interactions shape the pathogenesis of rheumatoid and psoriatic arthritis. *Ann. Rheum. Dis.* **81**, 1224–1242 (2022).
14. MacDonald, L. et al. Synovial tissue myeloid dendritic cell subsets exhibit distinct tissue-niche localization and function in health and rheumatoid arthritis. *Immunity* **57**, 2843–2862.e12 (2024).
15. Mulhearn, B., Barton, A. & Viatte, S. Using the immunophenotype to predict response to biologic drugs in rheumatoid arthritis. *J. Pers. Med.* **9**, 46 (2019).
16. Guthridge, J. M., Wagner, C. A. & James, J. A. The promise of precision medicine in rheumatology. *Nat. Med.* **28**, 1363–1371 (2022).
17. Clevers, H. et al. What is your conceptual definition of "Cell Type" in the context of a mature organism? *Cell Syst.* **4**, 255–259 (2017).
18. Dance, A. What is a cell type, really? The quest to categorize life's myriad forms. *Nature* **633**, 754–756 (2024).
19. McKinley, K. L., Castillo-Azofeifa, D. & Klein, O. D. Tools and concepts for interrogating and defining cellular identity. *Cell Stem Cell* **26**, 632–656 (2020).
20. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348–2357 (1986).
21. Szabo, S. J. et al. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* **100**, 655–669 (2000).
22. Zheng, W. & Flavell, R. A. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587–596 (1997).
23. Roederer, M. Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. *Cytometry* **45**, 194–205 (2001).
24. Bandura, D. R. et al. Mass cytometry: technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. *Anal. Chem.* **81**, 6813–6822 (2009).
25. Brestoff, J. R. & Frater, J. L. Contemporary challenges in clinical flow cytometry: small samples, big data, little time. *J. Appl. Lab. Med.* **7**, 931–944 (2022).
26. Saeys, Y., Van Gassen, S. & Lambrecht, B. N. Computational flow cytometry: helping to make sense of high-dimensional immunology data. *Nat. Rev. Immunol.* **16**, 449–462 (2016).
27. Morita, T. et al. The proportion of regulatory t cells in patients with rheumatoid arthritis: a meta-analysis. *PLoS ONE* **11**, e0162306 (2016).
28. Rafelski, S. M. & Theriot, J. A. Establishing a conceptual framework for holistic cell states and state transitions. *Cell* **187**, 2633–2651 (2024).
29. Ding, J., Sharon, N. & Bar-Joseph, Z. Temporal modelling using single-cell transcriptomics. *Nat. Rev. Genet.* **23**, 355–368 (2022).
30. Baysoy, A., Bai, Z., Satija, R. & Fan, R. The technological landscape and applications of single-cell multi-omics. *Nat. Rev. Mol. Cell Biol.* **24**, 695–713 (2023).
31. Weinand, K. et al. The chromatin landscape of pathogenic transcriptional cell states in rheumatoid arthritis. *Nat. Commun.* **15**, 4650 (2024).
32. Jonsson, A. H. et al. Granzyme K^+ CD8 T cells form a core population in inflamed human tissue. *Sci. Transl. Med.* **14**, eabo0686 (2022).
33. Donado, C. A. et al. Granzyme K activates the entire complement cascade. *Nature* https://doi.org/10.1038/s41586-025-08713-9 (2025).
34. Moon, J.-S. et al. Cytotoxic $CD8^+$ T cells target citrullinated antigens in rheumatoid arthritis. *Nat. Commun.* **14**, 319 (2023).
35. Jonsson, A. H. Granzyme K^+ CD8 T cells in autoimmunity. *Best. Pract. Res. Clin. Rheumatol.* **38**, 101930 (2024).
36. Dunlap, G. et al. Clonal associations between lymphocyte subsets and functional states in rheumatoid arthritis synovium. *Nat. Commun.* **15**, 4991 (2024).
37. Manzo, A. et al. Mature antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13 in the inflammatory environment of the rheumatoid joint. *Arthritis Rheum.* **58**, 3377–3387 (2008).
38. Rao, D. A. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017).
39. Yoshitomi, H. & Ueno, H. Shared and distinct roles of T peripheral helper and T follicular helper cells in human diseases. *Cell Mol. Immunol.* **18**, 523–527 (2021).
40. Bocharnikov, A. V. et al. PD-1hiCXCR5- T peripheral helper cells promote B cell responses in lupus via MAF and IL-21. *JCI Insight* **4**, e130062 (2019).
41. Lin, J., Yu, Y., Ma, J., Ren, C. & Chen, W. PD-1^+CXCR5-CD4+T cells are correlated with the severity of systemic lupus erythematosus. *Rheumatology* **58**, 2188–2192 (2019).
42. Pontarini, E. et al. Unique expansion of IL-21+ Tfh and Tph cells under control of ICOS identifies Sjögren's syndrome with ectopic germinal centres and MALT lymphoma. *Ann. Rheum. Dis.* **79**, 1588–1599 (2020).
43. Sasaki, T. et al. Clonal relationships between Tph and Tfh cells in patients with SLE and in murine lupus. *bioRxiv* 2025.01.27.635189 https://doi.org/10.1101/2025.01.27.635189 (2025).
44. Law, C. et al. Interferon subverts an AHR-JUN axis to promote $CXCL13^+$ T cells in lupus. *Nature* **631**, 857–866 (2024).
45. Pazhouhandeh, M. & Yu, D. Interferon disrupts immune and tissue homeostasis in SLE via CXCL13. *Nat. Rev. Rheumatol.* **20**, 745–746 (2024).
46. Mouat, I. C., Goldberg, E. & Horwitz, M. S. Age-associated B cells in autoimmune diseases. *Cell Mol. Life Sci.* **79**, 402 (2022).
47. Sanz, I. et al. Challenges and opportunities for consistent classification of human B cell and plasma cell populations. *Front. Immunol.* **10**, 2458 (2019).
48. Lutter, L. et al. Human regulatory T cells locally differentiate and are functionally heterogeneous within the inflamed arthritic joint. *Clin. Transl. Immunol.* **11**, e1420 (2022).
49. Simone, D. et al. Single cell analysis of spondyloarthritis regulatory T cells identifies distinct synovial gene expression patterns and clonal fates. *Commun. Biol.* **4**, 1–15 (2021).
50. Julé, A. M. et al. Disordered T cell-B cell interactions in autoantibody-positive inflammatory arthritis. *Front. Immunol.* **13**, 1068399 (2023).
51. Schnell, J. T. et al. The 'Treg paradox' in inflammatory arthritis. *Nat. Rev. Rheumatol.* **21**, 9–21 (2024).
52. Alivernini, S. et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. *Nat. Med.* **26**, 1295–1306 (2020).
53. Zhang, F. et al. IFN-γ and TNF-α drive a *CXCL10+ CCL2+* macrophage phenotype expanded in severe COVID-19 lungs and inflammatory diseases with tissue inflammation. *Genome Med.* **13**, 64 (2021).
54. Simmons, D. P. et al. SLAMF7 engagement super-activates macrophages in acute and chronic inflammation. *Sci. Immunol.* **7**, eabf2846 (2022).
55. Hanlon, M. M. et al. Loss of synovial tissue macrophage homeostasis precedes rheumatoid arthritis clinical onset. *Sci. Adv.* **10**, eadj1252 (2024).
56. Davidson, S. et al. Fibroblasts as immune regulators in infection, inflammation and cancer. *Nat. Rev. Immunol.* **21**, 704–717 (2021).
57. Croft, A. P. et al. Rheumatoid synovial fibroblasts differentiate into distinct subsets in the presence of cytokines and cartilage. *Arthritis Res. Ther.* **18**, 270 (2016).
58. Mizoguchi, F. et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat. Commun.* **9**, 789 (2018).
59. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
60. Korsunsky, I. et al. Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases. *Med* **3**, 481–518.e14 (2022).
61. Frank-Bertoncelj, M. et al. Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat. Commun.* **8**, 14852 (2017).
62. Elhai, M. et al. The long non-coding RNA HOTAIR contributes to joint-specific gene expression in rheumatoid arthritis. *Nat. Commun.* **14**, 8172 (2023).

63. Wei, K. et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* **582**, 259–264 (2020).
64. Smith, M. H. et al. Drivers of heterogeneity in synovial fibroblasts in rheumatoid arthritis. *Nat. Immunol.* **24**, 1200–1210 (2023).
65. Rauber, S. et al. CD200$^+$ fibroblasts form a pro-resolving mesenchymal network in arthritis. *Nat. Immunol.* **25**, 682–692 (2024).
66. Friedrich, M. et al. IL-1-driven stromal–neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nat. Med.* **27**, 1970–1981 (2021).
67. Öhlund, D. et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J. Exp. Med.* **214**, 579–596 (2017).
68. Tanner, S. D., Baranov, V. I., Ornatsky, O. I., Bandura, D. R. & George, T. C. An introduction to mass cytometry: fundamentals and applications. *Cancer Immunol. Immunother.* **62**, 955–965 (2013).
69. Trapnell, C. Defining cell types and states with single-cell genomics. *Genome Res.* **25**, 1491–1498 (2015).
70. Regev, A. et al. The human cell atlas. *Elife* **6**, e27041 (2017).
71. Tabula Sapiens, C. et al. The Tabula Sapiens: a multiple-organ, single-cell transcriptomic atlas of humans. *Science* **376**, eabl4896 (2022).
72. Zappia, L. & Theis, F. J. Over 1000 tools reveal trends in the single-cell RNA-seq analysis landscape. *Genome Biol.* **22**, 301 (2021).
73. Blondel, V. D., Guillaume, J.-L., Lambiotte, R. & Lefebvre, E. Fast unfolding of communities in large networks. *J. Stat. Mech.* **2008**, P10008 (2008).
74. Traag, V. A., Waltman, L. & van Eck, N. J. From Louvain to Leiden: guaranteeing well-connected communities. *Sci. Rep.* **9**, 5233 (2019).
75. Van Gassen, S. et al. FlowSOM: using self-organizing maps for visualization and interpretation of cytometry data. *Cytom. A* **87**, 636–645 (2015).
76. Weber, L. M. & Robinson, M. D. Comparison of clustering methods for high-dimensional single-cell flow and mass cytometry data. *Cytom. A* **89**, 1084–1096 (2016).
77. Liu, X. et al. A comparison framework and guideline of clustering methods for mass cytometry data. *Genome Biol.* **20**, 297 (2019).
78. Kiselev, V. Y., Andrews, T. S. & Hemberg, M. Challenges in unsupervised clustering of single-cell RNA-seq data. *Nat. Rev. Genet.* **20**, 273–282 (2019).
79. van der Maaten, L. & Hinton, G. Visualizing data using t-SNE. *J. Mach. Learn. Res.* **9**, 2579–2605 (2008).
80. McInnes, L., Healy, J. & Melville, J. UMAP: uniform manifold approximation and projection for dimension reduction. *arXiv* https://doi.org/10.48550/arXiv.1802.03426 (2018).
81. Fonseka, C. Y. et al. Mixed-effects association of single cells identifies an expanded effector CD4$^+$ T cell subset in rheumatoid arthritis. *Sci. Transl. Med.* **10**, eaaq0305 (2018).
82. Yi, H., Plotkin, A. & Stanley, N. Benchmarking differential abundance methods for finding condition-specific prototypical cells in multi-sample single-cell datasets. *Genome Biol.* **25**, 9 (2024).
83. Mulhearn, B. et al. Automated clustering reveals CD4$^+$ T cell subset imbalances in rheumatoid arthritis. *Front. Immunol.* **14**, 1094872 (2023).
84. Hrovatin, K. et al. Considerations for building and using integrated single-cell atlases. *Nat. Methods* **22**, 41–57 (2025).
85. Luecken, M. D. et al. Benchmarking atlas-level data integration in single-cell genomics. *Nat. Methods* **19**, 41–50 (2022).
86. Rood, J. E. et al. The Human Cell Atlas from a cell census to a unified foundation model. *Nature* **637**, 1065–1071 (2025).
87. Nowicka, M. et al. CyTOF workflow: differential discovery in high-throughput high-dimensional cytometry datasets. *F1000Res* **6**, 748 (2019).
88. Bendall, S. C. et al. Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development. *Cell* **157**, 714 (2014).
89. Szabo, P. A. et al. Single-cell transcriptomics of human T cells reveals tissue and activation signatures in health and disease. *Nat. Commun.* **10**, 4706 (2019).
90. Gaublomme, J. T. et al. Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. *Cell* **163**, 1400–1412 (2015).
91. Tietscher, S. et al. A comprehensive single-cell map of T cell exhaustion-associated immune environments in human breast cancer. *Nat. Commun.* **14**, 98 (2023).
92. Eizenberg-Magar, I. et al. Diverse continuum of CD4$^+$ T-cell states is determined by hierarchical additive integration of cytokine signals. *Proc. Natl Acad. Sci. USA* **114**, E6447–E6456 (2017).
93. Kiner, E. et al. Gut CD4$^+$ T cell phenotypes are a continuum molded by microbes, not by T_H archetypes. *Nat. Immunol.* **22**, 216–228 (2021).
94. Jameson, S. C. & Masopust, D. Understanding subset diversity in T cell memory. *Immunity* **48**, 214–226 (2018).
95. Nathan, A. et al. Single-cell eQTL models reveal dynamic T cell state dependence of disease loci. *Nature* **606**, 120–128 (2022).
96. Kang, J. B. et al. Mapping the dynamic genetic regulatory architecture of HLA genes at single-cell resolution. *Nat. Genet.* **55**, 2255–2268 (2023).
97. Watson, E. R., Mora, A., Taherian Fard, A. & Mar, J. C. How does the structure of data impact cell-cell similarity? Evaluating how structural properties influence the performance of proximity metrics in single cell RNA-seq data. *Brief. Bioinform* **23**, bbac387 (2022).
98. Chari, T. & Pachter, L. The specious art of single-cell genomics. *PLoS Comput. Biol.* **19**, e1011288 (2023).
99. Lause, J., Berens, P. & Kobak, D. The art of seeing the elephant in the room: 2D embeddings of single-cell data do make sense. *PLoS Comput. Biol.* **20**, e1012403 (2024).
100. Marx, V. Seeing data as t-SNE and UMAP do. *Nat. Methods* **21**, 930–933 (2024).
101. Stein-O'Brien, G. L. et al. Enter the matrix: factorization uncovers knowledge from omics. *Trends Genet.* **34**, 790–805 (2018).
102. Gutierrez-Arcelus, M. et al. Lymphocyte innateness defined by transcriptional states reflects a balance between proliferation and effector functions. *Nat. Commun.* **10**, 687 (2019).
103. Kotliar, D. et al. Identifying gene expression programs of cell-type identity and cellular activity with single-cell RNA-Seq. *Elife* **8**, e43803 (2019).
104. Stein-O'Brien, G. L. et al. Decomposing cell identity for transfer learning across cellular measurements, platforms, tissues, and species. *Cell Syst.* **8**, 395–411.e8 (2019).
105. Kotliar, D. et al. Reproducible single cell annotation of programs underlying T-cell subsets, activation states, and functions. *bioRxiv* https://doi.org/10.1101/2024.05.03.592310 (2024).
106. Maisog, J. M. et al. Assessing methods for evaluating the number of components in non-negative matrix factorization. *Mathematics* **9**, 2840 (2021).
107. Blei, D. M., Ng, A. Y. & Jordan, M. I. Latent Dirichlet allocation. *J. Mach. Learn. Res.* **3**, 993–1022 (2003).
108. Wu, X., Wu, H. & Wu, Z. Penalized latent Dirichlet allocation model in single-cell RNA sequencing. *Stat. Biosci.* **13**, 543–562 (2021).
109. Yang, Q. et al. An interpretable single-cell RNA sequencing data clustering method based on latent Dirichlet allocation. *Brief. Bioinform.* **24**, bbad199 (2023).
110. Reshef, Y. A. et al. Co-varying neighborhood analysis identifies cell populations associated with phenotypes of interest from single-cell transcriptomics. *Nat. Biotechnol.* **40**, 355–363 (2022).
111. Burkhardt, D. B. et al. Quantifying the effect of experimental perturbations at single-cell resolution. *Nat. Biotechnol.* **39**, 619–629 (2021).
112. Dann, E., Henderson, N. C., Teichmann, S. A., Morgan, M. D. & Marioni, J. C. Differential abundance testing on single-cell data using k-nearest neighbor graphs. *Nat. Biotechnol.* **40**, 245–253 (2022).
113. Trapnell, C. et al. The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells. *Nat. Biotechnol.* **32**, 381–386 (2014).
114. Tritschler, S. et al. Concepts and limitations for learning developmental trajectories from single cell genomics. *Development* **146**, dev170506 (2019).
115. Saelens, W., Cannoodt, R., Todorov, H. & Saeys, Y. A comparison of single-cell trajectory inference methods. *Nat. Biotechnol.* **37**, 547–554 (2019).
116. Cannoodt, R., Saelens, W. & Saeys, Y. Computational methods for trajectory inference from single-cell transcriptomics. *Eur. J. Immunol.* **46**, 2496–2506 (2016).
117. Weinreb, C., Rodriguez-Fraticelli, A., Camargo, F. D. & Klein, A. M. Lineage tracing on transcriptional landscapes links state to fate during differentiation. *Science* **367**, eaaw3381 (2020).
118. Welch, J. D., Hartemink, A. J. & Prins, J. F. MATCHER: manifold alignment reveals correspondence between single cell transcriptome and epigenome dynamics. *Genome Biol.* **18**, 138 (2017).
119. Sasaki, T. et al. Longitudinal immune cell profiling in early systemic lupus erythematosus. *Arthritis Rheumatol.* **74**, 1808–1821 (2022).
120. Thomas, T. et al. A longitudinal single-cell atlas of anti-tumour necrosis factor treatment in inflammatory bowel disease. *Nat. Immunol.* **25**, 2152–2165 (2024).
121. Orange, D. E. et al. RNA identification of PRIME cells predicting rheumatoid arthritis flares. *N. Engl. J. Med.* **383**, 218–228 (2020).
122. 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).
123. Rivellese, F. et al. Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials. *Lancet Rheumatol.* **5**, e648–e659 (2023).
124. Lewis, M. J. et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep.* **28**, 2455–2470.e5 (2019).
125. Humby, F. et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. *Ann. Rheum. Dis.* **78**, 761–772 (2019).
126. Lliso-Ribera, G. et al. Synovial tissue signatures enhance clinical classification and prognostic/treatment response algorithms in early inflammatory arthritis and predict requirement for subsequent biological therapy: results from the pathobiology of early arthritis cohort (PEAC). *Ann. Rheum. Dis.* **78**, 1642–1652 (2019).
127. Nerviani, A. et al. A pauci-immune synovial pathotype predicts inadequate response to TNFα-blockade in rheumatoid arthritis patients. *Front. Immunol.* **11**, 845 (2020).
128. Yasumizu, Y. et al. Single-cell transcriptome landscape of circulating CD4$^+$ T cell populations in autoimmune diseases. *Cell Genom.* **4**, 100473 (2024).
129. Moutsopoulos, H. M. Autoimmune rheumatic diseases: one or many diseases? *J. Transl. Autoimmun.* **4**, 100129 (2021).
130. Hosack, T. et al. Inflammation across tissues: can shared cell biology help design smarter trials? *Nat. Rev. Rheumatol.* **19**, 666–674 (2023).
131. Tchitchek, N. et al. Deep immunophenotyping reveals that autoimmune and autoinflammatory disorders are spread along two immunological axes capturing disease inflammation levels and types. *Ann. Rheum. Dis.* **83**, 638–650 (2024).
132. Pitzalis, C., Choy, E. H. S. & Buch, M. H. Transforming clinical trials in rheumatology: towards patient-centric precision medicine. *Nat. Rev. Rheumatol.* **16**, 590–599 (2020).
133. Duan, X.-P. et al. New clinical trial design in precision medicine: discovery, development and direction. *Sig Transduct. Target. Ther.* **9**, 1–29 (2024).

134. Perez, R. K. et al. Single-cell RNA-seq reveals cell type–specific molecular and genetic associations to lupus. *Science* **376**, eabf1970 (2022).
135. Hou, X. et al. Analysis of gene expression and TCR/B cell receptor profiling of immune cells in primary Sjögren's syndrome by single-cell sequencing. *J. Immunol.* **209**, 238–249 (2022).
136. Attrill, M. H. et al. The immune landscape of the inflamed joint defined by spectral flow cytometry. *Clin. Exp. Immunol.* **218**, 221–241 (2024).
137. Kuo, D. et al. HBEGF^{+} macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci. Transl. Med.* **11**, eaau8587 (2019).
138. Dolhain, R. J., van der Heiden, A. N., ter Haar, N. T., Breedveld, F. C. & Miltenburg, A. M. Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis. *Arthritis Rheum.* **39**, 1961–1969 (1996).
139. Miltenburg, A. M., van Laar, J. M., de Kuiper, R., Daha, M. R. & Breedveld, F. C. T cells cloned from human rheumatoid synovial membrane functionally represent the Th1 subset. *Scand. J. Immunol.* **35**, 603–610 (1992).
140. Bazzazi, H. et al. Th1–Th17 ratio as a new insight in rheumatoid arthritis disease. *Iran. J. Allergy Asthma Immunol.* **17**, 68–77 (2018).
141. Harrington, L. E. et al. Interleukin 17-producing CD4^{+} effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).
142. Taams, L. S. Interleukin-17 in rheumatoid arthritis: trials and tribulations. *J. Exp. Med* **217**, e20192048 (2020).
143. van Hamburg, J. P. et al. Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum.* **63**, 73–83 (2011).
144. Kim, K. W., Kim, H. R., Kim, B. M., Cho, M. L. & Lee, S. H. Th17 cytokines regulate osteoclastogenesis in rheumatoid arthritis. *Am. J. Pathol.* **185**, 3011–3024 (2015).
145. Niu, Q., Cai, B., Huang, Z. C., Shi, Y. Y. & Wang, L. L. Disturbed Th17/Treg balance in patients with rheumatoid arthritis. *Rheumatol. Int.* **32**, 2731–2736 (2012).
146. Wang, W. et al. The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. *Rheumatol. Int.* **32**, 887–893 (2012).
147. Zielinski, C. E. et al. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. *Nature* **484**, 514–518 (2012).
148. Antebi, Y. E. et al. Mapping differentiation under mixed culture conditions reveals a tunable continuum of T cell fates. *PLoS Biol.* **11**, e1001616 (2013).
149. Zielinski, C. E. T helper cell subsets: diversification of the field. *Eur. J. Immunol.* **53**, e2250218 (2023).
150. DuPage, M. & Bluestone, J. A. Harnessing the plasticity of CD4^{+} T cells to treat immune-mediated disease. *Nat. Rev. Immunol.* **16**, 149–163 (2016).
151. Xu, T. et al. Metabolic control of T_H17 and induced T_{reg} cell balance by an epigenetic mechanism. *Nature* **548**, 228–233 (2017).
152. Komatsu, N. et al. Pathogenic conversion of Foxp3^{+} T cells into T_H17 cells in autoimmune arthritis. *Nat. Med.* **20**, 62–68 (2014).
153. Ziegler-Heitbrock, L. et al. Nomenclature of monocytes and dendritic cells in blood. *Blood* **116**, e74–e80 (2010).
154. Ziegler-Heitbrock, L. & Hofer, T. P. J. Toward a refined definition of monocyte subsets. *Front. Immunol.* **4**, 23 (2013).
155. Rossol, M., Kraus, S., Pierer, M., Baerwald, C. & Wagner, U. The CD14^{bright}CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. *Arthritis Rheum.* **64**, 671–677 (2012).
156. Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J. & Hill, A. M. M-1/M-2 macrophages and the Th1/Th2 paradigm1. *J. Immunol.* **164**, 6166–6173 (2000).
157. Zhu, W. et al. Anti-citrullinated protein antibodies induce macrophage subset disequilibrium in RA patients. *Inflammation* **38**, 2067–2075 (2015).
158. Murray, P. J. et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41**, 14–20 (2014).
159. Nahrendorf, M. & Swirski, F. K. Abandoning M1/M2 for a network model of macrophage function. *Circ. Res.* **119**, 414 (2016).
160. Martinez, F. O. & Gordon, S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep.* **6**, 13 (2014).
161. Ambarus, C. A., Noordenbos, T., de Hair, M. J., Tak, P. P. & Baeten, D. L. Intimal lining layer macrophages but not synovial sublining macrophages display an IL-10 polarized-like phenotype in chronic synovitis. *Arthritis Res. Ther.* **14**, R74 (2012).
162. Mulder, K. et al. Cross-tissue single-cell landscape of human monocytes and macrophages in health and disease. *Immunity* **54**, 1883–1900.e5 (2021).
163. Kaul, A. et al. Systemic lupus erythematosus. *Nat. Rev. Dis. Prim.* **2**, 16039 (2016).
164. Odendahl, M. et al. Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J. Immunol.* **165**, 5970–5979 (2000).
165. Jacobi, A. M. et al. Correlation between circulating CD27^{high} plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* **48**, 1332–1342 (2003).
166. Wei, C. et al. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J. Immunol.* **178**, 6624–6633 (2007).
167. Jacobi, A. M. et al. Activated memory B cell subsets correlate with disease activity in systemic lupus erythematosus: delineation by expression of CD27, IgD, and CD95. *Arthritis Rheum.* **58**, 1762–1773 (2008).

Acknowledgements
The authors acknowledge the support received from Versus Arthritis (grant 21754) and the NIHR Manchester Biomedical Research Centre. The views expressed are those of the authors and not necessarily those of the NIHR. L.M. is funded by the Medical Research Council (MRC) through the MRC Doctoral Training Partnership (grant MR/W007428/1).

Author contributions
All authors contributed to all aspects of the preparation of this manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Rheumatology* thanks the anonymous reviewers for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01259-0

Review article

Check for updates

Fibroblasts in immune responses, inflammatory diseases and therapeutic implications

Angela E. Zou[1,3], Suppawat Kongthong[1,3], Alisa A. Mueller[1,2,4] & Michael B. Brenner[1,4]

Abstract

Once regarded as passive bystander cells of the tissue stroma, fibroblasts have emerged as active orchestrators of tissue homeostasis and disease. From regulating immunity and controlling tissue remodelling to governing cell growth and differentiation, fibroblasts assume myriad roles in guiding normal tissue development, maintenance and repair. By comparison, in chronic inflammatory diseases such as rheumatoid arthritis, fibroblasts recruit and sustain inflammatory leukocytes, become dominant producers of pro-inflammatory factors and catalyse tissue destruction. In other disease contexts, fibroblasts promote fibrosis and impair host control of cancer. Single-cell studies have uncovered striking transcriptional and functional heterogeneity exhibited by fibroblasts in both normal tissues and diseased tissues. In particular, advances in the understanding of fibroblast pathology in rheumatoid arthritis have shed light on pathogenic fibroblast states in other chronic diseases. The differentiation and activation of these fibroblast states is driven by diverse physical and chemical cues within the tissue microenvironment and by cell-intrinsic signalling and epigenetic mechanisms. These insights into fibroblast behaviour and regulation have illuminated therapeutic opportunities for the targeted deletion or modulation of pathogenic fibroblasts across many diseases.

Sections

[1]Division of Rheumatology, Inflammation and Immunity, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [2]Present address: Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA and Palo Alto Veterans Affairs Health Care System, Palo Alto, CA, USA. [3]These authors contributed equally: Angela E. Zou, Suppawat Kongthong. [4]These authors jointly supervised this work: Alisa A. Mueller and Michael B. Brenner. e-mail: mbrenner@bwh.harvard.edu

Key points

- Fibroblasts assume multifaceted roles in regulating immunity, guiding tissue architecture and remodelling and defining the functional organization of tissues.
- During disease, fibroblasts can acquire inflammatory, fibrogenic, tissue degradative or hyperplastic phenotypes that promote pathology and predominate in treatment resistance.
- Although long implicated in fibrotic disease, fibroblasts have emerged as important drivers and regulators of chronic inflammatory and malignant diseases.
- Single-cell profiling of diseased tissues has uncovered transcriptionally and functionally distinct fibroblast subpopulations, some of which are tissue specific or disease specific and others of which are shared across multiple tissues and diseases.
- Morphogen gradients, synergistic and autocrine cytokine signalling, adhesion molecules, mechanotransducers and epigenetic regulators are some of the key factors that promote the differentiation and activation of pathogenic fibroblast states.
- Insights into fibroblast pathogenicity are spurring the development of therapeutics to deplete, deactivate, neutralize or reprogram fibroblasts in fibrosis, cancer and chronic inflammatory diseases.

Introduction

Historically, fibroblasts were viewed as homogeneous, spindle-shaped cells that provided structural support to tissues[1]; however, the diverse phenotypes and functions of fibroblasts in both health and disease are becoming increasingly apparent. Although fibroblasts mostly originate from mesenchymal progenitors and typically express collagen, vimentin and platelet-derived growth factor receptor α (PDGFRα)[2–4], these markers are not expressed by all fibroblasts, nor do they fully distinguish fibroblasts from other cell types, such as epithelial, endothelial or haematopoietic cells[4–7]. Moreover, single-cell studies have revealed remarkable fibroblast heterogeneity across tissues, biological processes and disease states. Even within a single tissue, fibroblasts exhibit a range of fibroblast subsets and lineages, and they can readily change states in response to local cues.

During homeostasis, fibroblasts regulate immune responses, remodel tissues, define cellular niches within tissues and undergo controlled proliferation and differentiation. However, when dysregulated, fibroblasts can perpetuate tissue inflammation and damage to drive chronic inflammatory and fibrotic diseases and support cancer growth, metastasis and immune evasion. Critically, current molecular and immunological therapies fail to effectively target disease-associated fibroblasts. Pathogenic fibroblasts have now been linked to treatment resistance and poor prognosis across a range of diseases, including solid tumours, organ fibrosis, inflammatory bowel disease (IBD) and rheumatoid arthritis (RA)[8–13]. Thus, there is a major need to develop fibroblast-targeting therapeutic strategies. Although molecules such as fibroblast activation protein-α (FAPα) and cadherin-11 (CDH11) have been proposed as targetable markers of pathogenic fibroblasts[14,15], these molecules are also expressed at low levels during homeostasis and by other cell types[16–18], which adds to the challenge of devising safe, efficacious fibroblast-directed therapies.

In this Review, we describe the normal and pathogenic roles of fibroblasts, focusing on RA as a disease in which fibroblasts have been studied in detail and extending our discussion to other inflammatory diseases. RA is a chronic autoimmune disease marked by inflammation of the joint synovium, bone and cartilage destruction and progressive disability[19,20] (Box 1). DMARDs, including biologics such as TNF inhibitors, have transformed RA treatment; however, these therapies show limited efficacy. 50–60% of patients with RA on first-line DMARDs fail to meet treatment targets (≥70% reduction in disease activity, also referred to as ACR70, after 6 months of treatment)[20]. Moreover, among individuals who respond inadequately to any given DMARD, only 10–15% respond to a subsequent line of therapy, and 15–25% of all patients remain refractory to any therapeutic[20,21]. Strikingly, in the synovial tissue of treatment-refractory patients with RA, fibroblasts are the most highly enriched cell population[12,13]. Although healthy synovial fibroblasts structurally define the normal synovium and provide stability and lubrication to the joint[22,23], during RA these fibroblasts expand dramatically and acquire both pathogenic pro-inflammatory behaviours and tissue-invasive behaviours that enable them to drive chronic synovial inflammation and bone and cartilage destruction[19]. No RA therapies specifically target these pathogenic fibroblasts.

Here, we provide an overview of fibroblast biology that illustrates how homeostatic fibroblast functions are subverted during inflammatory diseases, fibrosis and cancer. We synthesize insights from single-cell studies to describe synovial fibroblast populations and cell states in RA. We describe how fibroblasts in RA compare with fibroblast states in other inflammatory contexts and examine the key mechanisms that drive fibroblast differentiation, activation and pathogenic behaviour. Finally, we discuss how these insights pave the way for therapeutic targeting of fibroblasts in RA and other diseases.

The roles of fibroblasts in health and inflammatory disease

In this section, we describe the prominent roles that fibroblasts assume in controlling immune responses, remodelling tissues, organizing niches within tissues and regulating their own proliferation and differentiation. We discuss how these functions are dysregulated in pathogenic fibroblasts that drive RA and other chronic inflammatory diseases, fibrosis and cancer.

Fibroblast-mediated immune regulation

To mount an effective immune response, antigens and immune cells must converge in specialized compartments within the lymph node, spleen and other secondary lymphoid tissues. Lymphoid tissue fibroblasts are frontline orchestrators of the complex cellular processes required for adaptive immunity. Understanding the roles that these fibroblasts assume in organizing lymphoid structures and regulating immune cells will provide insights into how pathogenic fibroblasts promote autoimmune and inflammatory diseases. Numerous lymphoid tissue fibroblast subpopulations, known as fibroblastic reticular cells (FRCs), have now been identified. Each FRC subpopulation is defined by its distinct location, marker expression and immunological function, as reviewed elsewhere[24–28]; here, we highlight a few prominent examples in the lymph node (Fig. 1a).

During embryonic development, FRC precursors regulate the formation and organization of the lymph node, a process highly dependent on lymphotoxin signalling[27,28]. As afferent lymph enters the mature

Box 1 | The synovium in health and rheumatoid arthritis

The synovium is a soft connective tissue that lines the inner surface of joints, acting as a physical and chemical barrier that protects the joint from mechanical and biological stressors[22,23,162]. Resident fibroblasts organize the synovium into a lining and sublining layer. The lining, which is located at the synovial surface, consists of a compact layer of lining fibroblasts and macrophages (which is 1–2 cells thick)[22]. Lining fibroblasts secrete hyaluronan and lubricin, which are key components of the synovial joint fluid that lubricates and minimizes friction along the joint space[22,162]. By contrast, the sublining is sparsely populated by fibroblasts, macrophages, adipocytes, T cells and B cells, which are loosely embedded within the tissue extracellular matrix[22,23,162,248].

In rheumatoid arthritis (RA), the synovium transforms into a highly inflamed and destructive tissue. The synovial lining expands to >10 cells in thickness and forms an invasive pannus that drives cartilage and bone destruction[22,162]. The sublining becomes even more hyperplastic and neovascularized than the lining and is characterized by a substantial infiltration of activated leukocytes, including monocytes, dendritic cells, T cells and B cells, which can organize into lymphoid aggregates and tertiary lymphoid structures[22,249].

RA synovitis arises from a complex interplay between genetic predisposition and environmental factors. Variants in the *HLA-DR* gene locus, along with exposures such as smoking and specific infections, are strongly linked to increased RA risk[19,250]. These factors are posited to predispose individuals to the development of autoreactive immune responses, particularly against post-translationally modified proteins such as citrullinated proteins[19]. Anti-citrullinated protein antibodies and pro-inflammatory cytokines are detectable prior to arthritis onset in a majority of patients with RA[19,251].

Subsequently, a 'second hit' that triggers symptomatic inflammation specifically within the joint synovium seems to be required[19,252]. In response to local pro-inflammatory stimuli, activated synovial fibroblasts and macrophages secrete a range of cytokines and chemokines to promote the influx of pathogenic leukocytes[22,249]. Synovia-infiltrating T cell populations are essential to disease progression, including $CD4^+$ and $CD8^+$ T cells, which recognize citrullinated proteins[253,254], peripheral $CD4^+$ helper T cells that facilitate local B cell proliferation and differentiation[255] and granzyme K-expressing $CD8^+$ T cells that activate the complement cascade[256]. Meanwhile, oligoclonally expanded B cells drive continued epitope spreading over the disease course[257]. Beyond these characteristic features, synovial architecture, cellular composition and transcriptomic profiles can vary substantially in patients with RA[13,105], suggesting that distinct routes of disease pathogenesis, progression and therapeutic intervention must be considered for each patient.

lymph node, it encounters a reticular meshwork formed by specialized FRCs known as marginal reticular cells, which capture and transport soluble antigens from the lymph to the B cell follicles[29,30]. Meanwhile, through chemokine secretion, other FRC subsets organize the trafficking, recruitment, compartmentalization and survival of diverse leukocyte populations throughout the lymph node. FRCs within the B cell follicles, termed follicular dendritic cells (FDCs), produce CXCL13 to attract CXCR5-expressing B cells and support their survival and proliferation within follicles through secretion of BAFF and CXCL12 (refs. 18,31). FDCs also capture soluble antigens that are delivered to the follicle by marginal reticular cells, present them to B cells using Fc receptors (such as CD32), and form and maintain germinal centres to help to generate affinity-matured plasma cells and memory B cells[32,33]. FRCs in the T cell zone, termed T cell zone reticular cells (TRCs), recruit CCR7-expressing naive T cells through the production of CCL19 and CCL21 and promote their survival by secreting IL-7 (refs. 18,34). TRCs also facilitate interactions between T cells and dendritic cells (DCs), expressing molecules such as podoplanin (PDPN) that enhance the motility and trafficking of DCs to the T cell zone via activation of C-type lectin receptor 2 (ref. 35).

In addition to activating immune responses, FRCs can suppress and resolve immune responses. For instance, TRCs can present self-antigens via MHC class II molecules to $CD4^+$ T cells to promote tolerance[18,36–39]. TRCs also produce immunosuppressive molecules, such as nitric oxide, that limit T cell expansion and priming by DCs once a pathogen or insult has been contained[40,41]. Thus, in health, FRCs successfully balance both the initiation and the resolution of adaptive immune responses.

Although not as comprehensively studied, under homeostatic conditions, fibroblasts in barrier tissues such as the gut and lung also exhibit antigen presentation and immunoregulatory properties that promote tolerance[42,43]. Notably, fibroblasts in the healthy synovial lining express the complement pathway inhibitor CD55 (also known as decay-accelerating factor[22]), which suggests a role in maintaining immunological quiescence in the synovium.

By contrast, during autoimmune diseases such as RA, systemic lupus erythematosus (SLE) and IBD, FRCs in the lymphoid tissues contribute to the breakdown of tolerance and generation of autoreactive T cell and B cell responses[44–46]. Lymph nodes from patients with autoimmunity are frequently enlarged, with prominent FDC-driven follicular hyperplasia and autoantibody production in activated germinal centres[47–50]. Additionally, fibroblasts in diseased peripheral tissues also become pathologically activated, adopting inflammatory, FRC-like states in response to stimuli such as TNF, IL-17, IL-1β, interferons and oncostatin M[51–53]. These inflammatory fibroblasts upregulate FAPα and produce substantial quantities of cytokines and chemokines, notably IL-6, IL-33, CXCL8, CXCL12, CCL19 and CCL21, to recruit an array of both innate and adaptive leukocytes to the site of inflammation[13,52–55] (Fig. 1a). Inflammatory fibroblasts also promote leukocyte activation and persistence within affected tissues and can even organize leukocytes into tertiary lymphoid structures (TLSs) that heavily resemble lymph nodes in structure and function. Additionally, compared with their homeostatic counterparts, inflammatory fibroblasts can exhibit substantially elevated expression of MHC class II molecules, which suggests an increased ability to present antigens to lymphocytes[56,57]. However, unlike FRCs, which initiate acute, inherently self-limited immune responses, fibroblasts in inflammatory diseases can engage in autocrine signalling through pro-inflammatory cytokine pathways[51,58] to drive a chronic inflammatory state marked by sustained leukocyte recruitment and activation. In tumours, similar populations of cancer-associated fibroblasts (CAFs) have also been observed. Among the identified CAF states, inflammatory CAFs influence immune cell trafficking and organization in tumours in ways analogous to lymph node FRCs[59]. Meanwhile, antigen-presenting CAFs, which express MHC

class II molecules, can either stimulate or inhibit antitumour immune responses depending on the tumour or tissue of origin[60–62].

Fibroblast-mediated tissue remodelling

Fibroblasts synthesize a wide array of extracellular matrix (ECM) molecules, including collagens, proteoglycans, laminins, glycoproteins and elastins, which provide structural scaffolding to tissues[63]. They actively modify ECM structure and composition through regulated production of lysyl oxidases, matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs) and MMP inhibitors that enable ECM crosslinking, glycosylation and proteolysis[63,64]. Fibroblasts also express a variety of integrins, cadherins, mechanoreceptors and cytoskeletal molecules, which enables them to sense mechanical cues from the ECM and neighbouring cells and also directly exert contractile force on the ECM[65–67]. Collectively, this balance of matrix sensing, synthesis and pruning by fibroblasts enables them to construct compositionally distinct ECM environments that are uniquely tuned to support specific anatomical, functional and biomechanical requirements across tissues (Fig. 1b).

In the synovium, production of lubricin (proteoglycan 4) and hyaluronan by fibroblasts is integral to proper joint movement and lubrication[68]. In the lymph nodes, FRC remodelling activities are similarly indispensable for constructing the conduits that transport immune cells and antigens and also serve as cellular scaffolding for leukocytes to migrate and interact[18,69]. Additionally, FRCs form adjacent connections with neighbouring FRCs through CDH11 and synthesize a combination of collagen type I and collagen type XIV, which enable mechanical regulation of channel diameter and tensile strength within the lymph node[18]. Importantly, during infection, FRCs promote relaxation and expansion of the lymph node architecture to support the ingress and clonal expansion of immune cells[35,70].

During tissue injury, fibroblasts mechanically sense local disruption to the ECM and become activated by cytokines (such as TGF-β and IL-11), leading to their differentiation into myofibroblasts, which are key mediators of tissue repair[67,71]. Myofibroblasts are marked by high expression of contractile proteins, including α-smooth muscle actin, and also deposit considerable quantities of ECM, which enables them to restore mechanical strength to the tissue[71]. Although myofibroblasts are transient in the context of acute injury and repair, activated myofibroblasts can persist during chronic injury and inflammation, producing excessive and disorganized ECM that disrupts normal tissue architecture and function and drives scarring and fibrosis[72,73] (Fig. 1b). The heart, lung, liver, colon and skin are common sites of fibrosis following chronic inflammation or injury[73]. Additionally, in cancers, a myofibroblast-like CAF population emerges in response to TGF-β activation and can suppress antitumour immunity by impeding the infiltration of immune cells into the tumour parenchyma[9,59].

By contrast, in RA and other inflammatory diseases, fibroblasts can acquire adverse ECM degradative properties (Fig. 1b). After stimulation by pro-inflammatory cytokines, such as TNF and IL-1β, synovial fibroblasts in RA produce excessive levels of MMPs and ADAMs and upregulate CDH11, which enables them to degrade the surrounding ECM and directly invade and destroy articular cartilage and bone[15,19,74]. Additionally, RA synovial fibroblasts highly express receptor activator of nuclear factor-κB ligand (RANKL), which activates nearby osteoclasts that resorb calcium and erode bone[19,75].

Fibroblast-mediated organization of tissue niches

Beyond producing ECM molecules, fibroblasts are also highly specialized in their ability to secrete molecules such as growth factors, cytokines and morphogens. As these factors are produced, their distribution and bioactivity are further modified by the ECM. This enables fibroblasts to signal to neighbouring cells in varied and elaborate ways and form unique niches that support key cell types and tissue functions (Fig. 2a). For instance, through spatially restricted secretion of CXCL12, CXCL13, CCL19 and CCL21, FRCs establish distinct B cell follicles and T cell zones in the lymph node[18,31,34]. In the gut, fibroblasts help to define the key Wnt and BMP signalling gradients that guide epithelial renewal and differentiation along the intestinal crypt to the villus[76–78]. At the crypt base, PDGFRA^{low} fibroblasts support the proliferation of nearby intestinal stem cells through secretion of canonical Wnt ligands, activators of Wnt signalling and inhibitors of BMP signalling, whereas at the villus, PDGFRA^{high} fibroblasts support intestinal epithelial cell differentiation through the production of BMP ligands and non-canonical Wnt ligands. In the skin, dermal fibroblasts that signal through the Hedgehog pathway secrete the TGF-β ligand SCUBE3 to activate hair follicles from resting to growth states[79]. Finally, in the synovium, fibroblasts expressing Notch receptors (such as NOTCH3) engage with neighbouring endothelial cells that express Notch ligands to establish perivascular niches that regulate local fibroblast differentiation[80].

Fibroblasts form specialized niches not only within a specific tissue but also across different anatomical regions. On the basis of their site of origin, dermal and synovial fibroblasts express distinct homeobox (HOX) transcription factors that direct site-specific cell differentiation and confer positional identity to the skin and joints[81,82]. Notably, HOXA13 is specifically expressed by fibroblasts in distal regions, such as the digits, and can induce the expression of Wnt ligands and other morphogens that are critical for the activation and maintenance of distal limb development and patterning[81,83].

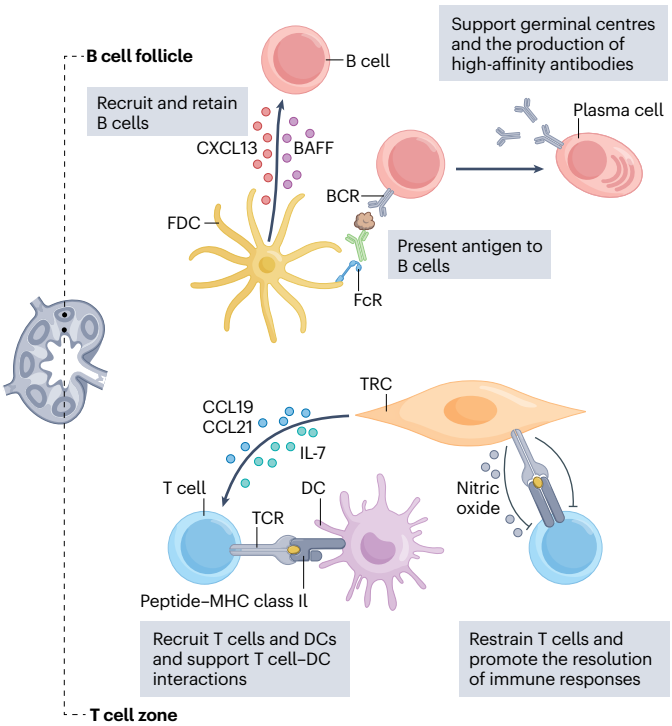
In tissues that are targeted by inflammatory and autoimmune diseases, such as the synovium in RA and the salivary glands in Sjögren syndrome, aberrantly activated inflammatory PDPN^{+}FAPα^{+} fibroblasts establish lymphoid aggregates and TLSs[84–86] (Fig. 2a). Much akin to the developing lymph node, TLSs are formed through lymphotoxin signalling by the local fibroblasts, as well as IL-13 and IL-22 derived from both stromal and immune cells[86]. TLSs resemble the lymph node in both structure and organization, with distinct T cell and B cell compartments, germinal centres and FDCs that promote the localized activation and persistence of autoreactive lymphocytes[87,88]. Meanwhile, through disordered production of ECM molecules and remodelling enzymes, pathogenic tissue remodelling fibroblasts can instigate the formation of an invasive pannus that degrades surrounding tissues, as is observed in RA[19,89], or drive the formation of fibroblastic foci and fibrotic zones as seen in fibrotic diseases[90]. Similarly, in cancer, inflammatory CAFs organize tumour-associated TLSs[91,92], whereas myofibroblast-like CAFs often perpetuate an immune cell-excluded tumour microenvironment (referred to as a 'immune desert'), which leads to the suppression of antitumour immune responses[8,9,59].

Regulation of fibroblast growth and differentiation

Fibroblast self-renewal, differentiation and proliferation are tightly controlled to preserve homeostatic tissue structure and function (Fig. 2b). Although FRCs rapidly proliferate at the onset of an immune response to facilitate lymph node expansion, they also readily contract in number as immune cells egress and the immune response resolves[35,93]. Similarly, the initial stages of tissue injury trigger fibroblast proliferation and conversion into myofibroblasts, but once tissue ECM and structural integrity are restored, myofibroblasts wane in number and can dedifferentiate into quiescent fibroblasts and adipocytes[94–96]. In healthy

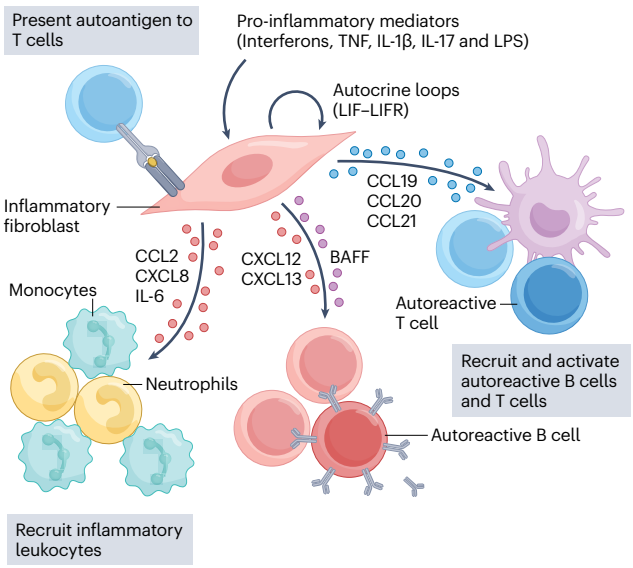
a

Adaptive immunity
Lymph node fibroblastic reticular cells



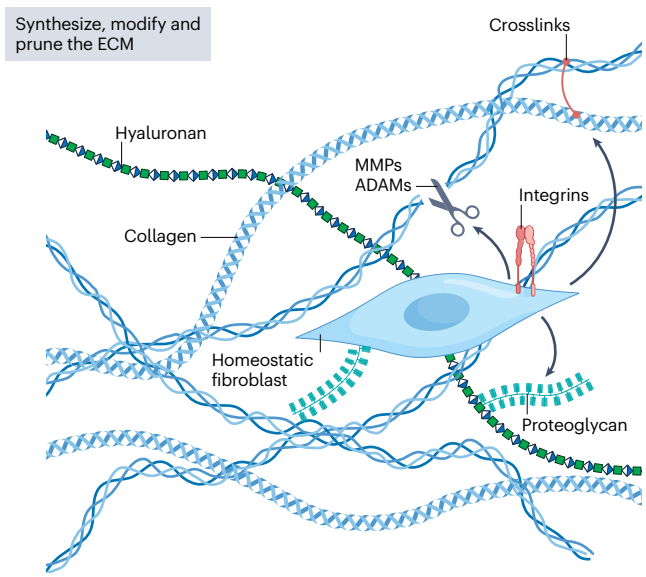
Chronic tissue inflamation
Inflammatory fibroblasts

Affected end-organs (such as the gut and joint)

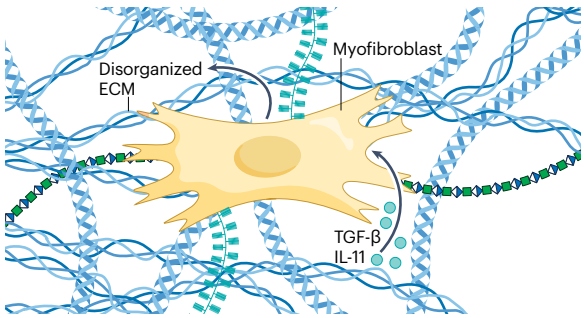


b

Balanced ECM remodelling
Homeostasis



Excessive ECM deposition
Fibrosis



Excessive ECM destruction
Invasion

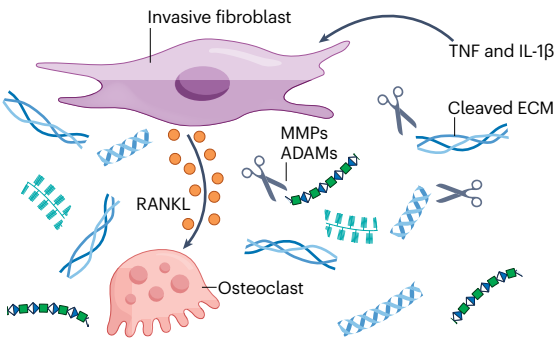


Fig. 1 | Fibroblasts regulate immunity and tissue remodelling. a, Left: orchestration of adaptive immunity by lymph node fibroblastic reticular cells. In the B cell follicle, follicular dendritic cells (FDCs) recruit B cells and support their activation, retention and maturation. In the T cell zone, T cell zone reticular cells (TRCs) enable T cell and dendritic cell (DC) recruitment and subsequent T cell–DC interactions. In addition, TRCs can suppress activated T cells at the conclusion of an immune response. Right: aberrant inflammatory signalling and leukocyte recruitment by peripheral tissue fibroblasts during chronic inflammation. Pathogenically activated fibroblasts are sustained by paracrine and autocrine pro-inflammatory stimuli, and these cells secrete an array of chemokines and cytokines to recruit, activate and retain inflammatory leukocytes in the diseased tissue. **b**, Left: coordinated extracellular matrix (ECM) production, modification and degradation by homeostatic fibroblasts (blue) to generate and maintain normal tissue structure and architecture. Right: fibroblasts can promote dysregulated ECM remodelling during disease. In tissue fibrosis, myofibroblasts (yellow) produce excessive ECM that is disorganized. In rheumatoid arthritis (RA), invasive fibroblasts (purple) cause ECM destruction and tissue degradation. ADAMs, a disintegrin and metalloproteinases; BCR, B cell receptor; LIF, leukaemia inhibitory factor; LIFR, LIF receptor; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; RANKL, receptor activator of nuclear factor-κB ligand.

synovium, lining and sparse sublining fibroblasts exist in tight equilibrium with other stromal and immune populations to maintain joint mechanics and immune homeostasis[22,97]; however, during chronic inflammatory diseases, pathogenic fibroblasts expand and persist, with emergence and enrichment of inflammatory subpopulations (Fig. 2b). For example, RA is marked by prominent hyperplasia of the synovial lining fibroblast layer, which expands from 1–2 cells to >10 cells in thickness, with sublining fibroblasts undergoing even greater expansion[53,98]. Fibrosis is characterized by the persistence of myofibroblasts (Fig. 2b), whereas fibrogenic or desmoplastic processes in tumours are driven by myofibroblast-like CAFs[59,73].

Collectively, through these prominent roles in immune regulation, tissue remodelling, niche formation, and growth and differentiation, fibroblasts have emerged as key contributors to both tissue homeostasis and disease.

Fibroblast populations in rheumatoid arthritis and other chronic inflammatory diseases

Synovial fibroblasts have historically been classified according to anatomical location in the lining or sublining[22,97]. Single-cell studies have greatly refined understanding of synovial fibroblast diversity and function. Here, we describe how sublining and lining fibroblasts differ not only anatomically but also transcriptionally and functionally. We discuss new layers of heterogeneity in the sublining fibroblast compartment and highlight studies that link synovial fibroblast populations to treatment-refractory RA. Finally, we describe parallels between RA synovial fibroblasts and pathogenic fibroblast states in other chronic inflammatory diseases.

Healthy and disease-associated synovial fibroblast subsets

Initial single-cell studies of synovial fibroblasts identified subpopulations that were pre-sorted by the surface expression of selected protein markers. In a study of human RA, synovial fibroblasts were sorted on the basis of THY1, PDPN, CDH11 and CD34 expression[98]. Sublining fibroblasts, which were defined as $THY1^{+}PDPN^{+}CDH11^{+}CD34^{-}$, were the main fibroblast population expanded in RA compared with osteoarthritis (OA)[98]. In transcriptomic and in vitro studies, this population exhibited enhanced cytokine secretion, proliferation and invasiveness, which suggests an active role in driving disease[98]. By contrast, $THY1^{-}$ fibroblasts, which represent lining fibroblasts, were expanded more in OA than in RA[98].

In a mouse experimental arthritis model, two fibroblast subsets defined by their expression of FAPα and THY1 were examined for their respective contributions to arthritis pathology[99]. $FAP\alpha^{+}THY1^{+}$ cells were mostly sublining fibroblasts and $FAP\alpha^{+}THY1^{-}$ cells were mostly lining fibroblasts. Adoptive transfer of sublining fibroblasts worsened the inflammatory severity of arthritis by increasing joint swelling and leukocyte infiltration, whereas adoptive transfer of lining fibroblasts promoted enhanced degradative disease by driving cartilage and bone joint damage[99]. These findings suggest that rather than simultaneously mediating both synovial inflammation and joint degradation, at least in mice, the lining and sublining fibroblasts predominantly assume distinct pathogenic roles.

Through large-scale single-cell studies of synovial biopsies from patients with RA and OA, consortium efforts from the Accelerating Medicines Partnership RA and SLE Network have substantially advanced the identification and functional annotation of synovial fibroblast subpopulations[13,53,100] (Fig. 3). An initial study of 1,844 sorted synovial fibroblasts identified a $CD55^{+}THY1^{-}$ lining fibroblast cluster, as well as three clusters of $THY1^{+}$ sublining fibroblasts that express HLA-DRA, CD34 and DKK3, respectively[53]. $HLA\text{-}DRA^{hi}$ sublining fibroblasts represent the main highly inflammatory, expanded fibroblast population in RA[53]. These fibroblasts account for nearly all of the IL-6 produced by fibroblasts in active RA, are dominant producers of CXCL12 and other chemokines and express high levels of CD74 (also known as the invariant chain), which functions as a chaperone for MHC class II molecules[53].

A second study by the AMP RA and SLE Network, which analysed 79,555 stromal cells from RA and OA synovial tissue, revealed additional synovial fibroblast heterogeneity by identifying two lining and seven sublining synovial fibroblast populations[13]. Notably, two sublining fibroblast populations, including the aforementioned $HLA\text{-}DRA^{hi}CD74^{hi}$ inflammatory cluster and a distinct $CXCL12^{+}SFRP1^{+}$ inflammatory cluster, are characterized by high IL-6 and CXCL12 production[13]. Meanwhile, the lining fibroblasts are enriched for matrix-degradative MMPs including MMP1 and MMP3 (ref. 13). These studies collectively suggest that inflammatory sublining fibroblasts dominate in RA and implicate lining fibroblasts in driving tissue destruction.

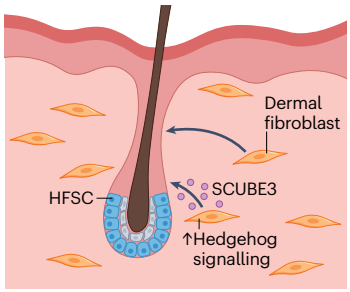
Insights have also been made into the functional roles of other sublining fibroblast populations and their relevance to RA pathogenesis. A $CD34^{+}$ sublining fibroblast subset, identified in both of the AMP RA and SLE Network studies[13,53], is expanded in RA and expresses high levels of *PI16* and *DPP4* (ref. 13). These markers define a population of fibroblasts found to be universally present across tissues[101]. Through lineage tracing studies in mice, these 'universal' fibroblasts have been proposed to function as a progenitor cell population[101]. Thus, $CD34^{+}$ synovial fibroblasts might serve as progenitors to other fibroblast subsets in the synovium.

The AMP RA and SLE Network studies also identified $NOTCH3^{+}$ perivascular and sublining fibroblast populations[13]. Organoid co-cultures revealed that the differentiation of these $NOTCH3^{+}$ fibroblasts was driven by endothelial cell-derived Notch ligands[80]. Importantly, genetic deletion or pharmacological blockade of NOTCH3 reduced inflammatory disease in a mouse model of inflammatory

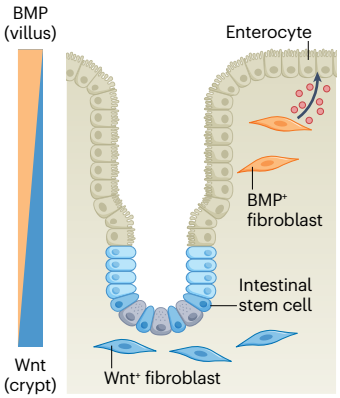
a

Homeostatic niche organization

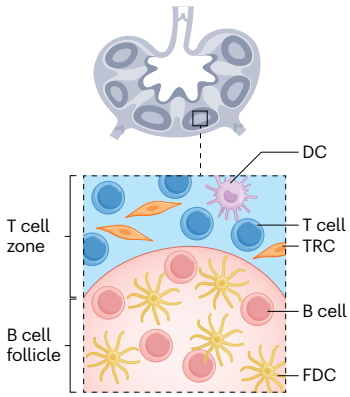
Hair follicle stem cell niche



Intestinal villus–crypt axis

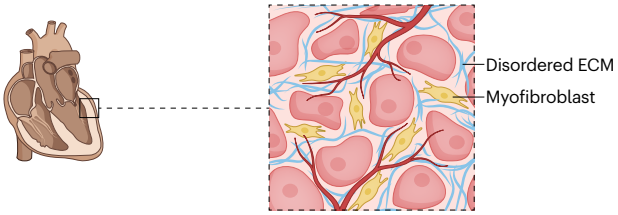


Lymph node T cell and B cell zones

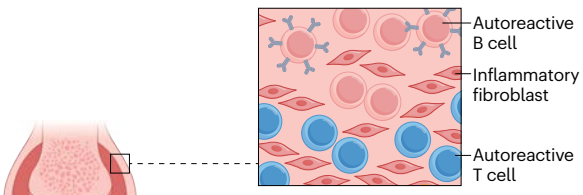


Dysregulated niche organization

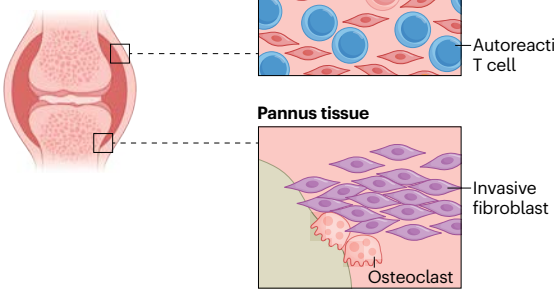
Fibrotic zone



Lymphoid aggregates



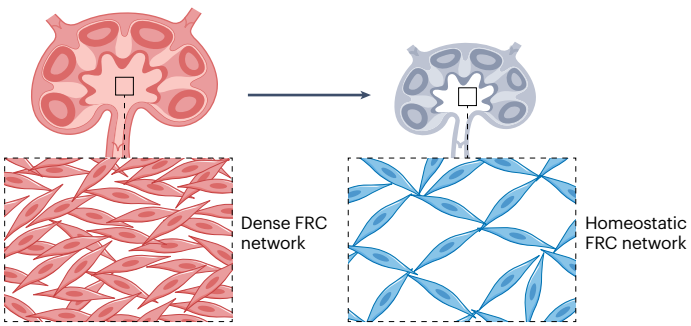
Pannus tissue



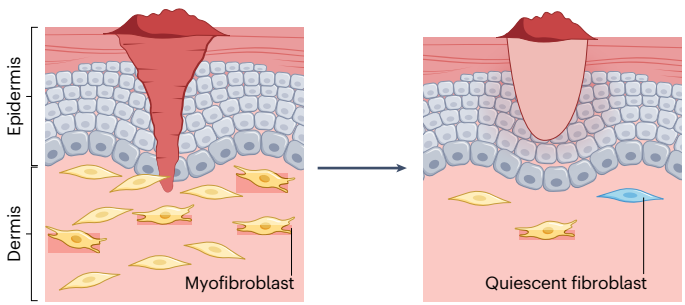
b

Homeostatic self-regulation

Resolving immune response
Lymph node fibroblast contraction



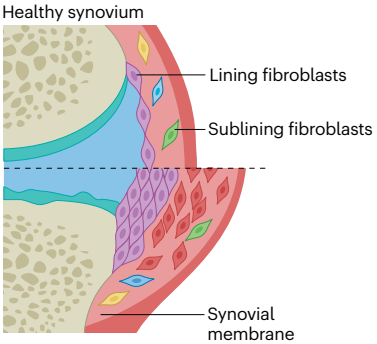
Wound healing
Myofibroblast dedifferentiation



Abnormal self-regulation

RA
Fibroblast hyperplasia

Healthy synovium



RA synovium (hyperplastic)

Fibrosis
Myofibroblast persistence

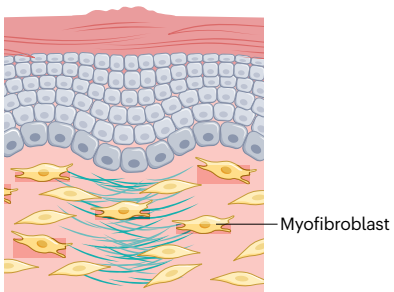


Fig. 2 | Fibroblasts organize tissue niches and undergo regulated proliferation. a, Left: fibroblasts organize cellular niches to ensure proper tissue form and function. Dermal fibroblasts regulate hair follicle stem cell (HFSC) activation (top). Intestinal fibroblasts at the crypt base and villus secrete a gradient of Wnt and BMP molecules to support intestinal stem-cell differentiation (bottom left). Lymph node fibroblastic reticular cells (FRCs) demarcate distinct T cell and B cell zones that guide the compartmentalized activation of B cell- and T cell-driven immune responses (bottom right). Right: pathogenic fibroblasts create dysfunctional cellular niches that promote disease. Persistently activated cardiac myofibroblasts create fibrotic tissue that disrupts myocardial function (top). In rheumatoid arthritis (RA), inflammatory fibroblasts organize lymphoid aggregates and tertiary lymphoid structures, sustaining autoreactive lymphocyte activity in the synovium (middle), whereas invasive fibroblasts organize pannus tissues that directly invade and destroy surrounding cartilage and bone (bottom). **b**, Left: fibroblasts undergo regulated proliferation and activation to support tissue homeostasis. Following an immune response, FRCs decrease in number to restore the lymph node to its homeostatic state (top). Myofibroblasts reduce in number and dedifferentiate to support the conclusion of successful wound healing (bottom). Right: pathogenic fibroblasts exhibit dysregulated proliferation and differentiation in disease; for example, synovial fibroblasts in RA become hyperplastic (top) and myofibroblasts persist and accumulate in fibrosis (bottom). BMP, bone morphogenetic protein; ECM, extracellular matrix; FDC, follicular dendritic cell; SCUBE3, signal peptide-CUB-EGF domain-containing protein 3; TRC, T cell zone reticular cell.

arthritis[80]. These studies highlight the importance of Notch signalling in determining fibroblast state and the potential relevance of targeting Notch signalling in RA.

The Wnt pathway has also been implicated in synovial fibroblast heterogeneity and pathology. In the AMP RA and SLE Network studies, two synovial fibroblast clusters derived from the sublining and intermediate lining–sublining regions were distinguished by the expression of the Wnt signalling regulators DKK3 and RSPO3, respectively[13]. *Dkk3*$^{+}$*Thy1*$^{+}$*Lrrc15*$^{+}$ fibroblasts are highly expanded in a mouse model of inflammatory arthritis and exhibit transcriptional signatures associated with inflammation and ECM remodelling[102]. Another study analysing human RA synovial tissue (available as a preprint at the time of this writing) revealed that the vast majority of Wnt receptors, ligands and regulators are expressed specifically in synovial fibroblasts compared with other cell types in the synovium, with *DKK3* and *RSPO3* defining a broad transcriptional gradient that extends across many synovial fibroblast populations[103]. These findings implicate synovial fibroblasts as the predominant cell population that engages in Wnt signalling during RA and suggest that Wnt activation drives a prominent axis of fibroblast heterogeneity. Strikingly, in mouse models of arthritis, injection of Wnt ligands worsened arthritis whereas pharmacological Wnt inhibition ameliorated arthritis[103], consistent with prior studies in which genetic deletion of Wnt5a in mice blunted arthritis severity[104]. Collectively, these studies demonstrate that Notch and Wnt signalling promote fibroblast differentiation and inflammatory activation, respectively, in inflammatory arthritis.

In RA, the proportions and types of fibroblasts vary across distinct synovial inflammatory phenotypes and correlate with response to therapy. Notably, RA synovia that exhibit a pauci-immune or fibroid pathotype, which is marked by histological enrichment of fibroblasts, are linked to higher rates of non-response to TNF inhibitors[105]. Furthermore, in a clinical trial (R4RA) comparing B cell depletion therapy (via rituximab) versus IL-6 receptor blockade therapy (via tocilizumab) in TNF inhibitor-refractory RA, bulk transcriptomic analyses revealed a predominance of fibroblast-derived genes in the synovial tissues of patients who failed to respond to all agents[12]. Deconvolution studies suggested a specific enrichment of DKK3^{+} fibroblasts in non-responders[12]. Meanwhile, work by the AMP RA and SLE Network has stratified RA synovial tissues into cell-type abundance phenotypes (CTAPs), each defined by the most abundant cell types or combinations of cell types enriched in synovial tissue[13]. For example, synovia enriched in fibroblasts, particularly lining fibroblasts and CD34^{+} sublining fibroblasts, are termed CTAP-F, whereas synovia enriched in T cells and B cells are designated CTAP-TB. The authors also applied CTAP designations to patient samples from the R4RA trial, and consistent with prior findings, synovial tissues from individuals refractory to anti-TNF therapy, rituximab and tocilizumab were significantly associated with CTAP-F, further linking synovial fibroblast enrichment to treatment resistance[13].

Not only do fibroblast subsets drive RA pathogenesis but they might also facilitate resolution of inflammation and disease remission (Fig. 3). In healthy human synovium, synovial fibroblasts, specifically, *APOD*$^{+}$ and *PLIN2*$^{+}$ populations, exhibit enhanced lipid metabolism and cortisol signalling signatures that diminish during disease[106]. Inhibition of cortisol signalling in mice through fibroblast-specific deletion of *Nr3c1* (which encodes the glucocorticoid receptor) worsened inflammatory arthritis[106]. In both humans and mice, CD200^{+}DKK3^{+} fibroblasts seem to be enriched during the resolving phase of arthritis, as well as after TNF inhibitor or IL-17 inhibitor treatment[107]. Expression of CD200 by these synovial fibroblasts might promote the activities of pro-resolving type 2 innate lymphoid cells, thereby fostering inflammatory resolution[107].

Fibroblast states shared across tissues and diseases

Cross-tissue and cross-disease fibroblast atlases in both humans and mice have generated further insights into fibroblast populations that are shared across multiple tissues and diseases. A study of fibroblast populations across four distinct diseases (RA, ulcerative colitis, Sjögren syndrome and interstitial lung disease) identified five fibroblast populations shared across tissues, with two specific populations shared across inflammatory states[108]. The first shared population associated with inflammation, CCL19^{+}CXCL10^{+} fibroblasts, most resembles the HLA-DRA^{hi} sublining fibroblasts described in RA. These cells directly interact with leukocytes through cytokine and chemokine signalling, antigen presentation and localization in leukocyte-rich cellular niches. The second shared inflammation-associated population, SPARC^{+}COL3A1^{+} fibroblasts, most resembles the THY^{hi}NOTCH3^{+} sublining fibroblast population identified in RA. These fibroblasts have a perivascular location, engage in crosstalk with endothelial cells and signal through Notch, Wnt, BMP and other developmental morphogens. Another cross-tissue analysis that focused on steady-state tissues in the mice identified two universal fibroblast populations, including the aforementioned *Pi16*$^{+}$ population, which adopts a progenitor-like phenotype, and a *Col15*$^{+}$ population, which secretes basement membrane proteins[101]. Extension of these analyses to human tissues revealed a universal human fibroblast population enriched for genes that define the murine *Pi16*$^{+}$ and *Col15*$^{+}$ populations, suggesting cross-species similarities[101]. The discovery of these shared populations indicates that there could be common programs of fibroblast differentiation and pathogenic inflammatory activation across diseases.

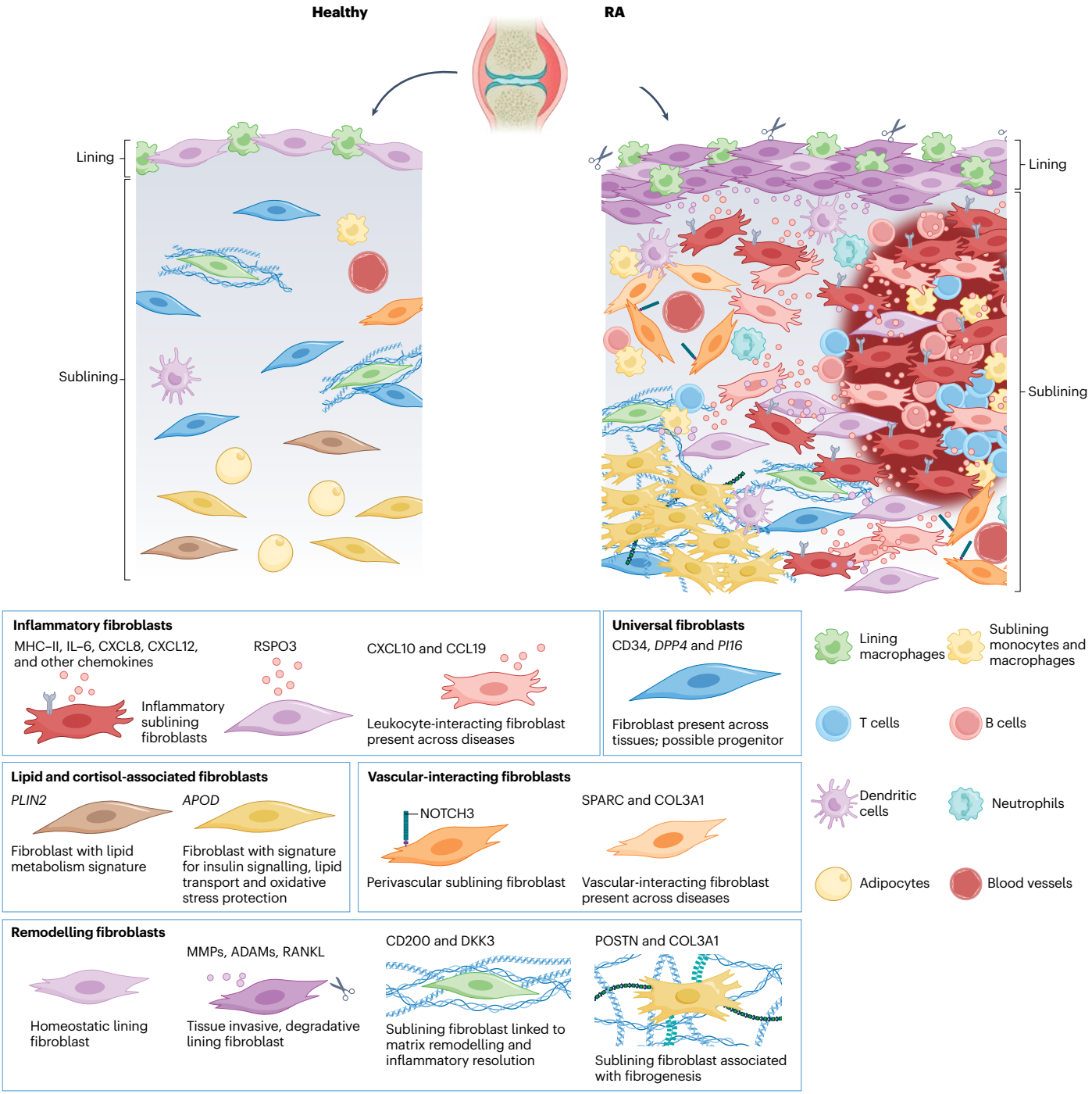


Fig. 3 | Fibroblast populations in rheumatoid arthritis and chronic inflammatory disease. In the healthy synovium (left), fibroblasts and synovial tissue-resident macrophages form a thin lining layer that produces hyaluronan and lubricin. In the sublining, scant fibroblasts produce extracellular matrix (ECM) and intermix with vascular structures and adipocytes. During RA (right), both lining and sublining fibroblasts become hyperplastic and adopt pathogenic activation states. Lining fibroblasts acquire tissue destructive and invasive abilities, whereas in the sublining, there is an emergence of inflammatory fibroblasts, ECM remodelling fibroblasts and an expansion of vascular-interacting fibroblasts. Inflammatory fibroblasts and vascular-interacting fibroblasts promote immune-cell infiltration into the synovium and can organize lymphoid aggregates and tertiary lymphoid structures. ECM remodelling fibroblasts are associated with both resolution of inflammation and synovial tissue fibrogenesis. ADAMs, a disintegrin and metalloproteinases; MMPs, matrix metalloproteinases; RANKL, receptor activator of nuclear factor-κB ligand.

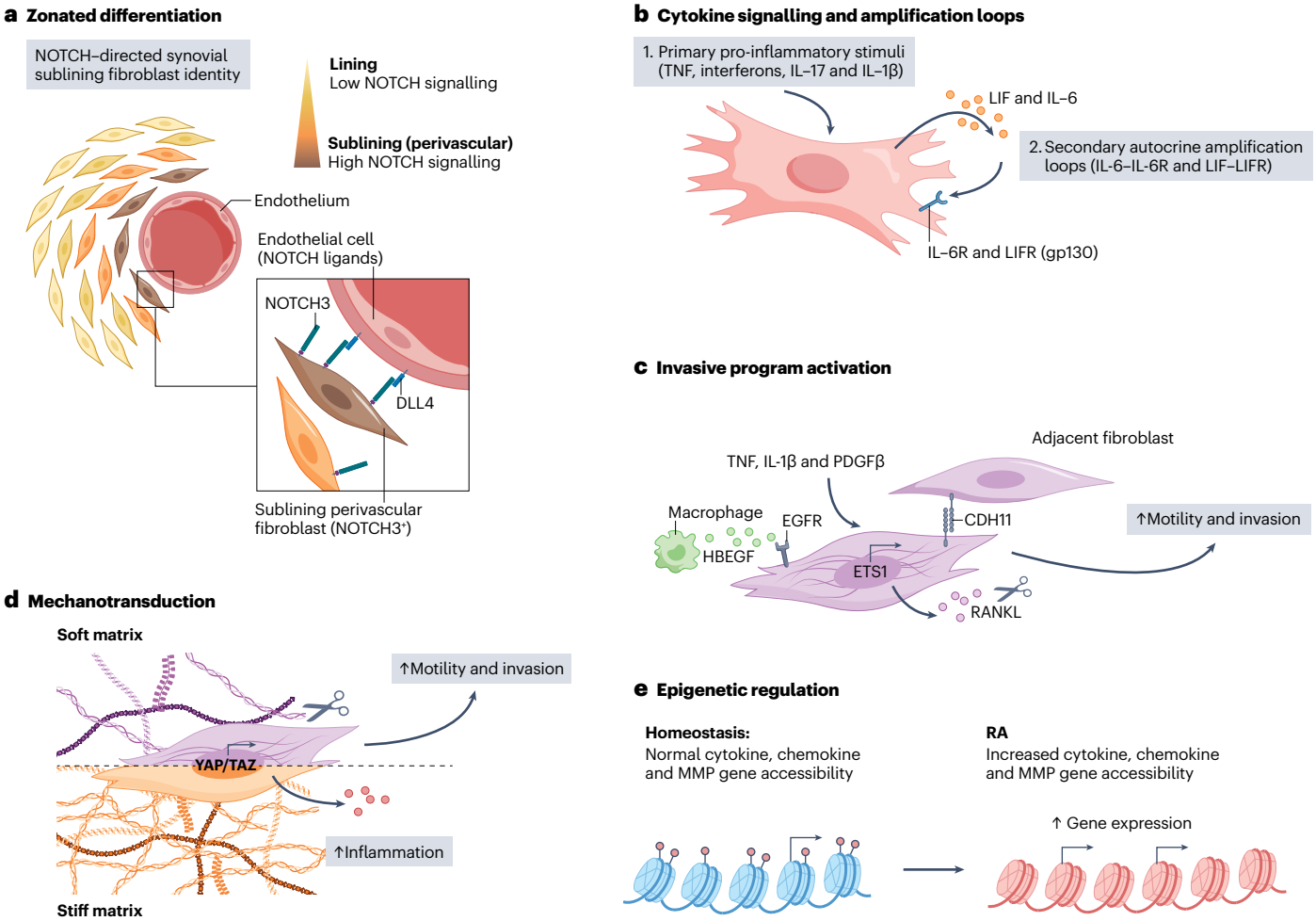


Fig. 4 | Mechanisms driving fibroblast heterogeneity and pathogenicity. **a**, Morphogens establish signalling gradients that are instrumental in regulating fibroblast positional identity; for example, the sublining synovial fibroblast state is driven by Notch signalling via interactions with neighbouring endothelial cells. **b**, Establishment of a chronic inflammatory fibroblast state occurs via a two-step process: primary pro-inflammatory stimuli activate the synovial fibroblast to produce IL-6 and LIF, which then signal in an autocrine fashion through IL-6R and LIFR to sustain and amplify the initial fibroblast inflammatory response. **c**, Synovial fibroblast-mediated tissue invasion and joint destruction can be activated through soluble stimuli, including macrophage-derived HBEGF, signalling through mesenchymal cadherins, including cadherin-11 (CDH11), and transcriptional programs, including ETS1-driven activation of RANKL. **d**, Through mechanosensing of local tissue microenvironments, fibroblasts respond dynamically to substrate stiffness by modulating inflammatory and invasive behaviours. **e**, In rheumatoid arthritis (RA) and other chronic inflammatory diseases, fibroblasts acquire epigenetic changes, including DNA hypomethylation and activating histone marks that increase the accessibility of pro-inflammatory and invasive gene loci. RANKL, receptor activator of nuclear factor-κB ligand; LIF, leukaemia inhibitory factor; LIFR, LIF receptor; HBEGF, heparin binding EGF-like growth factor; PDGFβ, platelet-derived growth factor β.

Mechanisms driving fibroblast heterogeneity and pathogenicity

In this section, we review mechanisms that promote fibroblast differentiation and pathogenicity. We discuss how the microanatomical zonation of fibroblasts regulates their differentiation, and how their subsequent activation and effector function can be dynamically shaped by exogenous stimuli and imprinted epigenetically.

Zonated differentiation of fibroblasts

The markedly distinct phenotypes of synovial lining and sublining fibroblasts suggests that their differentiation and function are regulated by their spatial zonation or positioning within the synovium. Indeed, in the sublining, fibroblast differentiation is directed by endothelial cells, which establish a positional gradient of Notch signalling in fibroblasts that extends outwards from perivascular zones[80] (Fig. 4a). Transcriptional analyses suggest that this Notch- and morphogen-driven fibroblast-endothelial crosstalk is conserved across other tissues and diseases and involves $SPARC^{+}COL3A1^{+}$ fibroblasts[108]. Meanwhile, a study of healthy human synovium found that the homeostatic synovial fibroblast state is maintained by cortisol derived from adipocytes immediately underlying the synovial sublining layer, a phenotype that is lost during inflammatory arthritis[106]. Collectively, the findings from these studies suggest that fibroblasts activated by Notch and other signalling pathways might form transcriptional gradients that correspond

to differential degrees of pathway activation, rather than discrete clusters or cell subsets. These gradients might correlate with the anatomical locations of fibroblasts within the tissue and their proximity to cues derived from neighbouring cell types. Similarly, it is tempting to speculate that in the lining, interactions between fibroblasts and nearby tissue-resident macrophages, along with their regulation by factors within synovial fluid, could be essential in driving the lining fibroblast phenotype.

Spatial transcriptomics technologies that enable transcriptome-level quantification of gene expression in intact tissues now have the potential to unlock major insights into fibroblast zonation, differentiation and specialization[109]. Initial spatial transcriptomics-based studies of tissues such as kidney[110,111], lung[112,113] and gut[114,115] have identified new cellular populations, niches and cell–cell interactions that are pivotal not only in tissue development and homeostasis but also in disease pathogenesis and progression. Similarly, spatial transcriptomics studies of the RA synovium (some of which are available as preprints at the time of this writing[116–118]) have begun to expand understanding of the cellular composition and spatial features of TLSs and perivascular niches[116–121]. Notably, sublining immune-interacting ITGA5^{+} fibroblasts and perivascular COMP^{hi} fibroblasts are proposed to drive local hubs of TGF-β signalling in the synovium that might be implicated in treatment-refractory disease[118,121]. Large-scale studies that are now underway, including efforts by the AMP Autoimmune and Immune-Mediated Disease Network, will provide additional insight into fibroblast heterogeneity and pathology in the full spatial context of the synovial tissue microenvironment.

Pathogenic signalling and amplification pathways in fibroblasts

Fibroblasts sense and respond to a range of cues, including cytokines, Toll-like receptor agonists, growth and differentiation factors, cell–cell contact and mechanical forces. In RA, synovial fibroblasts are activated by cytokines that include TNF, IL-17, interferons and IL-1β[51,54] (Fig. 4b). Notably, IFN-γ from infiltrating T cells and NK cells promotes the upregulation of MHC class II molecules and chemokines, which are highly expressed by the inflammatory HLA-DRA^{hi} fibroblast population that is expanded in the synovium in RA[120,122]. By comparison, TNF and IL-1β from T cells and myeloid cells can potently induce IL-6 and CXCL8 in inflammatory fibroblasts[51,120]. Fibroblasts also become highly activated after stimulation by endogenous Toll-like receptor ligands, such as heat shock proteins, fibrinogen and fibronectin extra domain A, which are enriched in inflamed tissues[123–125]. Finally, beyond classical pro-inflammatory stimuli, a study (available as a preprint at the time of this writing) reported the surprising role of Wnt proteins in driving pro-inflammatory cytokine and chemokine production by synovial fibroblasts[103], suggesting that molecules such as morphogens and growth factors might have additional functions in driving inflammatory fibroblast pathology.

In inflamed tissues, fibroblasts often encounter multiple stimulatory factors in combination, which together amplify the inflammatory response. Notably, IL-17 coupled with TNF or IL-1β stimulation synergistically enhances the production of IL-6 and other pro-inflammatory factors by fibroblasts[126,127]. In synovial fibroblasts, inflammatory gene induction by TNF and IL-17 co-stimulation is mediated by the transcriptional regulators cut-like homeobox 1 and IκBζ[128]. IL-17 also enhances the mRNA stability of pro-inflammatory cytokines and chemokines[129]. Similarly, in IBD, oncostatin M signals in concert with TNF and other cytokines to enhance, either additively or synergistically, the production of IL-6, CCL2, CXCL9 and other pro-inflammatory factors by fibroblasts[52].

The type of pathogenic fibroblast response depends on the nature of the cytokine stimulation. In particular, differences in the stimuli that promote inflammatory versus invasive fibroblast behaviours have emerged. For example, whereas IFN-γ, TNF and IL-1β have been extensively implicated in the inflammatory activation of fibroblasts, only TNF and IL-1β upregulate the production of MMPs and other ECM degradative factors that specifically mediate fibroblast invasiveness[130,131]. By comparison, TGF-β and IL-11 exert primarily fibrogenic effects on fibroblasts[72,132]. In addition to cytokines, growth factors such as PDGFs and epidermal growth factors (EGFs) can promote fibroblast migration and invasion. Notably, in the RA synovium, macrophages that secrete heparin-binding EGF-like growth factor activate EGF receptor signalling in synovial fibroblasts, which enhances the invasiveness of these fibroblasts but only minimally alters their inflammatory behaviour[133]. Differences in the transcriptional regulation of inflammatory versus invasive gene programs could also be important; notably, the transcription factor ETS1 promotes synovial fibroblast-mediated invasiveness and RANKL production but not chronic inflammation[134] (Fig. 4c).

Following primary activation, fibroblasts can initiate secondary autocrine cytokine signalling loops that powerfully sustain and augment the initial stimulus (Fig. 4b). An autocrine amplification loop mediated by leukaemia inhibitory factor (LIF) and its receptor (LIFR) promotes the prolonged upregulation of IL-6, CXCL8, IL-11 and other pro-inflammatory factors in fibroblasts. After primary stimulation, fibroblasts readily produce LIF, which in turn activates LIFR-expressing fibroblasts and engages STAT4 signalling to considerably amplify pro-inflammatory cytokine and chemokine transcription in fibroblasts[51]. The gp130 subunit for the LIF receptor is also the key signalling transducer for other IL-6 family receptors, including IL-6 and IL-11, both of which can signal in an autocrine fashion in activated fibroblasts as well. Notably, a positive feedback loop of IL-6–IL-6R signalling in fibroblasts contributes to arthritis and experimental autoimmune encephalitis in mice[58]. Meanwhile, autocrine LIF–LIFR and IL-11–IL-11RA signalling in TGFβ-stimulated fibroblasts sustains fibrogenic protein synthesis and promotes myofibroblast differentiation in cardiac and pulmonary fibrosis models[132,135].

Activated pathogenic fibroblasts also exhibit altered expression of non-coding RNAs such as microRNAs. These small RNA molecules further modify gene regulation in cells by catalysing the post-transcriptional silencing and degradation of target mRNAs[136]. Several microRNAs in synovial fibroblasts are directly induced by TNF or IL-1β[137–140] and can target matrix-degrading MMP genes[138,140] or factors such as TRAF6 and A20 (encoded by *TNFAIP3*), which regulate cytokine signalling pathways[137,141]. The wide range of microRNAs that are dysregulated in fibroblasts in RA and other inflammatory, fibrotic and malignant diseases has been reviewed comprehensively elsewhere[142–145]; however, the precise roles of many of these microRNAs in fibroblast pathology and disease pathogenesis remain unclear.

Beyond chemical cues, a range of physical cues from cell-surface receptors, the ECM and mechanotransducers combine to shape the activation and effector responses of fibroblasts (as reviewed in depth elsewhere[67,146,147]). Notably, although mesenchymal cadherins are conventionally thought to control cell adhesion and tissue morphogenesis and structure, studies have shown that they also regulate the pathogenic inflammatory and tissue remodelling behaviours of fibroblasts (Fig. 4c). CDH11 has been identified as a particularly important mediator of homotypic fibroblast-to-fibroblast

adhesion[65]; CDH11-deficient fibroblasts failed to organize a lining layer, indicating a key role for CDH11 in synovial morphogenesis during development[148]. Additionally, CDH11 is highly upregulated on RA synovial fibroblasts by pro-inflammatory stimuli, such as TNF, and promotes both their invasiveness[15,148] and their production of IL-6 and other pro-inflammatory cytokines[149,150]. In mouse models of inflammatory arthritis, synovial inflammation and cartilage damage were reduced in *Cdh11*-deficient mice or mice treated with an anti-CDH11 antibody[148]. Beyond regulating synovial fibroblast inflammation and invasion, CDH11 has also been implicated in skin, pulmonary and cardiac fibrosis; critically, CDH11 deficiency or blockade abolishes fibrosis[151–153].

The migratory and joint degradative behaviours of synovial fibroblasts are also potentiated by cell-surface receptor-type protein tyrosine phosphatase α (RPTPα), which activates focal adhesion kinase (FAK) and Src kinase signalling and in turn promotes cell motility and invasion[154,155]. In addition, fibroblasts continually respond to physical stiffness and mechanical cues within local tissue environments through mechanosensing via the Hippo pathway and its transcriptional effectors, YAP and TAZ (Fig. 4d). The motility and invasiveness of fibroblasts are generally enhanced on soft substrates compared with stiff substrates[156,157], whereas inflammatory and fibrogenic behaviours are increased on stiff substrates[158,159]. In a mouse model of inflammatory arthritis, synovial fibroblasts exposed to repeated mechanical loading expressed augmented levels of CXCL1 and CCL2 and promoted exacerbated disease[160]. These findings directly implicate mechanostimulation as a cause of inflammatory fibroblast activation.

Epigenetic regulation of pathogenic fibroblasts

Although fibroblast identity and function are heavily shaped by the local microenvironment, key aspects of their inflammatory and invasive behaviour persist both in ex vivo culture and in implantation and adoptive transfer experiments[99,161–163]. Moreover, mouse studies suggest that synovial fibroblasts can migrate from the inflamed synovium to spread disease to uninvolved joints[164], and in human studies, pre-inflammatory mesenchymal cells have been observed in the peripheral blood of patients with RA directly preceding disease flares[165]. These observations imply that beyond exogenous stimuli, cell-intrinsic mechanisms, such as epigenetic dysregulation, also contribute prominently to the development and maintenance of pathogenic fibroblast behaviours. Indeed, in RA synovial fibroblasts, a number of genes involved in inflammatory, invasive and differentiation pathways have now been found to be epigenetically altered (Fig. 4e). Targeted DNA methylation studies of synovial fibroblasts from individuals with RA compared with those from patients with OA identified hypomethylated loci in multiple cytokine signalling genes, including *IL6*, *CXCL12*, *STAT3*, *IL6R* and *CD74* (refs. 166–169). Meanwhile, *IL6*, *CXCL8* and several MMP genes also have altered histone modification profiles in RA-derived versus OA-derived synovial fibroblasts, including increased levels of activating histone marks (such as histone H3 trimethylation at lysine 4 and histone H3 acetylation at lysine 27) and decreased levels of repressive marks (such as histone H3 trimethylation at lysine 27)[170–172]. Collectively, these epigenetic changes have been proposed to confer an 'inflammatory memory' to RA synovial fibroblasts, priming them to exhibit amplified or prolonged expression of pro-inflammatory cytokines, chemokines and ECM remodelling factors after activation[171,173].

Chronic or repeated exposure to pro-inflammatory stimuli seems to be an important factor that promotes fibroblast priming[173,174]. Specifically, the complement component C3, which is upregulated in inflammatory fibroblasts, was shown to prime mouse synovial fibroblasts by increasing the chromatin accessibility of IL-6 and other pro-inflammatory cytokines and by polarizing fibroblasts to a more glycolytic and mTOR-activated metabolic state[174]. The ability of fibroblasts to undergo epigenetic and metabolic priming by cell-intrinsic factors such as C3 could be a key mechanism that explains why RA frequently recurs at the same joints.

Advances in single-cell profiling have begun to facilitate a deeper understanding of how specific fibroblast states in RA are epigenetically regulated. Paired assessment of single-cell chromatin accessibility (ATAC sequencing) and transcriptomic profiles (RNA sequencing) of the RA synovium has highlighted distinct chromatin states between resting and activated fibroblast populations and also revealed the presence of three chromatin 'superstates' that encompass multiple fine-grained synovial fibroblast transcriptional states[120,175]. Notably, inflammatory HLA-DRA^{hi}CD74^{hi} and CXCL12^{+}SFRP1^{+} sublining fibroblast populations identified by the AMP RA and SLE Network seem to share a common CXCL12^{+}HLA-DRA^{hi} chromatin superstate marked by enhanced accessibility of *CD74*, *HLA-DR*, *IL-6* and *CXCL12* gene loci[175]. In addition, beyond characterizing epigenetic regulation of known disease-related genes, the advent of multi-omic approaches that integrate genome-wide DNA methylation, chromatin accessibility and histone modification studies in RA synovial fibroblasts has identified entirely new genes as regulators of fibroblast pathology, including *HIP1*, which drives fibroblast invasiveness[176].

Many of these described epigenetic changes arise in early RA, potentially even prior to clinically detectable disease[177,178]. Moreover, these changes also differ by joint location[82,179,180]; for example, hip- and knee-derived synovial fibroblasts exhibit differential chromatin accessibility at *IL6*, and *JAK* and *STAT* genes that results in differential transcription of these factors and distinct sensitivity to Janus kinase (JAK) inhibitors[179,180]. Similar to dermal fibroblasts, which maintain highly anatomically compartmentalized HOX gene programs, synovial fibroblasts also exhibit joint-specific epigenetic regulation and expression of HOX genes that have been proposed to shape their functional development and pathogenic behaviours[82,179].

Fibroblasts as therapeutic targets

Historically, therapeutic targeting of pathogenic fibroblasts in fibrosis, cancer and inflammatory diseases has been hindered by a lack of drug targets that are both effective and safe. However, major insights into fibroblast heterogeneity and pathogenicity have expanded the repertoire of potential targets that are selectively expressed in pathogenic fibroblasts and reduced or absent in homeostatic fibroblasts. Furthermore, new therapeutic modalities such as chimeric antigen receptor T cells (CAR T cells) and T cell engagers (TCEs) could serve as innovative approaches to targeting pathogenic fibroblasts and modulating their biology. Here, we summarize the therapeutic landscape for targeting pathogenic fibroblasts, highlighting past clinical failures and successes and ongoing challenges in the development and translation of effective and safe therapies.

Depletion of pathogenic fibroblasts

Given the multifaceted roles of fibroblasts in disease, selective depletion of pathogenic fibroblasts or specific pathogenic fibroblast states is an attractive treatment strategy. Targeted cell depletion is traditionally achieved with monoclonal antibodies via antibody-dependent cellular cytotoxicity; however, immunoconjugates, which are monoclonal antibodies combined with drug payloads, such as cytotoxic compounds, small molecules or radioisotopes, have shown enhanced efficacy across

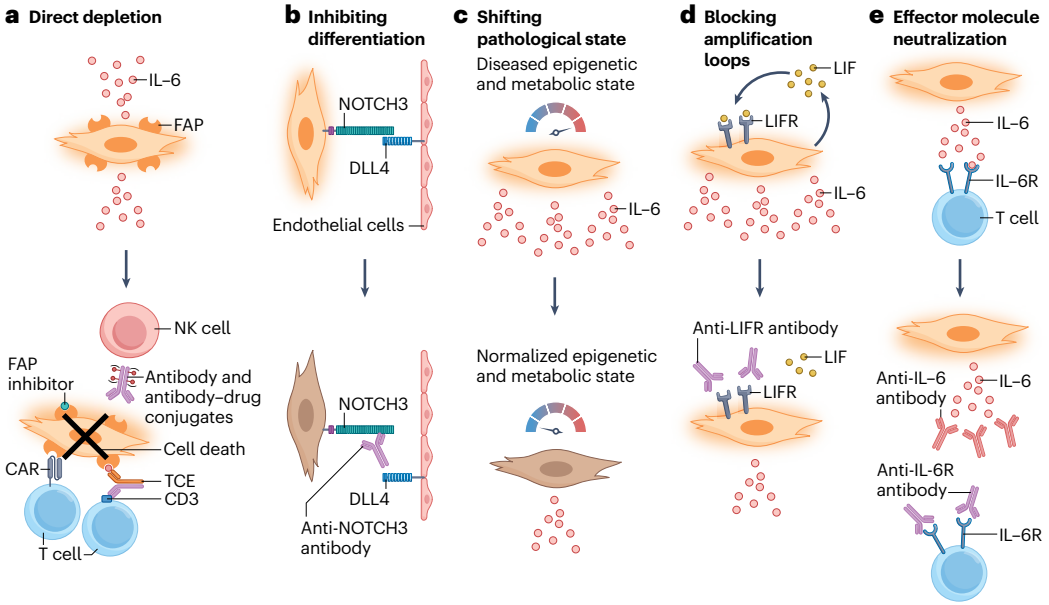


Fig. 5 | Fibroblast-targeting therapeutic strategies. a, Selective depletion of pathogenic fibroblast subsets via targeting of cell-surface activation markers such as fibroblast activation protein α (FAPα), either through antibody-mediated or through T cell-mediated depletion strategies. **b**, Targeting key pathways that drive fibroblast differentiation; for example, blocking NOTCH3 signalling to prevent the differentiation of inflammatory sublining fibroblasts during rheumatoid arthritis. **c**, Targeting the epigenetic or metabolic state of pathogenically activated fibroblasts. **d**, Blocking autocrine signalling pathways that amplify pathological fibroblast activation; for example, targeting autocrine LIF–LIFR signalling in fibroblasts, which augments and sustains their inflammatory responses. **e**, Targeting the activity of fibroblast-secreted factors, such as IL-6, to prevent fibroblast-mediated recruitment and activation of pathogenic leukocytes. CAR, chimeric antigen receptor; LIF, leukaemia inhibitory factor; LIFR, LIF receptor; NK, natural killer; TCE, T cell engager.

multiple cancers and autoimmune diseases[181]. Moreover, emerging modalities, such as CAR T cells and TCEs, can directly leverage T cell cytotoxicity for a deeper, more durable depletion of target pathogenic cells[182–184] (Fig. 5a). However, despite these therapeutic advances and an increased understanding of fibroblast biology, there are still no approved depletion strategies that specifically target fibroblasts. Selecting target molecules that are specific to pathogenic fibroblasts is critical to the success of fibroblast-depleting therapies.

FAPα, a type-II transmembrane protease, has been an appealing target owing to its low expression in healthy fibroblasts and broad upregulation by fibroblasts in conditions such as cancer, inflammatory diseases and fibrosis[14,185,186]. Initially developed to deplete CAFs in solid tumours, these FAPα-targeting strategies include unconjugated monoclonal antibodies, immunoconjugates, CAR T cells, vaccination and FAPα inhibitor–drug conjugates. Phase I–II clinical trials that assessed an anti-FAPα monoclonal antibody in cancer did not show efficacy, possibly owing to failure of the unconjugated monoclonal antibody to sufficiently induce cell death in CAFs[187]; however, newer approaches, such as CAR T cells, TCEs, vaccination and FAPI–drug conjugates, have demonstrated favourable results in cancer models and are now being explored in clinical trials (NCT04939610 and NCT01722149)[186,188–195]. On the basis of these promising findings, FAPα-based fibroblast depletion strategies are now being extended to disease indications beyond cancer. Notably, CAR T cells against FAPα could effectively treat cardiac fibrosis without clinical toxicity in mouse models[196,197]. Meanwhile, an anti-FAPα monoclonal antibody conjugated to IRDye-700DX, a photosensitizer that causes cell death after exposure to near-infrared light, reduced disease severity in a mouse model of arthritis[198]. As inflammatory fibroblasts, particularly subsets supporting TLS formation, express high levels of FAPα[13,86], targeting FAPα could represent an especially promising fibroblast depletion strategy to treat RA, Sjögren syndrome and other inflammatory conditions that are characterized by lymphoid aggregation and TLS persistence in target tissues. However, despite promising efficacy and tolerability, caution is warranted as FAPα is also expressed by healthy bone marrow stromal cells and other cell types, and systemic depletion of FAPα-expressing cells can cause cachexia and anaemia[16,17]. Additionally, as FAPα expression varies between patients, FAPα-targeting approaches might not be equally effective in all patients. Using molecular imaging techniques, such as FAPI tracers combined with PET imaging (known as FAPI PET), could help to identify specific patients with high fibroblast FAPα expression who would be more likely to benefit therapeutically from FAPα-based depletion[199].

In addition to FAPα, other potential cell-surface targets expressed by fibroblasts include THY1, PDPN, PDGFRA and CDH11 (refs. 4,18,65). Notably, CDH11 is highly upregulated by fibroblasts during inflammatory and fibrotic states, rendering it a promising target for direct depletion[15,148,149,151–153,200]; however, as CDH11 is also expressed by homeostatic lymph node FRCs[18], CDH11-based depletion strategies could also have undesirable adverse effects. Beyond thoughtful target selection and testing, the use of appropriate drug modalities that offer an acceptable risk–benefit ratio for patients is crucial (as reviewed extensively elsewhere[201–203]). For instance, although CAR T cell therapies can eliminate target cells more effectively than unconjugated monoclonal antibodies, they carry risks such as cytokine release syndrome and neurotoxicity[201–204]. In addition, prior to CAR T cell infusion, lymphodepletion must be performed. This process can affect fertility, posing a

tolerable risk for some patients with cancer but perhaps less acceptable for patients with autoimmunity, the majority of whom are women of childbearing age. To mitigate CAR T cell toxicities, preclinical studies have explored the use of transient CAR T cell therapeutic strategies, such as delivering lipid nanoparticles containing CAR constructs to T cells in vivo to transiently induce CAR expression and activity in T cells[197,205,206]. This approach not only bypasses the need for CAR T cell generation ex vivo but also reduces toxicity risks associated with long-term CAR T cell persistence in patients. As an alternative to CAR T cells, TCEs offer a promising, safer alternative with efficient depletion efficacy[203,207–209]. Additionally, natural killer (NK) cell-based therapies, such as CAR–NK cells or NK cell engagers, have emerged as potential alternatives to CAR T cells, as they are associated with a lower risk of cytokine release syndrome but still exhibit potent cytotoxicity[210].

Targeting the differentiation of pathogenic fibroblasts

As previously discussed, beyond transcriptomic insights into disease-associated fibroblasts, single-cell studies have also revealed pathways that potentiate the differentiation of fibroblasts into pathogenic subsets. Blocking pathogenic fibroblast differentiation thus represents another promising therapeutic strategy (Fig. 5b); for example, in mice, pharmacological inhibition of Notch signalling in fibroblasts with an anti-NOTCH3 monoclonal antibody ameliorated inflammatory fibroblast activation and arthritis severity[80]. As this perivascular fibroblast population (which expresses SPARC and COL3A1) is shared across multiple inflammatory diseases, targeting Notch signalling might be therapeutically beneficial beyond RA[108]. In fibrosis and cancer, several pathways, notably, TGF-β–SMAD signalling, can promote the differentiation of fibroblasts into myofibroblasts, as reviewed in detail elsewhere[8,9,211–213]. Blocking factors that promote myofibroblast differentiation might be a potential therapeutic strategy for these diseases; however, myofibroblast differentiation is biologically complex and mediated by multiple overlapping and redundant signalling mechanisms[72,73,211]. The successful therapeutic translation of preclinical fibrosis studies to patients has thus far been limited. Deeper analyses of omics-scale datasets could reveal more novel, promising therapeutic targets that can overcome these challenges.

Targeting pathogenic fibroblast state

Modulation of the dysregulated epigenetic and metabolic states of pathogenic fibroblasts has emerged as another potential therapeutic strategy (Fig. 5c). Targeting epigenetic regulators, such as the bromodomain histone-reading proteins, with I-BET151 suppresses the production of pro-inflammatory cytokines and MMPs by synovial fibroblasts in vitro[214]. Meanwhile, targeting DNA methylation curtails fibroblast invasiveness[215]. These strategies could extend to myofibroblasts and CAFs, as epigenetic dysregulation is also observed in these cells[216–218]. The metabolic regulation of fibroblasts is also altered in disease, presumably owing to the energetic and biosynthetic demands of sustained activation and the changes in nutrient and oxygen content in diseased tissues[219–225]. For example, in RA, fibroblasts depend on glycolysis, via glucose transporter 1 and hexokinase 2, and glutaminolysis, via glutaminase 1, and inhibiting these pathways can ameliorate arthritis in mouse models[226–230]. Additionally, targeting upstream pathways such as C3 signalling that potentiate both metabolic and epigenetic changes in synovial fibroblasts could be therapeutically beneficial[174]. Like inflammatory fibroblasts in RA, CAFs and myofibroblasts also exhibit increased glycolysis, and inhibition of glycolysis has shown therapeutic potential in mouse tumour and fibrosis models[231–234]. Unlike the prior approaches discussed, metabolic inhibition strategies can target shared pathways, such as increased glycolytic dependence, which are found in both pathogenic fibroblasts and immune cells; hence, this strategy could simultaneously dampen multiple disease-driving cell types. Tools such as Compass (which uses transcriptomic data to infer the metabolic states of cells) can deconvolute single-cell datasets to further identify and characterize shared pathogenic metabolic features[235].

Disruption of mediators that sustain fibroblast pathogenicity

A variety of external stimuli, including TNF, IFN-γ, IL-1β, IL-11 and TGF-β, can activate fibroblasts to drive inflammatory, degradative or fibrotic programs. Some current therapies work in part by blocking fibroblast activation, such as cytokine inhibitors (including TNF and IL-6 inhibitors) and signal transduction inhibitors (such as JAK inhibitors)[236,237]. However, as fibroblasts are highly responsive to many external cytokines, blocking any single activator will probably have only a partial effect. Mounting evidence shows that cytokine signalling induces autocrine amplification loops in fibroblasts, which heighten their pathogenic behaviours in autoimmune diseases, cancer and fibrosis. Targeting these autocrine amplification loops has the advantage of blocking fibroblast activation in response to multiple external activators (Fig. 5d). Notably, blocking the autonomous LIF–LIFR loop curtails fibroblast activation downstream of both inflammatory and

Glossary

Antibody-dependent cellular cytotoxicity
An immune response whereby antibodies tag a target cell, allowing immune cells, such as natural killer cells, to bind via their Fc receptors and release toxins to destroy the target cells.

Chimeric antigen receptor T cells
(CAR T cells). Genetically engineered T cells that are designed to recognize unique surface targets on diseased cells, which enables precise identification and elimination of target cells.

Cytokine release syndrome
A systemic inflammatory response caused by excessive cytokine release, which can be an adverse effect of CAR T cell therapy.

FAPα inhibitor–drug conjugates
(FAPI–drug conjugates). Small molecules or small peptides with an affinity for fibroblast activation protein α that are conjugated with drug payloads or with imaging agents such as radioisotopes.

Lymphodepletion
A chemotherapy or radiation-based regimen that reduces the number of circulating lymphocytes prior to CAR T cell and other adoptive cell therapies to improve the survival and persistence of the infused cells.

T cell engagers
(TCEs). Therapeutic molecules that simultaneously bind T cells via CD3 and target cells to more readily induce T cell-mediated cytotoxicity, using formats that range from small bispecific proteins (such as BiTEs) to larger immunoglobulin-like constructs.

Tolerance
A safeguarding property that prevents the immune system from mounting responses to self-derived antigens, which is achieved through mechanisms that inactivate or eliminate autoreactive T cells and B cells.

fibrogenic signals[51,135]. CDH11 engagement also amplifies fibroblast responses to inflammatory signals, and its blockade markedly reduces inflammatory, invasive and fibrotic fibroblast behaviour in in vitro co-culture and organoid models[15,148,149,151–153,200]. In a phase II clinical trial, however, a CDH11-blocking monoclonal antibody failed to show efficacy in patients with RA that was refractory to anti-TNF therapy[238]. Alternative CDH11-targeting therapeutic modalities or targeting of CDH11 in fibrosis could still be effective. Additionally, findings suggest that the Wnt pathway represents another promising therapeutic target for blocking fibroblast-driven synovial inflammation in RA[103]. Overall, the preclinical data suggest that targeting autocrine or paracrine amplifier loops downstream of multiple primary activators of fibroblasts could effectively inhibit pathogenic fibroblast effector functions without cell depletion. Further identification of factors that promote these amplification loops could be achieved using omics studies and analyses.

Neutralization of pathogenic fibroblast-derived effector molecules

The secretome of pathogenic fibroblasts includes mediators of tissue inflammation and ECM remodelling, such as cytokines, chemokines and MMPs. Targeting these soluble factors or their downstream pathways could have therapeutic potential (Fig. 5e). Indeed, inhibition of IL-6 signalling has revolutionized the treatment of multiple inflammatory diseases, including RA. Conceptually, this neutralization approach is straightforward, but the biology of these mediators is complex and highly tissue dependent or context dependent. For example, certain IL-6 family members (such as IL-6 and IL-11) are known to function via both *cis* and *trans* signalling pathways, each of which has a distinct role in maintaining homeostasis or perpetuating disease[239]. Non-selective blockade of both pathways could fail to achieve the desired therapeutic effect or could lead to adverse effects, such as the development of intestinal perforations with IL-6 inhibition in Crohn's disease[240–243]. These biological complexities are further demonstrated by the multiple failures to translate chemokine inhibition from animal studies into the clinic for inflammatory diseases, presumably owing to the functional redundancies between these chemokines[244–247]. Nevertheless, inhibition of the CXCL12–CXCR4 axis was efficacious in clinical cancer trials, highlighting an important, non-redundant role for fibroblast-derived CXCL12 in driving disease progression[247]. A more thorough understanding of these biological complexities is needed to identify both safe and therapeutically efficacious molecular targets.

Challenges and considerations

For any fibroblast-directed therapeutic approach, the major challenge will be to selectively deplete or modulate pathogenic fibroblasts but spare healthy fibroblasts. Moreover, it will be important to pinpoint the specific patients and disease contexts in which targeting fibroblasts provides the greatest therapeutic benefit. One approach would be to trial these therapies in patients with early-stage or established inflammatory disease, either alone or in combination with other therapies[11–13]. Alternatively, given the mounting evidence that fibroblasts predominate in treatment-refractory inflammatory diseases, fibroblast-targeting therapies could be particularly useful in patients who respond inadequately to standard leukocyte-targeting and immunosuppressive therapies.

Conclusions

Molecular and single-cell characterization of fibroblasts in diseased tissues has provided major insights into their heterogeneity and pathogenicity. Ongoing advances in single-cell technologies promise to yield an ever more refined view of pathogenic fibroblast states and cell–cell interactions. These data are shedding light on the mechanisms by which pathogenic fibroblasts arise through differentiation cues, aberrant signalling pathways and altered transcriptional and epigenetic regulation. Moreover, studies across multiple tissues and diseases have demonstrated that key pathogenic fibroblast subsets are shared, suggesting that approaches to depleting or modulating these fibroblast states could be beneficial across a range of disease indications.

Building on these findings, therapeutics that specifically target fibroblast deletion, activation or pathogenic behaviour show promise but are still in their nascency. Insights into pathogenic fibroblast activation and state have expanded the repertoire of potential therapeutic targets. Similarly, the development of innovative therapeutic modalities has greatly enhanced the efficiency and potency of fibroblast targeting. With these perspectives in mind, the field is primed, and emerging clinical trials across inflammatory, fibrotic and malignant diseases could be poised to reveal the next generation of effective, safe and durable fibroblast-targeting therapeutics.

Published online: 14 May 2025

References

1. Virchow, R. *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre: Pionierarbeit der Zellpathologie und Gewebelehre im 19. Jahrhundert*. (Good Press, 1858).
2. Dulbecco, R., Allen, R., Okada, S. & Bowman, M. Functional changes of intermediate filaments in fibroblastic cells revealed by a monoclonal antibody. *Proc. Natl Acad. Sci. USA* **80**, 1915–1918 (1983).
3. Chang, H. Y. et al. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc. Natl Acad. Sci. USA* **99**, 12877–12882 (2002).
4. Muhl, L. et al. Single-cell analysis uncovers fibroblast heterogeneity and criteria for fibroblast and mural cell identification and discrimination. *Nat. Commun.* **11**, 3953 (2020).
5. Mor-Vaknin, N., Punturieri, A., Sitwala, K. & Markovitz, D. M. Vimentin is secreted by activated macrophages. *Nat. Cell Biol.* **5**, 59–63 (2003).
6. Li, R. et al. Pdgfra marks a cellular lineage with distinct contributions to myofibroblasts in lung maturation and injury response. *eLife* **7**, e36865 (2018).
7. Chandrakanthan, V. et al. Mesoderm-derived PDGFRA$^+$ cells regulate the emergence of hematopoietic stem cells in the dorsal aorta. *Nat. Cell Biol.* **24**, 1211–1225 (2022).
8. Mariathasan, S. et al. TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* **554**, 544–548 (2018).
9. Krishnamurty, A. T. et al. LRRC15$^+$ myofibroblasts dictate the stromal setpoint to suppress tumour immunity. *Nature* **611**, 148 (2022).
10. Fan, D., Takawale, A., Lee, J. & Kassiri, Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenesis Tissue Repair* **5**, 15 (2012).
11. Friedrich, M. et al. IL-1-driven stromal-neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nat. Med.* **27**, 1970–1981 (2021).
12. Rivellese, F. et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. *Nat. Med.* **28**, 1256–1268 (2022).
13. Zhang, F. et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. *Nature* **623**, 616–624 (2023).
14. Kelly, T., Huang, Y., Simms, A. E. & Mazur, A. Fibroblast activation protein-α: a key modulator of the microenvironment in multiple pathologies. *Int. Rev. Cell Mol. Biol.* **297**, 83–116 (2012).
15. Kiener, H. P. et al. Cadherin-11 promotes invasive behavior of fibroblast-like synoviocytes. *Arthritis Rheum.* **60**, 1305–1310 (2009).
16. Roberts, E. W. et al. Depletion of stromal cells expressing fibroblast activation protein-α from skeletal muscle and bone marrow results in cachexia and anemia. *J. Exp. Med.* **210**, 1137–1151 (2013).
17. Tran, E. et al. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J. Exp. Med.* **210**, 1125–1135 (2013).
18. Malhotra, D. et al. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* **13**, 499–510 (2012).
19. Gravallese, E. M. & Firestein, G. S. Rheumatoid arthritis — common origins, divergent mechanisms. *N. Engl. J. Med.* **388**, 529–542 (2023).
20. Aletaha, D. & Smolen, J. S. Diagnosis and management of rheumatoid arthritis: a review. *JAMA* **320**, 1360–1372 (2018).
21. Smolen, J. S. & Aletaha, D. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. *Nat. Rev. Rheumatol.* **11**, 276–289 (2015).

22. Smith, M. D. The normal synovium. *Open Rheumatol. J.* **5**, 100–106 (2011).
23. Hochberg, M. C. et al. *Rheumatology* (Elsevier, 2023).
24. Fletcher, A. L., Acton, S. E. & Knoblich, K. Lymph node fibroblastic reticular cells in health and disease. *Nat. Rev. Immunol.* **15**, 350–361 (2015).
25. Perez-Shibayama, C., Gil-Cruz, C. & Ludewig, B. Fibroblastic reticular cells at the nexus of innate and adaptive immune responses. *Immunol. Rev.* **289**, 31–41 (2019).
26. Li, L., Wu, J., Abdi, R., Jewell, C. M. & Bromberg, J. S. Lymph node fibroblastic reticular cells steer immune responses. *Trends Immunol.* **42**, 723–734 (2021).
27. Onder, L. & Ludewig, B. A fresh view on lymph node organogenesis. *Trends Immunol.* **39**, 775–787 (2018).
28. van de Pavert, S. A. & Mebius, R. E. New insights into the development of lymphoid tissues. *Nat. Rev. Immunol.* **10**, 664–674 (2010).
29. Katakai, T. et al. Organizer-like reticular stromal cell layer common to adult secondary lymphoid organs. *J. Immunol.* **181**, 6189–6200 (2008).
30. Roozendaal, R. et al. Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. *Immunity* **30**, 264–276 (2009).
31. Cremasco, V. et al. B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. *Nat. Immunol.* **15**, 973–981 (2014).
32. Tew, J. G. et al. Follicular dendritic cells and presentation of antigen and costimulatory signals to B cells. *Immunol. Rev.* **156**, 39–52 (1997).
33. Qin, D. et al. Fcγ receptor IIB on follicular dendritic cells regulates the B cell recall response. *J. Immunol.* **164**, 6268–6275 (2000).
34. Link, A. et al. Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* **8**, 1255–1265 (2007).
35. Acton, SophieE. et al. Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2. *Immunity* **37**, 276–289 (2012).
36. Lee, J.-W. et al. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat. Immunol.* **8**, 181–190 (2007).
37. Magnusson, F. C. et al. Direct presentation of antigen by lymph node stromal cells protects against CD8 T-cell-mediated intestinal autoimmunity. *Gastroenterology* **134**, 1028–1037 (2008).
38. Fletcher, A. L. et al. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J. Exp. Med.* **207**, 689–697 (2010).
39. Baptista, A. P. et al. Lymph node stromal cells constrain immunity via MHC class II self-antigen presentation. *eLife* **3**, e04433 (2014).
40. Lukacs-Kornek, V. et al. Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. *Nat. Immunol.* **12**, 1096–1104 (2011).
41. Siegert, S. et al. Fibroblastic reticular cells from lymph nodes attenuate T cell expansion by producing nitric oxide. *PLoS ONE* **6**, e27618 (2011).
42. Pinchuk, I. V. et al. PD-1 ligand expression by human colonic myofibroblasts/fibroblasts regulates $CD4^+$ T-cell activity. *Gastroenterology* **135**, 1228–1237 (2008).
43. Owens, B. M. et al. $CD90^+$ stromal cells are non-professional innate immune effectors of the human colonic mucosa. *Front. Immunol.* **4**, 307 (2013).
44. Das, A. et al. Follicular dendritic cell activation by TLR ligands promotes autoreactive B cell responses. *Immunity* **46**, 106–119 (2017).
45. Zeng, Q. et al. Spleen fibroblastic reticular cell-derived acetylcholine promotes lipid metabolism to drive autoreactive B cell responses. *Cell Metab.* **35**, 837–854 e838 (2023).
46. Karouzakis, E. et al. Molecular characterization of human lymph node stromal cells during the earliest phases of rheumatoid arthritis. *Front. Immunol.* **10**, 1863 (2019).
47. Mellors, R. C., Heimer, R., Corcos, J. & Korngold, L. Cellular origin of rheumatoid factor. *J. Exp. Med.* **110**, 875–886 (1959).
48. Nosanchuk, J. S. & Schintzier, B. Follicular hyperplasia in lymph nodes from patients with rheumatoid arthritis. A clinicopathologic study. *Cancer* **24**, 343–354 (1969).
49. Shapira, Y., Weinberger, A. & Wysenbeek, A. J. Lymphadenopathy in systemic lupus erythematosus. Prevalence and relation to disease manifestations. *Clin. Rheumatol.* **15**, 335–338 (1996).
50. Cook, M. The size and histological appearances of mesenteric lymph nodes in Crohn's disease. *Gut* **13**, 970 (1972).
51. Nguyen, H. N. et al. Autocrine loop involving IL-6 family member LIF, LIF receptor, and STAT4 drives sustained fibroblast production of inflammatory mediators. *Immunity* **46**, 220–232 (2017).
52. West, N. R. et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nat. Med.* **23**, 579–589 (2017).
53. Zhang, F. et al. Defining inflammatory cell states in rheumatoid arthritis joint synovial tissues by integrating single-cell transcriptomics and mass cytometry. *Nat. Immunol.* **20**, 928–942 (2019).
54. Ivashkiv, L. B. Cytokine expression and cell activation in inflammatory arthritis. *Adv. Immunol.* **63**, 337–376 (1996).
55. Sponheim, J. et al. Inflammatory bowel disease-associated interleukin-33 is preferentially expressed in ulceration-associated myofibroblasts. *Am. J. Pathol.* **177**, 2804–2815 (2010).
56. Boots, A. M., Wimmers-Bertens, A. J. & Rijnders, A. W. Antigen-presenting capacity of rheumatoid synovial fibroblasts. *Immunology* **82**, 268–274 (1994).
57. Tran, C. N. et al. Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum.* **56**, 1497–1506 (2007).
58. Ogura, H. et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* **29**, 628–636 (2008).
59. Öhlund, D. et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. *J. Exp. Med.* **214**, 579–596 (2017).
60. Elyada, E. et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. *Cancer Discov.* **9**, 1102–1123 (2019).
61. Friedman, G. et al. Cancer-associated fibroblast compositions change with breast cancer progression linking the ratio of $100A4^+$ and $PDPN^+$ CAFs to clinical outcome. *Nat. Cancer* **1**, 692–708 (2020).
62. Kerdidani, D. et al. Lung tumor MHCII immunity depends on in situ antigen presentation by fibroblasts. *J. Exp. Med.* **219**, e20210815 (2022).
63. DeLeon-Pennell, K. Y., Barker, T. H. & Lindsey, M. L. Fibroblasts: the arbiters of extracellular matrix remodeling. *Matrix Biol.* **91–92**, 1–7 (2020).
64. Lu, P., Takai, K., Weaver, V. M. & Werb, Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* **3**, a005058 (2011).
65. Valencia, X. et al. Cadherin-11 provides specific cellular adhesion between fibroblast-like synoviocytes. *J. Exp. Med.* **200**, 1673–1679 (2004).
66. Eckes, B. et al. Mechanical tension and integrin α2β1 regulate fibroblast functions. *J. Investig. Dermatol. Symp. Proc.* **11**, 66–72 (2006).
67. Li, B. & Wang, J. H. Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *J. Tissue Viability* **20**, 108–120 (2011).
68. Levick, J. R. & McDonald, J. N. Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. *Ann. Rheum. Dis.* **54**, 417–423 (1995).
69. Gretz, J. E., Anderson, A. O. & Shaw, S. Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol. Rev.* **156**, 11–24 (1997).
70. Astarita, J. L. et al. The CLEC-2–podoplanin axis controls fibroblastic reticular cell contractility and lymph node microarchitecture. *Nat. Immunol.* **16**, 75–84 (2015).
71. Desmouliere, A., Chaponnier, C. & Gabbiani, G. Tissue repair, contraction, and the myofibroblast. *Wound Repair Regen.* **13**, 7–12 (2005).
72. Distler, J. H. W. et al. Shared and distinct mechanisms of fibrosis. *Nat. Rev. Rheumatol.* **15**, 705–730 (2019).
73. Zeisberg, M. & Kalluri, R. Cellular mechanisms of tissue fibrosis. 1. Common and organ-specific mechanisms associated with tissue fibrosis. *Am. J. Physiol. Cell Physiol.* **304**, C216–C225 (2013).
74. Tolboom, T. C. et al. Invasive properties of fibroblast-like synoviocytes: correlation with growth characteristics and expression of MMP-1, MMP-3, and MMP-10. *Ann. Rheum. Dis.* **61**, 975–980 (2002).
75. Tunyogi-Csapo, M. et al. Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: fibroblast-mediated pathologic bone resorption. *Arthritis Rheum.* **58**, 2397–2408 (2008).
76. Gregorieff, A. et al. Expression pattern of Wnt signaling components in the adult intestine. *Gastroenterology* **129**, 626–638 (2005).
77. McCarthy, N. et al. Distinct mesenchymal cell populations generate the essential intestinal BMP signaling gradient. *Cell Stem Cell* **26**, 391–402.e395 (2020).
78. Brugger, M. D., Valenta, T., Fazilaty, H., Hausmann, G. & Basler, K. Distinct populations of crypt-associated fibroblasts act as signaling hubs to control colon homeostasis. *PLoS Biol.* **18**, e3001032 (2020).
79. Liu, Y. et al. Hedgehog signaling reprograms hair follicle niche fibroblasts to a hyper-activated state. *Dev. Cell* **57**, 1758–1775.e1757 (2022).
80. Wei, K. et al. Notch signalling drives synovial fibroblast identity and arthritis pathology. *Nature* **582**, 259–264 (2020).
81. Rinn, J. L. et al. A dermal HOX transcriptional program regulates site-specific epidermal fate. *Genes Dev.* **22**, 303–307 (2008).
82. Frank-Bertoncelj, M. et al. Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat. Commun.* **8**, 14852 (2017).
83. Knosp, W. M., Scott, V., Bachinger, H. P. & Stadler, H. S. HOXA13 regulates the expression of bone morphogenetic proteins 2 and 7 to control distal limb morphogenesis. *Development* **131**, 4581–4592 (2004).
84. Dieu-Nosjean, M. C., Goc, J., Giraldo, N. A., Sautes-Fridman, C. & Fridman, W. H. Tertiary lymphoid structures in cancer and beyond. *Trends Immunol.* **35**, 571–580 (2014).
85. Barone, F. et al. Stromal fibroblasts in tertiary lymphoid structures: a novel target in chronic inflammation. *Front. Immunol.* **7**, 477 (2016).
86. Nayar, S. et al. Immunofibroblasts are pivotal drivers of tertiary lymphoid structure formation and local pathology. *Proc. Natl Acad. Sci. USA* **116**, 13490–13497 (2019).
87. Manzo, A. et al. Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis. *Eur. J. Immunol.* **35**, 1347–1359 (2005).
88. Pitzalis, C., Jones, G. W., Bombardieri, M. & Jones, S. A. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat. Rev. Immunol.* **14**, 447–462 (2014).
89. Kobayashi, I. & Ziff, M. Electron microscopic studies of the cartilage-pannus junction in rheumatoid arthritis. *Arthritis Rheum.* **18**, 475–483 (1975).
90. Kuhn, C. & McDonald, J. A. The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am. J. Pathol.* **138**, 1257–1265 (1991).
91. Rodriguez, A. B. et al. Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. *Cell Rep.* **36**, 109422 (2021).
92. Zhang, Y. et al. CCL19-producing fibroblasts promote tertiary lymphoid structure formation enhancing anti-tumor IgG response in colorectal cancer liver metastasis. *Cancer Cell* **42**, 1370–1385.e9 (2024).

93. Yang, C. Y. et al. Trapping of naive lymphocytes triggers rapid growth and remodeling of the fibroblast network in reactive murine lymph nodes. *Proc. Natl Acad. Sci. USA* **111**, E109–E118 (2014).
94. Hecker, L., Jagirdar, R., Jin, T. & Thannickal, V. J. Reversible differentiation of myofibroblasts by MyoD. *Exp. Cell Res.* **317**, 1914–1921 (2011).
95. Garrison, G. et al. Reversal of myofibroblast differentiation by prostaglandin E_2. *Am. J. Respir. Cell Mol. Biol.* **48**, 550–558 (2013).
96. Plikus, M. V. et al. Regeneration of fat cells from myofibroblasts during wound healing. *Science* **355**, 748–752 (2017).
97. Filer, A. & Buckley, C. D. et al. in *Rheumatology* (ed. Hochberg, M. C.) 1–7 (Elsevier, 2023).
98. Mizoguchi, F. et al. Functionally distinct disease-associated fibroblast subsets in rheumatoid arthritis. *Nat. Commun.* **9**, 789 (2018).
99. Croft, A. P. et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* **570**, 246–251 (2019).
100. Donlin, L. T. et al. Methods for high-dimensional analysis of cells dissociated from cryopreserved synovial tissue. *Arthritis Res. Ther.* **20**, 139 (2018).
101. Buechler, M. B. et al. Cross-tissue organization of the fibroblast lineage. *Nature* **593**, 575–579 (2021).
102. Armaka, M. et al. Single-cell multimodal analysis identifies common regulatory programs in synovial fibroblasts of rheumatoid arthritis patients and modeled TNF-driven arthritis. *Genome Med.* **14**, 78 (2022).
103. Mueller, A. A. et al. Wnt signaling drives stromal inflammation in inflammatory arthritis. Preprint at *bioRxiv* https://doi.org/10.1101/2025.01.06.631510 (2025).
104. MacLauchlan, S. et al. Genetic deficiency of Wnt5a diminishes disease severity in a murine model of rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 166 (2017).
105. Lewis, M. J. et al. Molecular portraits of early rheumatoid arthritis identify clinical and treatment response phenotypes. *Cell Rep.* **28**, 2455–2470.e2455 (2019).
106. Faust, H. J. et al. Adipocyte associated glucocorticoid signaling regulates normal fibroblast function which is lost in inflammatory arthritis. *Nat. Commun.* **15**, 9859 (2024).
107. Rauber, S. et al. $CD200^+$ fibroblasts form a pro-resolving mesenchymal network in arthritis. *Nat. Immunol.* **25**, 682–692 (2024).
108. Korsunsky, I. et al. Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases. *Med* **3**, 481–518.e14 (2022).
109. Tian, L., Chen, F. & Macosko, E. Z. The expanding vistas of spatial transcriptomics. *Nat. Biotechnol.* **41**, 773–782 (2023).
110. Lake, B. B. et al. An atlas of healthy and injured cell states and niches in the human kidney. *Nature* **619**, 585–594 (2023).
111. Wu, H. et al. High resolution spatial profiling of kidney injury and repair using RNA hybridization-based in situ sequencing. *Nat. Commun.* **15**, 1396 (2024).
112. Madissoon, E. et al. A spatially resolved atlas of the human lung characterizes a gland-associated immune niche. *Nat. Genet.* **55**, 66–77 (2023).
113. Vannan, A. et al. Spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis. *Nat. Genet.* **57**, 647–658 (2025).
114. Fawkner-Corbett, D. et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. *Cell* **184**, 810–826.e823 (2021).
115. Qi, J. et al. Single-cell and spatial analysis reveal interaction of FAP^+ fibroblasts and $SPP1^+$ macrophages in colorectal cancer. *Nat. Commun.* **13**, 1742 (2022).
116. Reis Nisa, P. et al. Spatial programming of fibroblasts promotes resolution of tissue inflammation through immune cell exclusion. Preprint at *bioRxiv* https://doi.org/10.1101/2024.09.20.614064 (2024).
117. Periyakoil, P. K. et al. Deep topic modeling of spatial transcriptomics in the rheumatoid arthritis synovium identifies distinct classes of ectopic lymphoid structures. Preprint at *bioRxiv* https://doi.org/10.1101/2025.01.08.631928 (2025).
118. Bhamidipati, K. et al. Spatial patterning of fibroblast TGFβ signaling underlies treatment resistance in rheumatoid arthritis. Preprint at *bioRxiv* https://doi.org/10.1101/2025.03.14.642821 (2025).
119. Vickovic, S. et al. Three-dimensional spatial transcriptomics uncovers cell type localizations in the human rheumatoid arthritis synovium. *Commun. Biol.* **5**, 129 (2022).
120. Smith, M. H. et al. Drivers of heterogeneity in synovial fibroblasts in rheumatoid arthritis. *Nat. Immunol.* **24**, 1200–1210 (2023).
121. Zheng, L. et al. $ITGA5^+$ synovial fibroblasts orchestrate proinflammatory niche formation by remodelling the local immune microenvironment in rheumatoid arthritis. *Ann. Rheum. Dis.* **84**, 232–252 (2025).
122. Zhao, S. et al. Effect of JAK inhibition on the induction of proinflammatory HLA–DR+CD90+ rheumatoid arthritis synovial fibroblasts by interferon-γ. *Arthritis Rheumatol.* **74**, 441–452 (2022).
123. Seibl, R. et al. Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. *Am. J. Pathol.* **162**, 1221–1227 (2003).
124. Kim, K. W. et al. Human rheumatoid synovial fibroblasts promote osteoclastogenic activity by activating RANKL via TLR-2 and TLR-4 activation. *Immunol. Lett.* **110**, 54–64 (2007).
125. Goh, F. G. & Midwood, K. S. Intrinsic danger: activation of Toll-like receptors in rheumatoid arthritis. *Rheumatology* **51**, 7–23 (2012).
126. Chabaud, M., Fossiez, F., Taupin, J. L. & Miossec, P. Enhancing effect of IL-17 on IL-1-induced IL-6 and leukemia inhibitory factor production by rheumatoid arthritis synoviocytes and its regulation by Th2 cytokines. *J. Immunol.* **161**, 409–414 (1998).
127. Zrioual, S. et al. Genome-wide comparison between IL-17A- and IL-17F-induced effects in human rheumatoid arthritis synoviocytes. *J. Immunol.* **182**, 3112–3120 (2009).
128. Slowikowski, K. et al. CUX1 and IκBζ (NFKBIZ) mediate the synergistic inflammatory response to TNF and IL-17A in stromal fibroblasts. *Proc. Natl Acad. Sci. USA* **117**, 5532–5541 (2020).
129. Hartupee, J., Liu, C., Novotny, M., Li, X. & Hamilton, T. IL-17 enhances chemokine gene expression through mRNA stabilization. *J. Immunol.* **179**, 4135–4141 (2007).
130. MacNaul, K. L., Chartrain, N., Lark, M., Tocci, M. J. & Hutchinson, N. I. Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts. Synergistic effects of interleukin-1 and tumor necrosis factor-α on stromelysin expression. *J. Biol. Chem.* **265**, 17238–17245 (1990).
131. Ainola, M. M. et al. Pannus invasion and cartilage degradation in rheumatoid arthritis: involvement of MMP-3 and interleukin-1β. *Clin. Exp. Rheumatol.* **23**, 644–650 (2005).
132. Schafer, S. et al. IL-11 is a crucial determinant of cardiovascular fibrosis. *Nature* **552**, 110–115 (2017).
133. Kuo, D. et al. $HBEGF^+$ macrophages in rheumatoid arthritis induce fibroblast invasiveness. *Sci. Transl. Med.* **11**, eaau8587 (2019).
134. Yan, M. et al. ETS1 governs pathological tissue-remodeling programs in disease-associated fibroblasts. *Nat. Immunol.* **23**, 1330–1341 (2022).
135. Nguyen, H. N. et al. Leukemia inhibitory factor (LIF) receptor amplifies pathogenic activation of fibroblasts in lung fibrosis. *Proc. Natl Acad. Sci. USA* **121**, e2401899121 (2024).
136. Xiao, Y. & MacRae, I. J. Toward a comprehensive view of microRNA biology. *Mol. Cell* **75**, 666–668 (2019).
137. Trenkmann, M. et al. Tumor necrosis factor α-induced microRNA-18a activates rheumatoid arthritis synovial fibroblasts through a feedback loop in NF-κB signaling. *Arthritis Rheum.* **65**, 916–927 (2013).
138. Stanczyk, J. et al. Altered expression of microRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* **58**, 1001–1009 (2008).
139. Nakasa, T. et al. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. *Arthritis Rheum.* **58**, 1284–1292 (2008).
140. Long, L. et al. Upregulated microRNA-155 expression in peripheral blood mononuclear cells and fibroblast-like synoviocytes in rheumatoid arthritis. *J. Immunol. Res.* **2013**, 296139 (2013).
141. Saferding, V. et al. MicroRNA-146a governs fibroblast activation and joint pathology in arthritis. *J. Autoimmun.* **82**, 74–84 (2017).
142. Churov, A. V., Oleinik, E. K. & Knip, M. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. *Autoimmun. Rev.* **14**, 1029–1037 (2015).
143. Iborra, M., Bernuzzi, F., Invernizzi, P. & Danese, S. MicroRNAs in autoimmunity and inflammatory bowel disease: crucial regulators in immune response. *Autoimmun. Rev.* **11**, 305–314 (2012).
144. O'Reilly, S. MicroRNAs in fibrosis: opportunities and challenges. *Arthritis Res. Ther.* **18**, 11 (2016).
145. Aprelikova, O. & Green, J. E. MicroRNA regulation in cancer-associated fibroblasts. *Cancer Immunol. Immunother.* **61**, 231–237 (2012).
146. Eckes, B. et al. Fibroblast-matrix interactions in wound healing and fibrosis. *Matrix Biol.* **19**, 325–332 (2000).
147. Janmey, P. A., Fletcher, D. A. & Reinhart-King, C. A. Stiffness sensing by cells. *Physiol. Rev.* **100**, 695–724 (2020).
148. Lee, D. M. et al. Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* **315**, 1006–1010 (2007).
149. Chang, S. K. et al. Cadherin-11 regulates fibroblast inflammation. *Proc. Natl Acad. Sci. USA* **108**, 8402–8407 (2011).
150. Vandooren, B. et al. Tumor necrosis factor α drives cadherin 11 expression in rheumatoid inflammation. *Arthritis Rheum.* **58**, 3051–3062 (2008).
151. Schneider, D. J. et al. Cadherin-11 contributes to pulmonary fibrosis: potential role in TGF-β production and epithelial to mesenchymal transition. *FASEB J.* **26**, 503–512 (2012).
152. Wu, M. et al. Identification of cadherin 11 as a mediator of dermal fibrosis and possible role in systemic sclerosis. *Arthritis Rheumatol.* **66**, 1010–1021 (2014).
153. Schroer, A. K. et al. Cadherin-11 blockade reduces inflammation-driven fibrotic remodeling and improves outcomes after myocardial infarction. *JCI Insight* **4**, e131545 (2019).
154. Stanford, S. M. et al. Receptor protein tyrosine phosphatase α–mediated enhancement of rheumatoid synovial fibroblast signaling and promotion of arthritis in mice. *Arthritis Rheumatol.* **68**, 359–369 (2016).
155. Sendo, S. et al. Clustering of phosphatase RPTPα promotes Src signaling and the arthritogenic action of synovial fibroblasts. *Sci. Signal.* **16**, eabn8668 (2023).
156. Lo, C.-M., Wang, H.-B., Dembo, M. & Wang, Y.-l. Cell movement is guided by the rigidity of the substrate. *Biophys. J.* **79**, 144–152 (2000).
157. Gu, Z. et al. Soft matrix is a natural stimulator for cellular invasiveness. *Mol. Biol. Cell* **25**, 457–469 (2014).
158. Liu, F. et al. Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **308**, L344–L357 (2015).
159. Southern, B. D. et al. Matrix-driven myosin II mediates the pro-fibrotic fibroblast phenotype. *J. Biol. Chem.* **291**, 6083–6095 (2016).
160. Cambré, I. et al. Mechanical strain determines the site-specific localization of inflammation and tissue damage in arthritis. *Nat. Commun.* **9**, 4613 (2018).
161. Müller-Ladner, U. et al. Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *Am. J. Pathol.* **149**, 1607–1615 (1996).

162. Bartok, B. & Firestein, G. S. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol. Rev.* **233**, 233–255 (2010).
163. Whitaker, J. W. et al. An imprinted rheumatoid arthritis methylome signature reflects pathogenic phenotype. *Genome Med.* **5**, 40 (2013).
164. Lefevre, S. et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat. Med.* **15**, 1414–1420 (2009).
165. Orange, D. E. et al. RNA identification of PRIME cells predicting rheumatoid arthritis flares. *N. Engl. J. Med.* **383**, 218–228 (2020).
166. Nile, C. J., Read, R. C., Akil, M., Duff, G. W. & Wilson, A. G. Methylation status of a single CpG site in the *IL6* promoter is related to *IL6* messenger RNA levels and rheumatoid arthritis. *Arthritis Rheum.* **58**, 2686–2693 (2008).
167. Karouzakis, E. et al. DNA methylation regulates the expression of CXCL12 in rheumatoid arthritis synovial fibroblasts. *Genes. Immun.* **12**, 643–652 (2011).
168. Nakano, K., Whitaker, J. W., Boyle, D. L., Wang, W. & Firestein, G. S. DNA methylome signature in rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 110–117 (2013).
169. de la Rica, L. et al. Identification of novel markers in rheumatoid arthritis through integrated analysis of DNA methylation and microRNA expression. *J. Autoimmun.* **41**, 6–16 (2013).
170. Araki, Y. et al. Histone methylation and STAT-3 differentially regulate interleukin-6-induced matrix metalloproteinase gene activation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheumatol.* **68**, 1111–1123 (2016).
171. Lee, A. et al. Tumor necrosis factor α induces sustained signaling and a prolonged and unremitting inflammatory response in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum.* **65**, 928–938 (2013).
172. Loh, C. et al. TNF-induced inflammatory genes escape repression in fibroblast-like synoviocytes: transcriptomic and epigenomic analysis. *Ann. Rheum. Dis.* **78**, 1205–1214 (2019).
173. Sohn, C. et al. Prolonged tumor necrosis factor alpha primes fibroblast-like synoviocytes in a gene-specific manner by altering chromatin. *Arthritis Rheumatol.* **67**, 86–95 (2015).
174. Friščić, J. et al. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity* **54**, 1002–1021.e10 (2021).
175. Weinand, K. et al. The chromatin landscape of pathogenic transcriptional cell states in rheumatoid arthritis. *Nat. Commun.* **15**, 4650 (2024).
176. Ai, R. et al. Comprehensive epigenetic landscape of rheumatoid arthritis fibroblast-like synoviocytes. *Nat. Commun.* **9**, 1921 (2018).
177. Ai, R. et al. DNA methylome signature in synoviocytes from patients with early rheumatoid arthritis compared to synoviocytes from patients with longstanding rheumatoid arthritis. *Arthritis Rheumatol.* **67**, 1978–1980 (2015).
178. Karouzakis, E. et al. Analysis of early changes in DNA methylation in synovial fibroblasts of RA patients before diagnosis. *Sci. Rep.* **8**, 7370 (2018).
179. Ai, R. et al. Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. *Nat. Commun.* **7**, 11849 (2016).
180. Hammaker, D. et al. Joint location–specific JAK-STAT signaling in rheumatoid arthritis fibroblast-like synoviocytes. *ACR Open Rheumatol.* **1**, 640–648 (2019).
181. Carter, P. J. & Lazar, G. A. Next generation antibody drugs: pursuit of the 'high-hanging fruit. *Nat. Rev. Drug. Discov.* **17**, 197–223 (2018).
182. Cappell, K. M. & Kochenderfer, J. N. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat. Rev. Clin. Oncol.* **20**, 359–371 (2023).
183. van de Donk, N. & Zweegman, S. T-cell-engaging bispecific antibodies in cancer. *Lancet* **402**, 142–158 (2023).
184. Fenis, A., Demaria, O., Gauthier, L., Vivier, E. & Narni-Mancinelli, E. New immune cell engagers for cancer immunotherapy. *Nat. Rev. Immunol.* **24**, 471–486 (2024).
185. Busek, P., Mateu, R., Zubal, M., Kotackova, L. & Sedo, A. Targeting fibroblast activation protein in cancer — prospects and caveats. *Front. Biosci.* **23**, 1933–1968 (2018).
186. Xin, L. et al. Fibroblast activation protein-α as a target in the bench-to-bedside diagnosis and treatment of tumors: a narrative review. *Front. Oncol.* **11**, 648187 (2021).
187. Scott, A. M. et al. A phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. *Clin. Cancer Res.* **9**, 1639–1647 (2003).
188. McConathy, J. et al. 671P LuMIERE: a phase I/II study evaluating safety, dosimetry, and preliminary activity of [177Lu]Lu-FAP-2286 in patients with advanced solid tumors. *Ann. Oncol.* **35**, S526 (2024).
189. Shahvali, S., Rahiman, N., Jaafari, M. R. & Arabi, L. Targeting fibroblast activation protein (FAP): advances in CAR-T cell, antibody, and vaccine in cancer immunotherapy. *Drug. Deliv. Transl. Res.* **13**, 2041–2056 (2023).
190. Chai, X. P. et al. Tumor-targeting efficacy of a BF211 prodrug through hydrolysis by fibroblast activation protein-α. *Acta Pharmacol. Sin.* **39**, 415–424 (2018).
191. Cui, X. Y. et al. Covalent targeted radioligands potentiate radionuclide therapy. *Nature* **630**, 206–213 (2024).
192. Loureiro, L. R. et al. Immunotheranostic target modules for imaging and navigation of UniCAR T-cells to strike FAP-expressing cells and the tumor microenvironment. *J. Exp. Clin. Cancer Res.* **42**, 341 (2023).
193. de Sostoa, J. et al. Targeting the tumor stroma with an oncolytic adenovirus secreting a fibroblast activation protein-targeted bispecific T-cell engager. *J. Immunother. Cancer* **7**, 19 (2019).
194. Hiltbrunner, S. et al. Local delivery of CAR T cells targeting fibroblast activation protein is safe in patients with pleural mesothelioma: first report of FAPME, a phase I clinical trial. *Ann. Oncol.* **32**, 120–121 (2021).
195. Bocci, M. et al. In vivo activation of FAP-cleavable small molecule-drug conjugates for the targeted delivery of camptothecins and tubulin poisons to the tumor microenvironment. *J. Control. Rel.* **367**, 779–790 (2024).
196. Aghajanian, H. et al. Targeting cardiac fibrosis with engineered T cells. *Nature* **573**, 430–433 (2019).
197. Rurik, J. G. et al. CAR T cells produced in vivo to treat cardiac injury. *Science* **375**, 91–96 (2022).
198. Dorst, D. N. et al. Targeting of fibroblast activation protein in rheumatoid arthritis patients: imaging and ex vivo photodynamic therapy. *Rheumatology* **61**, 2999–3009 (2022).
199. Mori, Y. et al. FAPI PET: fibroblast activation protein inhibitor use in oncologic and nononcologic disease. *Radiology* **306**, e220749 (2023).
200. Chavula, T., To, S. & Agarwal, S. K. Cadherin-11 and its role in tissue fibrosis. *Cell Tissues Organs* **212**, 293–303 (2023).
201. Schett, G. et al. Advancements and challenges in CAR T cell therapy in autoimmune diseases. *Nat. Rev. Rheumatol.* **20**, 531–544 (2024).
202. Chung, J. B., Brudno, J. N., Borie, D. & Kochenderfer, J. N. Chimeric antigen receptor T cell therapy for autoimmune disease. *Nat. Rev. Immunol.* **24**, 830–845 (2024).
203. Michaelson, J. S. & Baeuerle, P. A. CD19-directed T cell-engaging antibodies for the treatment of autoimmune disease. *J. Exp. Med.* **221**, e20240499 (2024).
204. Shah, K. et al. Disrupting B and T-cell collaboration in autoimmune disease: T-cell engagers versus CAR T-cell therapy? *Clin. Exp. Immunol.* **217**, 15–30 (2024).
205. Parayath, N. N., Stephan, S. B., Koehne, A. L., Nelson, P. S. & Stephan, M. T. In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo. *Nat. Commun.* **11**, 6080 (2020).
206. Zhao, Y. et al. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. *Cancer Res.* **70**, 9053–9061 (2010).
207. Bucci, L. et al. Bispecific T cell engager therapy for refractory rheumatoid arthritis. *Nat. Med.* **30**, 1593–1601 (2024).
208. Hagen, M. et al. BCMA-targeted T-cell-engager therapy for autoimmune disease. *N. Engl. J. Med.* **391**, 867–869 (2024).
209. Alexander, T., Kronke, J., Cheng, Q., Keller, U. & Kronke, G. Teclistamab-induced remission in refractory systemic lupus erythematosus. *N. Engl. J. Med.* **391**, 864–866 (2024).
210. Heipertz, E. L. et al. Current perspectives on "off-the-shelf" allogeneic NK and CAR-NK cell therapies. *Front. Immunol.* **12**, 732135 (2021).
211. Younesi, F. S., Miller, A. E., Barker, T. H., Rossi, F. M. V. & Hinz, B. Fibroblast and myofibroblast activation in normal tissue repair and fibrosis. *Nat. Rev. Mol. Cell Biol.* **25**, 617–638 (2024).
212. Bhattacharya, M. & Ramachandran, P. Immunology of human fibrosis. *Nat. Immunol.* **24**, 1423–1433 (2023).
213. Zhao, M. et al. Targeting fibrosis, mechanisms and clinical trials. *Signal. Transduct. Target. Ther.* **7**, 206 (2022).
214. Klein, K. et al. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. *Ann. Rheum. Dis.* **75**, 422–429 (2016).
215. Neidhart, M., Karouzakis, E., Jungel, A., Gay, R. E. & Gay, S. Inhibition of spermidine/spermine N1-acetyltransferase activity: a new therapeutic concept in rheumatoid arthritis. *Arthritis Rheumatol.* **66**, 1723–1733 (2014).
216. Kehrberg, R. J., Bhyravbhatla, N., Batra, S. K. & Kumar, S. Epigenetic regulation of cancer-associated fibroblast heterogeneity. *Biochim. Biophys. Acta Rev. Cancer* **1878**, 188901 (2023).
217. Ulukan, B., Sila Ozkaya, Y. & Zeybel, M. Advances in the epigenetics of fibroblast biology and fibrotic diseases. *Curr. Opin. Pharmacol.* **49**, 102–109 (2019).
218. Liu, Y. et al. Epigenetics as a versatile regulator of fibrosis. *J. Transl. Med.* **21**, 164 (2023).
219. Fearon, U., Hanlon, M. M., Wade, S. M. & Fletcher, J. M. Altered metabolic pathways regulate synovial inflammation in rheumatoid arthritis. *Clin. Exp. Immunol.* **197**, 170–180 (2019).
220. Hu, Z. et al. Metabolic changes in fibroblast-like synoviocytes in rheumatoid arthritis: state of the art review. *Front. Immunol.* **15**, 1250884 (2024).
221. Sahai, E. et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* **20**, 174–186 (2020).
222. Zhang, F. et al. Cancer associated fibroblasts and metabolic reprogramming: unraveling the intricate crosstalk in tumor evolution. *J. Hematol. Oncol.* **17**, 80 (2024).
223. Hamanaka, R. B. & Mutlu, G. M. Metabolic requirements of pulmonary fibrosis: role of fibroblast metabolism. *FEBS J.* **288**, 6331–6352 (2021).
224. Wang, S., Liang, Y. & Dai, C. Metabolic regulation of fibroblast activation and proliferation during organ fibrosis. *Kidney Dis.* **8**, 115–125 (2022).
225. Noom, A., Sawitzki, B., Knaus, P. & Duda, G. N. A two-way street — cellular metabolism and myofibroblast contraction. *NPJ Regen. Med.* **9**, 15 (2024).
226. Takahashi, S. et al. Glutaminase 1 plays a key role in the cell growth of fibroblast-like synoviocytes in rheumatoid arthritis. *Arthritis Res. Ther.* **19**, 76 (2017).
227. Song, G. et al. Inhibition of hexokinases holds potential as treatment strategy for rheumatoid arthritis. *Arthritis Res. Ther.* **21**, 87 (2019).
228. Ahmed, S. et al. Dual inhibition of glycolysis and glutaminolysis for synergistic therapy of rheumatoid arthritis. *Arthritis Res. Ther.* **25**, 176 (2023).
229. Garcia-Carbonell, R. et al. Critical role of glucose metabolism in rheumatoid arthritis fibroblast-like synoviocytes. *Arthritis Rheumatol.* **68**, 1614–1626 (2016).

230. Koedderitzsch, K., Zezina, E., Li, L., Herrmann, M. & Biesemann, N. TNF induces glycolytic shift in fibroblast like synoviocytes via GLUT1 and HIF1A. *Sci. Rep.* **11**, 19385 (2021).
231. Becker, L. M. et al. Epigenetic reprogramming of cancer-associated fibroblasts deregulates glucose metabolism and facilitates progression of breast cancer. *Cell Rep.* **31**, 107701 (2020).
232. Broz, M. T. et al. Metabolic targeting of cancer associated fibroblasts overcomes T-cell exclusion and chemoresistance in soft-tissue sarcomas. *Nat. Commun.* **15**, 2498 (2024).
233. Cho, S. J., Moon, J. S., Lee, C. M., Choi, A. M. & Stout-Delgado, H. W. Glucose transporter 1-dependent glycolysis is increased during aging-related lung fibrosis, and phloretin inhibits lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* **56**, 521–531 (2017).
234. Yin, X. et al. Hexokinase 2 couples glycolysis with the profibrotic actions of TGF-β. *Sci. Signal.* **12**, eaax4067 (2019).
235. Wagner, A. et al. Metabolic modeling of single Th17 cells reveals regulators of autoimmunity. *Cell* **184**, 4168–4185.e21 (2021).
236. Huynh, N. C.-N. et al. Oncostatin M-driven macrophage-fibroblast circuits as a drug target in autoimmune arthritis. *Inflamm. Regen.* **44**, 36 (2024).
237. Tsaltskan, V. & Firestein, G. S. Targeting fibroblast-like synoviocytes in rheumatoid arthritis. *Curr. Opin. Pharmacol.* **67**, 102304 (2022).
238. Finch, R. et al. Op0224 results of a phase 2 study of Rg6125, an anti-cadherin-11 monoclonal antibody, in rheumatoid arthritis patients with an inadequate response to anti-TNFalpha therapy. *Ann. Rheum. Dis.* **78**, 189–189 (2019).
239. Scheller, J., Grötzinger, J. & Rose-John, S. Updating interleukin-6 classic-and trans-signaling. *Signal. Transduct.* **6**, 240–259 (2006).
240. Choy, E. H. et al. Translating IL-6 biology into effective treatments. *Nat. Rev. Rheumatol.* **16**, 335–345 (2020).
241. Rose-John, S., Jenkins, B. J., Garbers, C., Moll, J. M. & Scheller, J. Targeting IL-6 trans-signalling: past, present and future prospects. *Nat. Rev. Immunol.* **23**, 666–681 (2023).
242. Ruderman, E. M. Rheumatoid arthritis: IL-6 inhibition in RA-deja vu all over again? *Nat. Rev. Rheumatol.* **11**, 321–322 (2015).
243. Danese, S. et al. Randomised trial and open-label extension study of an anti-interleukin-6 antibody in Crohn's disease (ANDANTE I and II). *Gut* **68**, 40–48 (2019).
244. Haringman, J. J. et al. A randomized controlled trial with an anti-CCL2 (anti-monocyte chemotactic protein 1) monoclonal antibody in patients with rheumatoid arthritis. *Arthritis Rheum.* **54**, 2387–2392 (2006).
245. Vergunst, C. E. et al. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. *Arthritis Rheum.* **58**, 1931–1939 (2008).
246. Szekanecz, Z. & Koch, A. E. Successes and failures of chemokine-pathway targeting in rheumatoid arthritis. *Nat. Rev. Rheumatol.* **12**, 5–13 (2016).
247. Cambier, S., Gouwy, M. & Proost, P. The chemokines CXCL8 and CXCL12: molecular and functional properties, role in disease and efforts towards pharmacological intervention. *Cell Mol. Immunol.* **20**, 217–251 (2023).
248. Kurowska-Stolarska, M. & Alivernini, S. Synovial tissue macrophages in joint homeostasis, rheumatoid arthritis and disease remission. *Nat. Rev. Rheumatol.* **18**, 384–397 (2022).
249. Hitchon, C. A. & El-Gabalawy, H. S. The synovium in rheumatoid arthritis. *Open Rheumatol. J.* **5**, 107–114 (2011).
250. Raychaudhuri, S. et al. Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat. Genet.* **44**, 291–296 (2012).
251. Nielen, M. M. et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* **50**, 380–386 (2004).
252. Firestein, G. S. & McInnes, I. B. Immunopathogenesis of rheumatoid arthritis. *Immunity* **46**, 183–196 (2017).
253. Snir, O. et al. Identification and functional characterization of T cells reactive to citrullinated vimentin in HLA-DRB1*0401-positive humanized mice and rheumatoid arthritis patients. *Arthritis Rheum.* **63**, 2873–2883 (2011).
254. Moon, J. S. et al. Cytotoxic $CD8^+$ T cells target citrullinated antigens in rheumatoid arthritis. *Nat. Commun.* **14**, 319 (2023).
255. Rao, D. A. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017).
256. Donado, C. A. et al. Granzyme K activates the entire complement cascade. *Nature* https://doi.org/10.1038/s41586-025-08713-9 (2025).
257. Kongpachith, S. et al. Affinity maturation of the anti-citrullinated protein antibody paratope drives epitope spreading and polyreactivity in rheumatoid arthritis. *Arthritis Rheumatol.* **71**, 507–517 (2019).

Acknowledgements
The authors thank the members of the Brenner laboratory for helpful discussions. A.E.Z. received grant support from the US National Institutes of Health (NIH) (F30AI174699, T32GM007753 and T32GM144273). A.A.M. received grant support from the NIH (K08AR083513, T32AR007530 and P30AR070253) and the Rheumatology Research Foundation. M.B.B. received grant support from the NIH (R01AR0637039 and P01AI148102).

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

Competing interests
M.B.B. is on the scientific advisory board of GSK and Moderna, is a consultant to 4F0 Ventures and is a founder of Mestag Therapeutics. However, this Review does not discuss any of the therapeutics of their pipelines.

Additional information
Peer review information *Nature Reviews Rheumatology* thanks George Kalliolias, Samuel Kemble and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

nature reviews rheumatology

https://doi.org/10.1038/s41584-025-01252-7

Review article

Check for updates

Arterial and venous thrombosis in systemic and monogenic vasculitis

Federica Bello[1,15], Filippo Fagni[2,3,15], Giacomo Bagni[4], Catherine L. Hill[5,6], Aladdin J. Mohammad[7,8], Sergey Moiseev[9], Iacopo Olivotto[1,10], Emire Seyahi[11] & Giacomo Emmi[12,13,14]

Abstract

Systemic vasculitis, common forms of which include anti-neutrophil cytoplasmic antibody-associated small-vessel vasculitis, large-vessel vasculitis and Behçet syndrome, are frequently complicated by arterial or venous thrombotic events (AVTEs). Newly identified entities such as DADA2 (deficiency of adenosine deaminase 2) and VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, which are driven by genetic mutations, also exhibit vasculitic features and are associated with a high risk of AVTEs. AVTEs in systemic vasculitis, including monogenic forms of vasculitis, are due to the complex interaction of inflammation and coagulation. New insights into the pathogenetic mechanisms implicate endothelial dysfunction, immune complex deposition and the interplay of pro-inflammatory cytokines with prothrombotic factors, which collectively promote thrombus formation. AVTEs impose a substantial disease burden, complicate diagnosis and negatively affect prognosis by increasing the risk of morbidity and mortality. Early diagnosis and treatment are crucial to prevent lasting damage. Management strategies should target both thrombosis and underlying inflammation. Antithrombotic therapies, including low-dose aspirin, or oral anticoagulants should be used on the basis of individual thrombotic risk assessment. Immunosuppressive therapy is the cornerstone of treatment for arterial and venous thrombosis, particularly in Behçet syndrome, in which vascular inflammation has a crucial role in thrombotic complications.

Sections

A full list of affiliations appears at the end of the paper. e-mail: giacomo.emmi@units.it

Key points

- Systemic vasculitis, including anti-neutrophil cytoplasmic antibody-associated vasculitis, large-vessel vasculitis and Behçet syndrome, is frequently complicated by arterial or venous thrombotic events.
- Newly identified monogenic entities such as DADA2 (deficiency of adenosine deaminase 2) and VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome also exhibit vasculitic features and are associated with a high risk of thrombosis.
- Thrombosis in systemic and monogenic vasculitis is a result of intertwining inflammatory processes and coagulation abnormalities.
- Arterial and venous events negatively affect the prognosis of systemic and monogenic vasculitis, and represent a diagnostic challenge in clinical practice.
- Therapeutic strategies should be aimed at both managing thrombosis and downregulating the underlying inflammatory response.

Introduction

The connection between haemostasis and inflammation has been extensively investigated, especially in the past 30 years, but is still far from being fully elucidated. Procoagulant and anticoagulant factors are tightly balanced within the bloodstream; however, chronic inflammation can disrupt this balance, favouring a prothrombotic state[1]. Furthermore, thrombotic events contribute to a pro-inflammatory environment via effects on leukocyte trafficking and by sustaining the production of cytokines and chemokines[2,3].

In clinical practice, this connection becomes evident in various pathological situations, such as bacterial sepsis, viral infections (for example, SARS-CoV-2) and cancer[4–6]. A growing body of evidence has linked autoimmune diseases, particularly systemic vasculitis, to an increased incidence of arterial and venous thrombotic events (AVTEs), namely myocardial infarction, stroke, cardiovascular death and venous thromboembolic complications[7,8] (Box 1).

The optimization of immunosuppressive therapies has considerably improved the survival rates of patients with systemic vasculitis[9]. Thus, the primary concern in the management of these patients had shifted from increasing survival towards minimizing organ damage and enhancing overall quality of life. In this regard, recognizing AVTEs as the presenting manifestation of a previously unknown vasculitis, and preventing and managing AVTEs and their complications, has become a priority for clinicians, including (but not limited to) rheumatologists, immunologists, internists and nephrologists.

This Review is aimed at updating physicians on the most recent and relevant findings in the management of systemic vasculitis, focusing on anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and large-vessel vasculitis (LVV), presenting the latest clinical and preclinical insights into arterial and venous thrombosis. We also describe the latest findings in Behçet syndrome, which has so far been considered as the disease model of inflammation-induced thrombosis. Additionally, we discuss the impact of AVTEs in newly identified forms of monogenic vasculitis, such as DADA2 (deficiency of adenosine deaminase 2) and VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome.

Other systemic vasculitides, including cryoglobulinaemic vasculitis, Kawasaki disease, and IgA vasculitis, have been associated with thrombotic events[10–12]. However, owing to the limited availability of literature and data on thrombotic mechanisms and their clinical implications, these conditions will not be extensively discussed in this Review.

Clinical manifestations of systemic vasculitis

This section explores the epidemiology and clinical manifestations of vascular involvement in the various types of systemic vasculitis, as well as the risk factors associated with arterial and venous thrombosis in systemic vasculitis and their impact on morbidity and mortality (Fig. 1).

Anti-neutrophil cytoplasmic antibody-associated vasculitis

AAV comprises rare systemic diseases, including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA), that are characterized by small-vessel inflammation and affect various organ systems[13]. Clinical symptoms of AAV reflect areas in which blood vessels are affected, commonly featuring necrotizing glomerulonephritis with renal impairment and respiratory tract manifestations[14].

The prognosis of AAV has dramatically improved with the introduction of cytotoxic drugs and biologic DMARDs (bDMARDs)[15]. However, affected individuals still have elevated rates of comorbidities, including AVTEs, compared with the general population[16–18].

Individuals with AAV have a higher rate of mortality due to cardiovascular disease (CVD)[19] and have a 2.7-fold increased risk of death compared with the general population[20], with CVD accounting for 26% of mortality[21].

Arterial involvement. The data on the risk of AVTEs in people with EGPA are limited and are frequently pooled with data from other forms of AAV. A 2021 retrospective study showed that the risk of AVTEs was higher in patients with EGPA ($n = 573$) than in a population-based reference cohort, particularly around the time of EGPA diagnosis[22]. Venous events, although less frequent than arterial ones, were associated with a higher age-standardized event ratio than arterial events (3.32 (95% CI 2.35–4.70) versus 1.64 (95% CI 1.22–2.22))[22]. The study suggested that use of immunosuppressants might exert a protective effect, whereas the role of anticoagulant and antiplatelet agents was unclear. A high risk of AVTEs in EGPA that was comparable with that in GPA and MPA was also shown in other retrospective studies[23].

As well as acute thrombotic events, individuals with AAV (especially GPA and MPA) are at an increased risk of CVD owing to accelerated atherosclerosis, which can be attributed to traditional risk factors (such as older age, dyslipidaemia, hypertension, type 2 diabetes mellitus, etc.), organ damage (kidney in particular), prolonged use of glucocorticoids and disease-specific mechanisms, mostly related to persistent inflammation[24]. In the general population, the risk of cardiovascular complications and death rapidly increases with declining kidney function and is particularly high in individuals undergoing dialysis[25]. Renal involvement presenting in patients with AAV can vary in severity and chronic kidney disease stage and a substantial proportion of these patients still ultimately reach end-stage kidney disease despite the use of new remission induction and maintenance treatment regimens[26].

The incidence of CVD also increases with increasing age at AAV diagnosis[27,28], especially when associated with a high level of disease activity at diagnosis. In addition to accelerated atherosclerosis and inflammatory hypercoagulability, CVD can present with cardiac arrhythmias such as atrial fibrillation or ventricular tachycardia –

secondary to hyperinflammation status — early during disease onset[29]. The trigger for accelerated atherosclerosis could be the high level of inflammatory activity at disease onset, long-term exposure to glucocorticoids, or both[28,30].

Venous involvement. The rate of venous thromboembolism (VTE) in patients with GPA and MPA (up to 10%) is 10–20 times higher than in the general population[31]. Most VTEs occur within the first 6 months following diagnosis, although the risk remains elevated for the first year[27,28]. Inflammation is the most important factor contributing to an increased risk of VTE in individuals with AAV, probably by enhancing the hypercoagulable state and increasing endothelial damage[32,33]. A thrombogenic effect of glucocorticoids can also be important[34].

Unlike other forms of AAV, specific cardiac involvement characterized by eosinophil-rich inflammatory infiltrates and myocardial fibrosis is frequently seen in EGPA (up to 40% of cases), particularly ANCA-negative EGPA[35]. Eosinophilic myocarditis is the most common cardiac abnormality in EGPA, and heart disease could be attributable to both eosinophilic infiltration and vasculitis[36]. Eosinophilic infiltration can lead to irreversible cardiac damage manifested by dilated cardiomyopathy (a restrictive pattern can also be present) and/or intracardiac thrombosis, with low left ventricular ejection fraction and subsequent heart failure[37] (Fig. 2). Signs and symptoms of cardiac involvement in EGPA, such as chest pain or rhythm disorders, are non-specific and should be differentiated from coronary artery disease[37].

Large-vessel vasculitis

Giant-cell arteritis (GCA) and Takayasu arteritis are two distinct forms of systemic LVV that primarily affect the aorta and its major branches, are characterized by a chronic granulomatous inflammation of the arterial walls and are associated with an increased risk of arterial thrombotic events[7,38]. Despite similarities in their pathophysiology, GCA and Takayasu arteritis differ substantially in their demographic presentation and clinical course[39]. GCA predominantly affects individuals over the age of 50 years and can preferentially affect the supra-aortic branches[40]. By contrast, Takayasu arteritis occurs primarily in younger individuals, typically women <40 years of age, and mainly causes inflammation of the aorta and of its larger branches[41] (Fig. 2).

Arterial involvement. Ischaemic cerebrovascular events are frequent in GCA, the most common being anterior ischaemic optic neuropathy, which can be observed in around 20–30% of cases[42]. Posterior circulation stroke involving the vertebral and basilar arteries also has an increased prevalence in GCA compared with the general population, with a reported incidence of about 2.8% over a 27-year follow-up in a large cohort of individuals with GCA[43]. A French population-based study reached similar conclusions, estimating the prevalence of GCA-related stroke to be 7.0% (ref. 44). Disease-associated factors can increase the likelihood of developing arterial thrombosis in GCA. Notably, one large Italian cohort study suggested that a lack of systemic symptoms and an absence of high C-reactive protein (CRP) levels were associated with increased odds of cranial ischaemic events[45]. Furthermore, a 2023 retrospective study involving a large cohort of patients with GCA found an association between GCA-related cerebrovascular events, lower BMI and involvement of vertebral, intracranial and axillary arteries[46]. In addition, many traditional cardiovascular risk factors can be exacerbated by glucocorticoid therapy, further increasing the risk of ischaemia[47]. In forms of GCA with preponderant extracranial symptoms[48], the formation of aortic aneurysms and ischaemia of the upper and lower extremities, resulting from vasculitic and atherothrombotic occlusion of the supplying arteries, are more frequent than cranial GCA[49]. Aortic aneurysm is especially common in GCA and is found in an estimated 10–20% of patients[50]. Structural complications such as aneurysm rupture, fistula and thrombotic occlusion are rare but lethal late-stage complications of GCA[51]. Importantly, acute ischaemic events in LVV are driven primarily by inflammation, whereas atherosclerotic ischaemic events tend to develop later in the disease course as a result of endothelial damage, the effects of glucocorticoids and in general owing to increased cardiovascular risk.

In contrast to GCA, in Takayasu arteritis inflammatory stenoses and aneurysms of large arteries are commonly observed and usually coexist[52]. Stenotic lesions are predominant in Takayasu arteritis and can be found in more than 90% of patients[53]; aneurysms are reportedly observed in 20–40% of patients with Takayasu arteritis[54]. Aortic aneurysms have been described in ~4% of patients with Takayasu arteritis but can reach high levels of severity and involve complications that include aortic dissection and mural thrombus, frequently requiring surgical treatment[55]. The symptoms of Takayasu arteritis are highly variable

Box 1 | Definitions of arterial and venous thrombotic events

Arterial thrombotic events

Cardiovascular
- Acute coronary syndromes
- Left ventricular thrombus
- Aneurysm rupture

Cerebrovascular
- Ischaemic stroke and transient ischaemic attack
- Retinal artery occlusion

Other organ arterial thrombosis
- Mesenteric ischaemia
- Renal artery stenosis and thrombosis
- Pulmonary vein thrombosis

Peripheral
- Acute limb ischaemia due to arterial occlusion
- Chronic peripheral artery disease
- Arterial embolism

Venous thrombotic events

Cerebral
- Cerebral venous sinus thrombosis (superior sagittal sinus, transverse sinus)
- Cortical venous thrombosis

Pulmonary
- Acute pulmonary embolism
- Chronic thromboembolic pulmonary embolism

Other organ venous thrombosis
- Portal vein thrombosis
- Hepatic vein thrombosis (Budd–Chiari syndrome)
- Mesenteric venous thrombosis

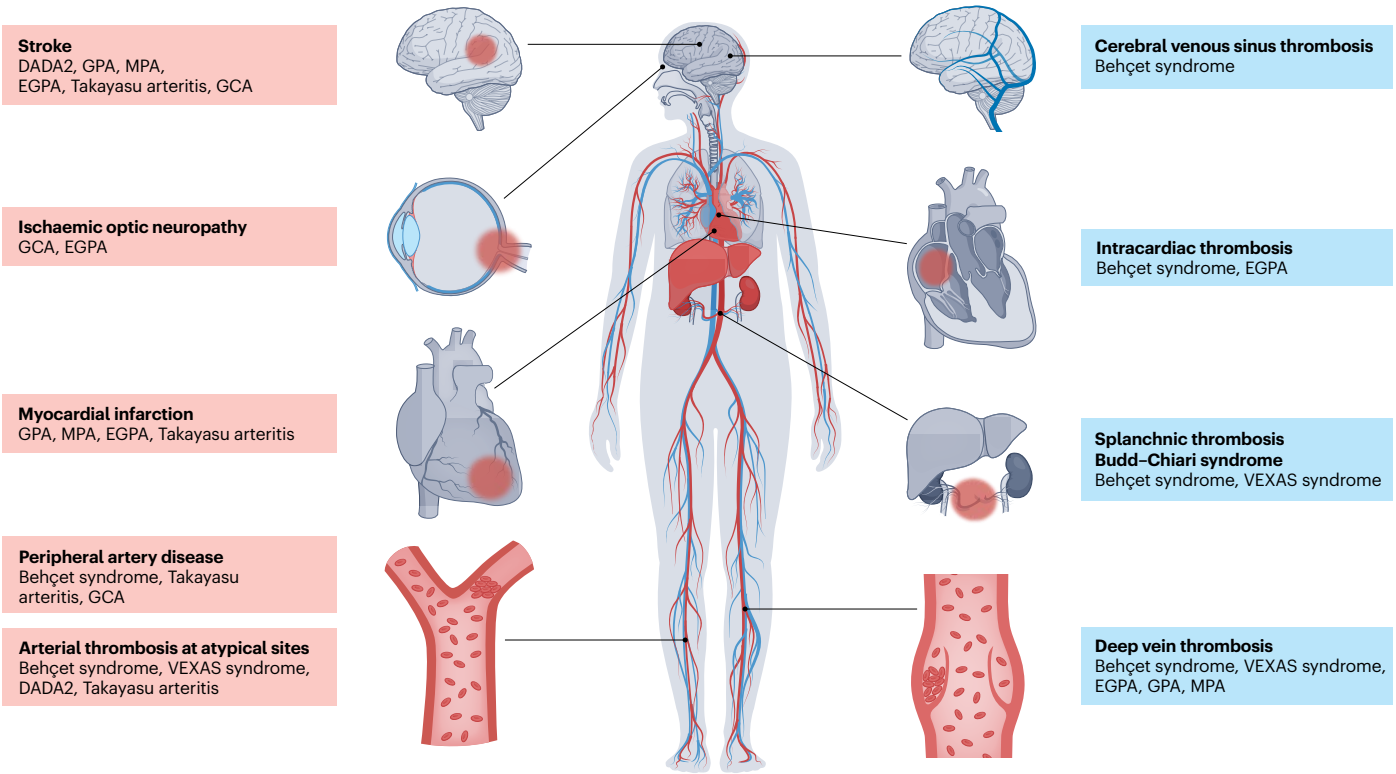


Fig. 1 | Thrombotic manifestations associated with systemic and monogenic vasculitis. The anatomical figure illustrates the distribution of the main thrombotic complications across distinct vasculitic syndromes. The left side of the figure shows arterial vascular manifestations. Stroke is observed mainly in DADA2 (deficiency of adenosine deaminase 2), granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA), Takayasu arteritis and giant-cell arteritis (GCA). Ischaemic optic neuropathy is linked with GCA and EGPA. Myocardial infarction is usually associated with GPA, MPA, EGPA and Takayasu arteritis. Peripheral artery disease is predominantly reported in Behçet syndrome, Takayasu arteritis and GCA. Arterial thrombosis at atypical sites is observed in Behçet syndrome, VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, DADA2 and Takayasu arteritis. On the right side of the figure are the venous vascular manifestations. Cerebral venous sinus thrombosis is typically linked with Behçet syndrome. Budd–Chiari syndrome and splanchnic thrombosis are seen in Behçet syndrome and VEXAS syndrome. Intracardiac thrombosis occurs in Behçet syndrome and EGPA. Deep vein thrombosis is associated with Behçet syndrome, VEXAS syndrome, EGPA, GPA and MPA.

and usually involve the vascular district distal to the inflamed arterial branch[56]. In general, the pathophysiology of ischaemia in Takayasu arteritis involves vasculitic as well as thromboembolic mechanisms, owing to the coexistence of inflammatory infiltration of the vascular wall and increased susceptibility of the endothelium to atheroma formation[38,57]. Accordingly, the most common regional symptom of Takayasu arteritis is claudication of one or both limbs, with reduction or loss of a palpable pulse owing to vasculitic stenosis and possibly atherothrombosis of the aorta and its proximal branches, most frequently involving the subclavian, axillary, brachial, iliac or femoral arteries[54,56]. Renal ischaemic disease is frequent in Takayasu arteritis, as almost 40% of patients present with renal artery stenosis and ~5% have complete renal artery occlusion[58].

Atherosclerosis in Takayasu arteritis is an important contributor to cardiovascular morbidity; it is characterized by increased carotid intima-media thickness, atherosclerotic plaques, arterial stiffness and circumferential vascular calcifications, particularly at sites of vasculitic involvement[59–61].

Ischaemic stroke or transient ischaemic attack is common in Takayasu arteritis and is observed in 10–20% of patients[62,63]. One large retrospective study found that ~52% of individuals with Takayasu arteritis who experienced an ischaemic stroke were first diagnosed with Takayasu arteritis after admission for a suspected stroke event[64].

Venous involvement. VTE is less prevalent in GCA and Takayasu arteritis than in other forms of vasculitis such as Behçet syndrome, in which both arterial inflammation and venous inflammation are well documented. In LVV, vessel inflammation is primarily arterial, and venous involvement remains largely understudied; consequently, its pathogenesis could be different to that of arterial involvement. Nonetheless, some reports suggest an increased prevalence of venous involvement in individuals with LVV compared with the general population. A large population-based study in a North American cohort found an increased risk of VTE of about 3.5- to 4-fold, which was increased to about 7-fold in the first months after diagnosis, compared with individuals without GCA[65]. Similar results also emerged from a large mixed cohort of GCA and polymyalgia rheumatica, in which a 3.88-fold risk of deep vein thrombosis (DVT) and 4.21-fold risk of pulmonary embolism were found compared with age- and sex-matched individuals with osteoarthritis[66].

There is also evidence to suggest that glucocorticoid therapy contributes to increasing the risk of DVT in GCA[67]; however, a large

study demonstrated a temporal trend in the incidence of VTE in GCA, with a 10-fold increase within 3 months of GCA diagnosis but also an almost fourfold increase among patients with imminent GCA who had not been exposed to oral glucocorticoids, indicating a role for inflammation-associated thrombosis that is independent of glucocorticoid use[68].

A 5-year prospective study investigated the influence of disease-specific factors on the incidence of DVT over time in GCA and found that age, number of comorbidities, frequency of hospital admission and thrombocytosis were significantly associated with an increased risk of developing VTE[69]. Despite suggestive evidence, however, this topic is still largely under-investigated and no precise risk estimates can be made at the present time.

Behçet syndrome

Behçet syndrome is a systemic variable-vessel vasculitis of unknown aetiology, characterized by different manifestations that vary from patient to patient and overlap over time[70]. Oral and genital ulcers and uveitis are the hallmarks of the disease, but major organ involvement (for example, neurological, gastrointestinal and vascular manifestations) can also be present[70]. Disease onset is usually in the third decade of life, without a clear gender predominance, even if the disease course is usually more severe in men than in women[70,71].

Vascular involvement in Behçet syndrome varies considerably in prevalence, ranging from 6% to 52% (refs. 72,73), yet it remains the leading cause of morbidity and mortality[74]. Like ocular involvement, vascular Behçet syndrome is more commonly observed in young men[72], often presenting early in the disease course with relapsing episodes, systemic inflammation and fever[75,76]. Patients with vascular Behçet syndrome tend to have genital ulcers and uveitis less frequently[73,77] and approximately one-third present with isolated vascular manifestations without typical mucocutaneous lesions[75]. Venous involvement is more frequent than arterial disease, although the latter, including aneurysms and in situ thrombosis, can also occur; notably, the coexistence of both venous and arterial thrombosis is a unique feature of Behçet syndrome[78] (Fig. 2).

Arterial involvement. Pulmonary artery involvement is the most common form of arterial involvement in Behçet syndrome. It is characterized by pulmonary artery aneurysms or in situ pulmonary artery thrombosis, which are usually bilateral, multiple and confined mostly to the lower lobes[79].

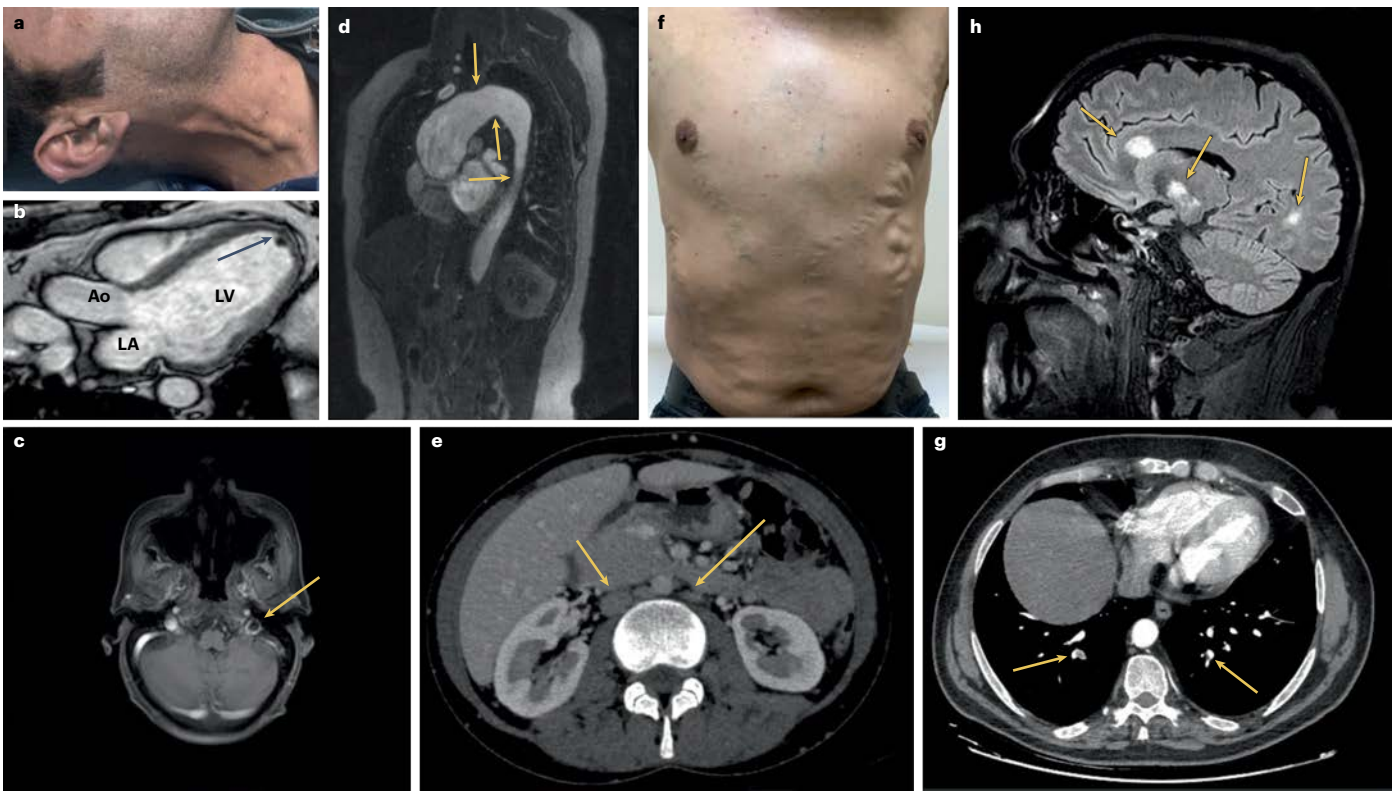


Fig. 2 | Arterial and venous manifestations in patients with systemic and monogenic vasculitis. a, Extended neck veins in a man with superior vena cava thrombosis due to Behçet syndrome. **b**, Cardiac MRI in a 42-year-old man with EGPA. Cine-3 chamber view. An apical thrombus is visible (grey arrow). **c**, Contrast-enhanced brain CT showing thrombosis of the left jugular vein (yellow arrow) in a patient with Behçet syndrome. **d**, Contrast-enhanced MRI angiography in a 44-year-old woman with Takayasu arteritis, showing pronounced vasculitic thickening of the arterial wall of the ascending and descending aorta, with small focal areas of partial thrombosis at the level of the aortic arch and in the descending aorta (yellow arrows). **e**, Contrast-enhanced abdomen CT showing inferior vena cava thrombosis (shorter yellow arrow), as well as thrombosis on the left renal vein extending to the inferior vena cava (longer yellow arrow). **f**, Collateral veins on the chest and abdomen in a man with superior vena cava syndrome due to Behçet syndrome. **g**, Contrast-enhanced chest CT showing acute thrombosis on the bilateral lower lobe segmentary branches in a patient with Behçet syndrome (yellow arrows). **h**, Multiple and diffuse lacunar lesions and ischaemic lesions (yellow arrows) at the level of the basal ganglia/corpus callosum in a 34-year-old man with DADA2. Ao, aorta; DADA2, deficiency of adenosine deaminase 2; EGPA, eosinophilic granulomatosis with polyangiitis; LA, left atrium; LV, left ventricle. Figure 2h, image courtesy of Roberto Padoan.

In this regard, Hughes-Stovin syndrome is a rare systemic condition that is considered to be part of the Behçet syndrome spectrum, and is characterized by widespread venous thrombosis in combination with bilateral pulmonary artery aneurysms[80]. Unlike DVT, pulmonary artery aneurysm is pathognomonic for Behçet syndrome[81]. Within the past 2 years, studies demonstrated that presentation with isolated pulmonary artery thrombosis has become the dominant type of arterial involvement in Behçet syndrome in contrast to what has been documented in earlier reports[82,83]. Massive bleeding caused by aneurysm rupture into bronchi is the major complication of pulmonary artery aneurysms. Isolated forms of pulmonary artery thrombosis have been associated with better outcomes than forms that coexist with pulmonary artery aneurism[83]. Recurrence rates range from 20% to 30% and mortality rates are between 11% and 26% (refs. 79,83). Furthermore, the outcome can rarely be complicated by pulmonary arterial hypertension, bronchial artery enlargement, pneumothorax or solitary giant aneurysm requiring lobectomy[79,84,85]. Extra-pulmonary arterial involvement, usually a late event, includes aneurysms, thrombotic occlusions, stenosis and aortitis and frequently affects the abdominal aorta, iliac, femoral and carotid arteries[86]. Cardiac involvement, although rare, can cause intracardiac thrombosis[87], typically on the right side of the heart, which is strongly associated with pulmonary artery involvement and can potentially progress to endomyocardial fibrosis[87] (Fig. 1).

Venous involvement. Venous involvement in Behçet syndrome manifests clinically as recurrent thrombotic attacks. The thrombus is thick, inflammatory and strongly adherent to the underlying inflamed endothelium, so that the risk of pulmonary emboli is quite low[78].

Vein thrombosis is the most frequent vascular manifestation, accounting for 70–80% of vascular events[75,77]. It typically affects both deep and superficial veins of the lower legs, often bilaterally. Recurrence occurs in up to 45% of affected individuals within 1 year[76]. Poor recanalization, residual thrombi and lack of immunosuppressive therapy are important predictors of relapse[76,88]. Thrombotic events frequently lead to post-thrombotic syndrome, which can include recalcitrant stasis ulcers[89]. DVT could extend to the inferior vena cava via ilio-femoral vein thrombosis, often progressing insidiously, leading to collateral vein formation and post-thrombotic syndrome[90]. Hepatic and supra-hepatic inferior vena cava involvement, accompanied by hepatic vein thrombosis, leads to Budd–Chiari syndrome (BCS)[90]. Mortality in BCS associated with Behçet syndrome is significantly higher than that in BCS from non-Behçet syndrome causes[91,92]. Superior vena cava thrombosis is less common than involvement of the inferior vena cava[90]. It is often associated with upper-extremity vein thrombosis with potential extension to the jugular and subclavian veins. Superior vena cava thrombosis generally has a more favourable outcome and a less relapsing course than inferior vena cava involvement, although complications such as haemoptysis, sleep apnoea and pleural effusions can occur[90]. Cerebral venous sinus thrombosis (CVST), commonly involving the superior sagittal and transverse sinuses, can be the sole vascular manifestation in juvenile patients and young female patients[93]. Presentation is typically sub-acute or chronic, with fewer neurological deficits and seizures than CVST of non-Behçet syndrome causes[94]). Although relapses of CVST are rare, complications such as irreversible optic atrophy (5%), visual field defects, residual headaches and arteriovenous fistulas have been reported[94].

Monitoring of subclinical venous involvement. Growing evidence indicates that vein wall thickness (VWT) of the lower-extremity veins, as measured with Doppler ultrasonography, is increased in Behçet syndrome compared with that found in several inflammatory diseases and in healthy individuals[95,96]. VWT was found to be particularly increased among those with Behçet syndrome who had no apparent vascular involvement, suggesting that VWT could be an early indication of subclinical vascular inflammation[95,97]. However, most studies of VWT as an imaging marker of disease have been conducted in Turkey and should be validated in other regions before it can be established as a standard technique for disease monitoring. By contrast, a 2024 study from Brazil, a region with a low prevalence of Behçet syndrome, evaluated the utility of VWT as a diagnostic tool for Behçet syndrome and found that although VWT was increased in patients with vascular involvement, its diagnostic accuracy was limited and insufficient for clinical use[98].

Main mechanisms of thrombosis in systemic vasculitis

This section explores the cellular and humoral mechanisms that drive endothelial dysfunction, thrombosis and tissue damage in systemic vasculitis. For each type of vasculitis, different immune-mediated processes can lead to a prothrombotic state (Fig. 3).

Cellular and humoral inducers of endothelial dysfunction

Endothelial dysfunction is the common outcome of different immune-mediated processes across all types of vasculitis and constitutes the main switch to a prothrombotic state[7,99]. However, a wide array of mechanisms of damage and of aggravating factors can be observed, depending on the type of systemic vasculitis.

Thrombosis in AAV, and especially GPA and MPA, is the culmination of humoral- and neutrophil-driven inflammation at the level of the endothelium[18]. The main mechanism causing endothelial dysfunction and thrombosis in AAV is reactive oxygen species-mediated vascular damage caused by neutrophils that become overactivated when surface-expressed myeloperoxidase (MPO) and proteinase 3 (PR3) antigens are bound by circulating ANCAs[100]. Similar to LVV, the adhesion of activated neutrophils to the vascular wall is the first step leading to thrombosis. In the case of AAV, however, the increased expression of intracellular adhesion molecules (ICAMs) and selectins occurs in response to systemic inflammatory stimuli mediated by the release of the cytokines TNF and IL1-β or through local triggers such as bacterial lipopolysaccharides[101]. Differently from LVV, neutrophil-rich vessel infiltration in AAV is particularly pronounced (that is, 'neutrophil swarming'), leading to the formation of granulomas through the interaction of neutrophils with resident monocytes and the abundant release of neutrophil extracellular traps (NETs)[102]. This 'overshooting' activation of neutrophils leads to the release of granular proteinases en masse, leading to oxidative bursts with extensive generation of reactive oxygen species[103]). These processes cause the overexpression of ANCA antigens, including MPO and PR3, on the surface of intravascular neutrophils, which is considered the major contributor to the loss of local tolerance[104]. The binding of circulating ANCAs to surface-expressed MPO and PR3 ultimately leads to the amplification of vasculitis by indefinitely sustaining neutrophil activation and local reactive oxygen species generation, and by triggering the release of the complement fraction C5a from neutrophils[105]. Notably, in the presence of TNF, primed neutrophils increase their secretion of C5a, creating a synergistic pro-inflammatory loop that involves TNF, ANCAs, the complement system and the endothelium. ANCAs and neutrophil activity have also been suggested to interfere with coagulation pathways. In a prospective study, patients with active AAV had elevated

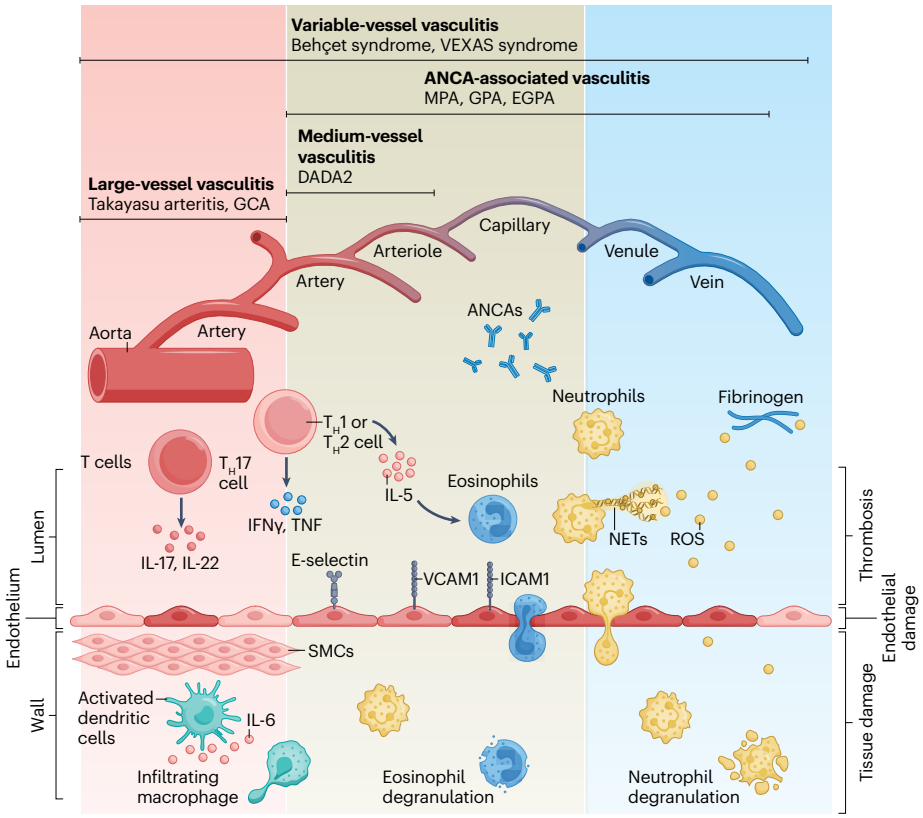


Fig. 3 | Vessel size and cellular mechanism associated with thrombotic manifestation in vasculitis. The figure depicts vessel sizes and the associated cellular and molecular mechanisms implicated in thrombotic manifestations across different types of vasculitis. Key processes involved in promoting thrombotic events in vasculitis include the upregulation of adhesion molecules (such as vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)), and the infiltration and hyperactivation of different cell sub-types, such as neutrophils, macrophages and eosinophils. Neutrophil-derived elements, including neutrophil extracellular traps (NETs), reactive oxygen species (ROS) and anti-neutrophil cytoplasmic antibodies (ANCAs), are additional important mediators of inflammation-induced thrombosis in vasculitis. The figure highlights the distinct pathophysiological pathways in small, medium and large vessels, providing a detailed overview of how immune dysregulation, endothelial injury and cellular activation collectively contribute to thrombotic events in systemic vasculitis and monogenic forms of vasculitis. DADA2, deficiency of adenosine deaminase 2; EGPA, eosinophilic granulomatosis with polyangiitis; GCA, giant-cell arteritis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; VEXAS, vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic. The schematic in the top part of the figure is adapted with permission from ref. 199, John Wiley & Sons.

levels of circulating thrombin–antithrombin complexes, D-dimers and coagulation factors in complex with their natural inhibitors of the intrinsic coagulation pathway, and the abundance of these markers of hypercoagulability correlated with disease activity, ANCA titre, CRP level and proteinuria[32]. In line with these findings, Von Willebrand factor antigen levels are increased in childhood-onset AAV and correlate with disease activity[33].

In EGPA, eosinophils provide a further pro-thrombotic stimulus; these cells have prominent prothrombotic effects that are synergic but independent from neutrophil-mediated inflammation[37]. In general, the thrombogenic properties of eosinophils are mediated by the production of reactive oxygen species and granule protein and by intrinsic procoagulatory properties[106]. In particular, activated eosinophils degranulate high quantities of NADPH-oxidase and eosinophil peroxidase, which generate a quantity of reactive oxygen species substantially higher than neutrophils, thus sustaining endothelial damage. Eosinophil-specific granule proteins, namely eosinophilic cationic protein and major basic protein, can directly interfere with the coagulation cascade, in part through a Factor XII-dependent mechanism[107]. Adding to these effects, activated intravasal eosinophils can provide a procoagulant oxidized aminophospholipid scaffold that supports the exposure of tissue factor, facilitating thrombin formation and platelet adhesion[108]. Finally, experimental evidence has shown that fibrin clots of individuals with EGPA are less permeable and more lysis resistant than those of healthy individuals, possibly owing to post-translational redox modification to the secondary structure of fibrinogen[108].

In LVV, dendritic-cell activation in the adventitial layer represents the main triggering factor for vascular inflammation[109]. Vascular dendritic cells in LVV severely under-express programmed cell death 1 ligand 1 (ref. 110). This immune checkpoint dysfunction causes the suppression of inhibitory signalling from dendritic cells, which leads to uncontrolled T cell and macrophage infiltration into the arterial wall[111]. The increased secretion of pro-inflammatory cytokines escalates the inflammatory process by shifting T cells towards a T helper 1 (T_H1) and T_H17 phenotype with upregulation of TNF, IFNγ, IL-6, IL-17 and IL-21, which sustain macrophage activity and vascular neutrophil infiltration[112].

This cascade activates the two main processes that synergically lead to thrombosis in LVV: vessel-wall hypertrophy and endothelial damage. Macrophage degranulation of matrix metalloproteases (MMPs) such as MMP-9 and reactive oxygen species in the vascular wall facilitates collagen degradation and disruption of the endothelial cell layer[113,114]. Endothelial disruption is further exacerbated by the respiratory burst of infiltrating neutrophils, which is ultimately the major final effector of endothelial and vascular smooth muscle cell damage in LVV. When exposed to reactive oxygen species, endotheliocytes and smooth muscle cells switch to a prothrombotic proangiogenic phenotype that promotes atheroma formation by increasing the expression of ICAMs such as ICAM-1 and ICAM-2, vascular cell adhesion molecule 1, E-selectin and P-selectin, as well vascular hyperplasia through the release of PDGF and VEGF[115]. The totality of these changes ultimately results in increased blood turbulence and increased platelet adhesion at sites of inflammation, which sets the basis for both increased chronic atherothrombosis and acute ischaemia in active LVV. Nonetheless, the clinical expression of LVV can also be influenced by non-immune factors, such as homozygosity for the *PLA2* allele of the platelet glycoprotein receptor IIIA in

GCA, which has been associated with increased susceptibility to the development of optic ischaemia[116] (Fig. 2).

Behçet syndrome as a model of inflammatory thrombosis

Behçet syndrome represents a model disease for inflammation-induced thrombosis and specifically for neutrophil-induced thromboinflammation. As observed in other forms of systemic vasculitis, T_H1 and T_H17 polarization leads to increased vascular infiltration of activated neutrophils, which are the final effectors of endothelial damage and platelet activation in Behçet syndrome[117]. Notably, Behçet syndrome has a markedly neutrophil-dependent pathogenesis, even more so than other forms of vasculitis[118,119]. In Behçet syndrome, the extensive activation of vessel-infiltrating neutrophils causes an indiscriminate release of their cytotoxic granules and reactive oxygen species both in the target tissue and in the bloodstream[118,120]. The release of these mediators leads to a major change in the systemic redox state, which directly interferes with cellular and molecular processes at the vascular level, in particular causing endothelial dysfunction, impairment of coagulation and pathological fibrinogen polymerization[118,120,121]. Experimental evidence shows that patients with Behçet syndrome have increased serum levels of lipid peroxidation markers[122], increased formation of NETs[119] and reduced levels of antioxidant enzymes such as glutathione peroxidase and catalase[123], and that these redox parameters correlate closely with disease activity[124]. At the cellular level, this shift of the redox state towards increased oxidation is the result of the hyperactivation of neutrophils, with respiratory burst and increase of NADPH-oxidase activity leading to extensive generation of neutrophil-derived reactive oxygen species at vascular sites[121]. Accordingly, a decreased production of nitric oxide, a vasodilator and key antithrombotic factor, and increased levels of endothelial injury markers have been reported in active Behçet syndrome[124]. As well as directly damaging the endothelium and inducing a prothrombotic phenotype, reactive oxygen species induce oxidative modifications of fibrinogen that affect its secondary structure and function of fibrin[120]. Oxidation impaired the capacity of fibrinogen to form a fibrin clot and reduced the susceptibility of fibrin networks to plasmin-induced lysis, resulting in a shifted prothrombotic balance[120]. Interestingly, increased oxidation was observed only in fibrinogen samples incubated with neutrophils, and not with monocytes and lymphocytes, from patients with Behçet syndrome[120]. Moreover, a dose-dependent relationship between neutrophil activity and NETosis and DVT has been described[125], which could be further exacerbated in Behçet syndrome (Fig. 3).

Management of arterial and venous thrombotic events in systemic vasculitis

In this section, we review the current evidence regarding the clinical management of cardiovascular risk in patients with systemic vasculitis. Additionally, we discuss the standard of care for managing acute arterial and venous thrombotic events across different types of vasculitis (Table 1).

Anti-neutrophil cytoplasmic antibody-associated vasculitis

Guidelines for primary or secondary prevention of CVD in AAV that take into consideration the added risk factors related to vasculitis are lacking. Concerning traditional cardiovascular risk factors, physicians caring for patients with AAV should follow the existing guidelines for the prevention of CVD in the general population[15]. Periodic assessment of individual cardiovascular risk profiles and modification of preventive treatments are recommended[15]. A recent study showed that approximately one third of 144 AAV patients from the Netherlands and Canada had indications for blood pressure or lipid-lowering therapy, but were either not treated or not treated at target levels[126]. Whether more stringent therapeutic targets and follow-up should be adopted in AAV, such as a serum LDL target level of <55 mg/dl, remains a matter of debate, as to our knowledge no study thus far has specifically addressed this issue[126].

Some data suggest that statins inhibit neutrophil activation by ANCAs[127] and might even be beneficial for the prevention of AAV relapses[128]. As the available evidence is limited, however, the anti-inflammatory effect of statins in autoimmune diseases, including AAV, should not be overestimated.

Tight control of AAV activity can reduce cardiovascular risk and is essential to prevent accrual of damage, for example, progressive impairment of renal function that increases the risk of CVD[129]. According to emerging data from randomized controlled trials (RCTs), more rapid tapering of glucocorticoids (such as with the PEXIVAS regimen[130]) or the use of newly available drugs for treating AAV (for example, avacopan instead of glucocorticoids for induction of remission)[131] constitute potential strategies to limit exposure to glucocorticoids, agents that have atherogenic and pro-thrombotic properties[15].

Routine anticoagulation is not recommended in AAV despite the high rate of VTE, given the unpredictable risk of bleeding related to necrotizing vascular lesions, although this risk could probably be diminished by postponing anticoagulation until stabilization of vasculitis is achieved[15]. Antiplatelet agents are not a viable alternative to anticoagulants in active AAV, as they can also cause bleeding and have low efficacy in the prevention of DVT[132].

Increased eosinophil count has been associated with an elevated risk of CVD and represents an independent predictor of major cardiovascular events[106]. In this context, patients presenting with AVTEs in conjunction with blood hypereosinophilia warrant careful evaluation for a variety of potential differential diagnoses other than EGPA, such as idiopathic hypereosinophilic syndrome[133].

Currently, no established guidelines or RCTs specifically address the treatment of thrombotic events in EGPA. Although immunosuppressive therapy might potentially reverse endothelial dysfunction, the role of treatment — particularly the use of corticosteroids — remains controversial. Given the evidence that eosinophils directly contribute to the pathogenesis of thrombosis, therapies targeting eosinophils, such as anti-IL-5 and anti-IL-5 receptor antibodies, might help to prevent further thrombotic complications[134].

For patients with venous thrombosis, no consensus exists regarding the optimal duration of anticoagulation therapy once eosinophilia has been normalized. Several case reports have documented the recurrence of thromboembolic events despite adequate anticoagulation[135]. However, once eosinophil counts have normalized and thrombi have resolved, discontinuation of anticoagulation can be considered in the absence of other risk factors[136]. Further research is needed to identify subgroups of individuals who might benefit from prolonged anticoagulation (Table 1).

Large-vessel vasculitis

Patients with GCA and Takayasu arteritis are at an increased risk of developing cardiovascular and cerebrovascular events, including myocardial infarction, stroke, aneurysm rupture and peripheral vascular disease[137–139], and these events are associated with substantially increased mortality[140]. The indication for antiplatelet therapy in LVV, however, is still debated. Two relatively small retrospective studies —

Table 1 | Treatment strategies according to different vascular involvement in systemic vasculitis

Vascular manifestation	Immunosuppressants	Antiplatelet agents	Anticoagulants	Statins
ANCA-associated vasculitis				
Primary prophylaxis	Oral glucocorticoids alone or in combination with csDMARDs for induction and maintenance of remission might reduce thrombotic risk[15,22]	Antiplatelet therapy did not protect against major cardiovascular events in patients with AAV[190] At present not recommended	Not recommended[15]	In vitro studies demonstrated that statins are able to reduce neutrophil degranulation induced by ANCA[191]; RCTs and real-world studies on the use of statins in AAV are lacking; furthermore, cases of statin-induced AAV have been reported[192,193]
Venous involvement	N/A	Not recommended[15]	Treat according to current guidelines for venous thrombosis[15]; duration of anticoagulation therapy in patients with AAV and VTE is controversial; ideally, anticoagulation should be continued if vasculitis is active[194]	N/A
Arterial involvement	N/A	Treat according to current guidelines for cardiovascular events[15]	Treat according to current guidelines for cardiovascular events[15]	N/A
Large-vessel vasculitis				
Primary prophylaxis	Oral glucocorticoids alone or in combination with traditional immunosuppressants for induction and maintenance of remission might reduce thrombotic risk[145]	In GCA, antiplatelet therapy is not associated with reduced severe ischaemic complications; a marginal benefit may exist when used with corticosteroids Limit antiplatelet use to patients with cardiovascular indications or high ischaemic risk[143,145] In Takayasu arteritis, the use of antiplatelets as primary prophylaxis showed discordant results; EULAR recommends use of antiplatelets after individual evaluation, considering vessel stenosis and cardiovascular risk factors[145,195]	N/A	No benefit in the use of statins in GCA[148]; in Takayasu arteritis, discordant results have been observed; at present, statins are not recommended for primary prophylaxis[195–197]
Arterial involvement	N/A	Antiplatelet agents should be used lifelong in patients undergoing endovascular procedures[148]	Treat according to current guidelines for cardiovascular events[148]	Treat according to current guidelines for cardiovascular events[148]
Behçet syndrome				
DVT	Oral glucocorticoids alone or in combination with csDMARDs for first unprovoked DVT; glucocorticoids plus bDMARDs for relapses[78,150]	N/A	Anticoagulation for DVT is controversial in Behçet syndrome; treatment does not seem to affect relapse rate, presence of post-thrombotic syndrome or residual thrombosis; optimal duration of anticoagulant therapy is unknown[78,89]	Statins might improve endothelial dysfunction in Behçet syndrome[198]; however, statins are not routinely used in DVT in Behçet syndrome
Budd–Chiari syndrome and splanchnic thrombosis	High-dose intravenous methylprednisolone and csDMARDs (cyclophosphamide) or bDMARDs (anti-TNF) for induction of remission; azathioprine for remission maintenance[78,150]	N/A	Vitamin K antagonists can be used in combination with immunosuppressants[78,150]	Statins might improve endothelial dysfunction in Behçet syndrome[198]; however, statins are not routinely used in Budd–Chiari syndrome and splanchnic thrombosis in Behçet syndrome
Pulmonary or aortic aneurysms or major arterial thrombotic events	High-dose glucocorticoids in combination with anti-TNF or cyclophosphamide; azathioprine for small-sized aneurysms; tocilizumab for refractory disease[78,150]	Treat according to current guidelines for cardiovascular events or arterial thrombosis[150]	Anticoagulation is contraindicated in the presence of pulmonary artery aneurysms It is not recommended for arterial events in Behçet syndrome	Statins might improve endothelial dysfunction in Behçet syndrome[198]; however, statins are not routinely used in the management of neither pulmonary and aortic aneurysms nor major arterial thrombotic events in Behçet syndrome

Table 1 (continued) | Treatment strategies according to different vascular involvement in systemic vasculitis

Vascular manifestation	Immunosuppressants	Antiplatelet agents	Anticoagulants	Statins
DADA2				
Ischaemic stroke	Glucocorticoids and/or anti-TNF therapy for remission induction Anti-TNF therapy should be considered for the prevention of further strokes[178]	Not recommended owing to the potential risk of brain haemorrhage[178]	Not recommended owing to the potential risk of brain haemorrhage[178]	N/A
VEXAS syndrome				
Venous thrombosis	Oral glucocorticoids alone or in combination with JAK inhibitors[189]	N/A	Recommended, but no studies have compared heparin, vitamin K antagonists and direct oral anticoagulants in VEXAS syndrome Optimal duration of anticoagulation therapy is unknown[189]	N/A

ANCA, anti-neutrophil cytoplasmic antibody; AAV, ANCA-associated vasculitis; bDMARD, biologic DMARD; csDMARD, conventional synthetic DMARD; DVT, deep venous thrombosis; EGPA, eosinophilic granulomatosis with polyangiitis; EULAR, European Alliance of Associations for Rheumatology; GCA, giant-cell arteritis; JAK, Janus kinase; N/A, not applicable; RCT, randomized controlled trial; VEXAS, vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic; VTE, venous thromboembolism.

the results of which would later fail to be replicated — found that the addition of low-dose aspirin to the therapy regimens of GCA was associated with a significantly lower incidence of cardiovascular events, including stroke and optic ischaemia[141,142]. However, a growing number of studies and meta-analyses from multiple GCA cohorts worldwide did not confirm the benefits of low-dose aspirin in preventing vascular ischaemic events in LVV[143,144].

Owing to this conflicting evidence, and in consideration of the known bleeding risk associated with antiplatelet therapy, the most recent (2018) European Alliance of Associations for Rheumatology recommendations no longer recommend routine prescription of low-dose aspirin in GCA, limiting its use to cases with overlapping clinical indications for primary or secondary prevention of thrombotic arterial events[145]. As evidence for the use of antiplatelet agents in Takayasu arteritis remains only anecdotal, the same principles as in GCA should be applied. In general, lifelong use of antiplatelet agents is recommended following endovascular or surgical interventions in patients with GCA or Takayasu arteritis[146].

Currently, interest is growing in other candidate drugs to address the increased cardiovascular risk in LVV, such as statins. Statins can have immunomodulatory properties at the level of atherosclerotic plaque by eliciting vasodilatory and anti-inflammatory effects through increased local expression of nitric oxide synthase[147]. However, the evidence so far is scarce and contradictory[148], and there does not seem to be a relevant influence of statins on the clinical course and on the glucocorticoid requirements of patients with LVV[145] (Table 1). Specific RCTs should be designed to answer these questions.

Regarding immunosuppressive therapy, glucocorticoid-sparing conventional synthetic DMARDs (csDMARDs) and bDMARDs can limit the increased cardiovascular risk connected with long-term use of glucocorticoids, which are routinely prescribed in LVV. Notably, however, early results from a phase III trial have demonstrated the efficacy of the Janus kinase (JAK) inhibitor upadacitinib as a therapeutic option for GCA[149]. The entry of JAK inhibitors into the therapeutic armamentarium for LVV will require discussion of the balance between the need for glucocorticoid sparing and the potentially increased risk of cardiovascular events linked to JAK inhibitors in elderly patients.

Behçet syndrome

The management of vascular Behçet syndrome varies according to the vascular territory involved and the type and severity of the event[78]. Given the pathogenetic mechanisms sustaining thrombosis in Behçet syndrome, glucocorticoids, immunosuppressants and bDMARDs are the treatments of choice, whereas the use of anticoagulants is highly debated[78,150].

According to the 2018 European Alliance of Associations for Rheumatology recommendations for the management of Behçet syndrome, patients experiencing a first acute venous event at a typical site (that is, DVT of the upper or lower limbs) should be treated with glucocorticoids and immunosuppressive agents (primarily azathioprine)[150], whereas anticoagulation is not routinely recommended in Behçet syndrome-associated DVT. Individuals with refractory VTE should undergo treatment with TNF inhibitors (primarily infliximab or adalimumab)[151–153], possibly in combination with csDMARDs. Of note, a 2024 phase II multicentre randomized study found that, in patients with major vascular or central nervous system involvement, first-line therapy with infliximab was associated with better efficacy and a lower incidence of adverse events than cyclophosphamide[153]. IFN-α has also shown efficacy, especially in patients with recurrent disease, despite treatment with azathioprine, and should be considered in selected cases[154]. Within the past few years, a few studies from China have suggested the potential effectiveness of the JAK inhibitors baricitinib and tofacitinib in patients with refractory Behçet syndrome with vascular involvement[155–157]. Four retrospective observational studies have shown that the addition of anticoagulants to immunosuppressants does not seem to significantly decrease the recurrence of vascular events compared with treatment with DMARDs alone[151,158–160]. However, the combination of anticoagulants with immunosuppressants is recommended for large thrombotic events at atypical sites, such as thrombosis of the vena cava, BCS, intracardiac thrombi or cerebral venous sinus thrombosis[78,150].

Patients with large pulmonary or aortic aneurysms or major arterial thrombotic events should be treated with high-dose glucocorticoids in combination with TNF blockers (especially infliximab) or cyclophosphamide[153,161], whereas csDMARDs (mainly azathioprine) could be used for small aneurysms and pseudoaneurysms[162]. The anti-IL-6 receptor antibody tocilizumab has been suggested for major arterial involvement in patients whose condition is refractory to multiple treatments; however, its use requires further studies[163,164] (Table 1).

Monogenic vasculitis

In this section, we review the epidemiology, pathophysiology and clinical features of the major forms of monogenic vasculitis, with a particular focus on their cardiovascular manifestations. We also discuss the current approaches to managing arterial and venous thrombosis associated with these conditions.

Deficiency of adenosine deaminase 2

DADA2 is an autosomal-recessive disorder caused by loss-of-function mutations in the *ADA2* gene[165]. First described in 2014, DADA2 is characterized by a broad spectrum of clinical manifestations. Common features include inflammatory constitutional symptoms, haematological abnormalities and vascular manifestations involving small-to-medium-sized vessels[165]. Vascular manifestations mostly present as ischaemic strokes[166,167]. However, vascular involvement in DADA2 can extend to multiple other organs, including the liver, spleen, kidneys, heart and bowel[167].

Ischaemic strokes in DADA2 affect nearly 50% of patients and can be the presenting feature in approximately 30% of cases[166,168,169]. Neurological events typically occur at a very young age and are frequently associated with inflammatory flares[170]. Haemorrhagic strokes are an additional complication of DADA2, reported in approximately 12–17% of cases[166,167,171]. Intracranial haemorrhages can occur either in isolation or concomitantly with ischaemic strokes[166,169] (Fig. 2).

Cerebral MRI findings in patients with DADA2 typically reveal multiple small ischaemic infarcts in regions such as the nucleo-capsular, mesencephalic and thalamic areas, underscoring the predominant involvement of deep perforating arteries[172]. Notably, these findings are frequently observed in the absence of detectable features of cerebral vasculitis on angiographic studies, such as vessel narrowing or wall thickening[166].

The histopathology of DADA2 is characterized by necrotizing transmural inflammation of medium-sized arteries with prominent leukocyte infiltration. The mechanisms by which DADA2 leads to vascular inflammation remain unclear. It has been hypothesized that DADA2 might shift monocyte polarization towards the pro-inflammatory 'M1-like' phenotype[165,170]. Additionally, DADA2 has been implicated in promoting the formation of NETs from activated neutrophils[170,173,174]. Both these processes could lead to systemic immune activation and increased production of pro-inflammatory cytokines, in particular TNF[170].

In individuals with DADA2 who present with acute stroke, immunosuppressive treatments, including glucocorticoids and/or TNF inhibitors, should be promptly initiated[175]. Although comparative data on the efficacy of glucocorticoids versus TNF inhibitors in this setting remain scarce, TNF inhibitors have been shown to effectively prevent the recurrence of strokes[176,177]. Secondary haemorrhagic stroke conversion has been documented in some individuals with DADA2 treated with antiplatelet agents, anticoagulants and/or other antithrombotic therapies[169,178]. Given the potential risk of haemorrhagic complications and the limited evidence supporting their efficacy, the use of antithrombotic therapies in patients with DADA2 is not recommended[169,178] (Table 1).

Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic syndrome

VEXAS syndrome, first identified in 2020, is a late-onset autoinflammatory condition caused by somatic mutations in the *UBA1* gene, which affects myeloid precursors[179]. Clinical manifestations of VEXAS syndrome include recurrent fevers, neutrophilic dermatoses, haematological abnormalities involving the myeloid lineage (including the presence of bone marrow vacuoles), ear and nose chondritis, vasculitis and pulmonary and cardiac involvement[179,180]. Rates of thrombosis are significantly elevated in VEXAS syndrome, with VTE reported in 35–56% of cases[181]. Arterial events, such as transient ischaemic attack, ischaemic stroke and myocardial infarction, are less frequent, occurring in 1–25% of patients, according to various case series, and might be influenced by other factors, such as advanced age or the high prevalence of atherosclerotic risk factors in the VEXAS syndrome population[181–183].

Nearly 60% of AVTEs occur within the first 2 years of disease onset. These events are typically unprovoked and coincide with active inflammation[181,184]. Recurrence of AVTEs occurs in 40% of patients, with 20% experiencing recurrence despite anticoagulation therapy[181,184].

Risk factors for thrombosis include cardiac and pulmonary manifestations, which could indicate a more severe disease phenotype; however, despite its high incidence, thrombosis in VEXAS syndrome is not associated with increased mortality[181].

Decreased ubiquitylation is thought to be an important promoter of thrombogenesis in VEXAS syndrome[181,182]. Loss-of-function mutations in *UBA1* result in reduced cytoplasmic ubiquitylation of proteins destined for degradation by proteasomes, leading to cellular stress, activation of inflammatory pathways and excess cytokine production, which contribute to a prothrombotic state[179,184]. Transcriptomic analysis of neutrophils in VEXAS syndrome has shown upregulation of gene networks associated with thromboinflammation, such as those involving TNF, IL-6 and IFN-γ[179]. Additionally, neutrophils in individuals with VEXAS syndrome demonstrate exaggerated spontaneous NETosis. Activated monocytes, which express tissue factor, can also contribute to thromboinflammation by playing a critical role in coagulation[185]. Persistent positivity for lupus anticoagulant and elevated factor VIII levels have been reported in several studies of VEXAS syndrome and might further enhance the prothrombotic profile of these patients[179,181].

Although screening for *UBA1* mutations in men with a first episode of VTE did not identify new cases of VEXAS syndrome, *UBA1* mutation testing can be considered in men with DVT or superficial thrombophlebitis if inflammatory markers are persistently elevated, especially when macrocytosis or other systemic inflammatory features are present[186].

Currently, no RCTs are available to guide the treatment of VEXAS syndrome, and information on the use of therapies beyond glucocorticoids is limited to observational reports, case series and cohort studies. Among therapeutic options, JAK inhibitors have elicited both clinical and biochemical responses in cases of VEXAS syndrome[187,188]. Anticoagulation is generally recommended for treating venous thrombosis, especially if JAK inhibitor therapy is being considered or used, as these drugs themselves confer an increased risk of thrombosis[184]. However, no studies have directly compared the efficacy of the three main types of anticoagulants — low-molecular-weight heparin, vitamin K antagonists or direct oral anticoagulants — in this setting. Routine anticoagulation is not recommended for patients on JAK inhibitors who do not have a history of thrombosis[189] (Table 1).

Conclusions

A growing body of evidence highlights the considerable incidence of AVTEs in both systemic and monogenic vasculitis. These complications not only contribute to morbidity but also add to the complexity of disease management. Understanding the underlying mechanisms, risk factors and optimal management strategies is crucial for improving outcomes. Future research should focus on better defining the thrombotic risk in various forms of vasculitis and developing targeted interventions to mitigate these risks. Enhanced awareness among clinicians regarding the association between vasculitis and increased thrombotic risk is essential for timely diagnosis and treatment, ultimately leading to improved patient care.

Published online: 6 May 2025

References

1. Stark, K. & Massberg, S. Interplay between inflammation and thrombosis in cardiovascular pathology. *Nat. Rev. Cardiol.* **18**, 666–682 (2021).
2. Esmon, C. T. Molecular circuits in thrombosis and inflammation. *Thromb. Haemost.* **109**, 416–420 (2013).
3. Engelmann, B. & Massberg, S. Thrombosis as an intravascular effector of innate immunity. *Nat. Rev. Immunol.* **13**, 34–45 (2012).
4. Khorana, A. A. et al. Cancer-associated venous thromboembolism. *Nat. Rev. Dis. Prim.* **8**, 1–18 (2022).
5. Mackman, N. et al. Coagulation abnormalities and thrombosis in patients infected with SARS-CoV-2 and other pandemic viruses. *Arterioscler. Thromb. Vasc. Biol.* **40**, 2033–2044 (2020).
6. Rittirsch, D., Flierl, M. A. & Ward, P. A. Harmful molecular mechanisms in sepsis. *Nat. Rev. Immunol.* **8**, 776–787 (2008).
7. Emmi, G. et al. Thrombosis in vasculitis: from pathogenesis to treatment. *Thromb. J.* **13**, 15 (2015).
8. Sherer, Y. & Shoenfeld, Y. Mechanisms of disease: atherosclerosis in autoimmune diseases. *Nat. Clin. Pract. Rheumatol.* **2**, 99–106 (2006).
9. Phillip R., Luqmani, R. Mortality in systemic vasculitis: a systematic review. *Clin. Exp. Rheumatol.* **26**, S94–104 (2008).
10. Della Rossa, A., Tavoni, A. & Bombardieri, S. Hyperviscosity syndrome in cryoglobulinemia: clinical aspects and therapeutic considerations. *Semin. Thromb. Hemost.* **29**, 473–477 (2003).
11. Kuramochi, Y. et al. Hemodynamic factors of thrombus formation in coronary aneurysms associated with Kawasaki disease. *Pediatrics Int.* **42**, 470–475 (2000).
12. Du, L. et al. Multisystemic manifestations of IgA vasculitis. *Clin. Rheumatol.* **40**, 43–52 (2021).
13. Kitching, A. R. et al. ANCA-associated vasculitis. *Nat. Rev. Dis. Prim.* **6**, 71 (2020).
14. Rathmann, J. et al. Stable incidence but increase in prevalence of ANCA-associated vasculitis in southern Sweden: a 23-year study. *RMD Open* **9**, e002949 (2023).
15. Hellmich, B. et al. EULAR recommendations for the management of ANCA-associated vasculitis: 2022 update. *Ann. Rheum. Dis.* **83**, 30–47 (2024).
16. Moiseev, S. et al. Traditional and disease-specific risk factors for cardiovascular events in antineutrophil cytoplasmic antibody-associated vasculitis: a multinational retrospective study. *J. Rheumatol.* **50**, 1145–1151 (2023).
17. Mohammad, A. J. et al. Rate of comorbidities in giant cell arteritis: a population-based study. *J. Rheumatol.* **44**, 84–90 (2017).
18. Kronbichler, A. et al. Comorbidities in ANCA-associated vasculitis. *Rheumatology* **59** (Suppl. 3), iii79–iii83 (2020).
19. Sánchez Álamo, B. et al. Long-term outcomes and prognostic factors for survival of patients with ANCA-associated vasculitis. *Nephrol. Dial. Transplant.* **38**, 1655–1665 (2023).
20. Tan, J. A. et al. Mortality in ANCA-associated vasculitis: a meta-analysis of observational studies. *Ann. Rheum. Dis.* **76**, 1566–1574 (2017).
21. Flossmann, O. et al. Long-term patient survival in ANCA-associated vasculitis. *Ann. Rheum. Dis.* **70**, 488–494 (2011).
22. Bettiol, A. et al. Risk of acute arterial and venous thromboembolic events in eosinophilic granulomatosis with polyangiitis (Churg–Strauss syndrome). *Eur. Respir. J.* **57**, 2004158 (2021).
23. Allenbach, Y. et al. High frequency of venous thromboembolic events in Churg-Strauss syndrome, Wegener's granulomatosis and microscopic polyangiitis but not polyarteritis nodosa: a systematic retrospective study on 1130 patients. *Ann. Rheum. Dis.* **68**, 564–567 (2009).
24. Clifford, A. H. & Cohen Tervaert, J. W. Cardiovascular events and the role of accelerated atherosclerosis in systemic vasculitis. *Atherosclerosis* **325**, 8–15 (2021).
25. Go, A. S. Cardiovascular disease consequences of CKD. *Semin. Nephrol.* **36**, 293–304 (2016).
26. Moiseev, S. et al. End-stage renal disease in ANCA-associated vasculitis. *Nephrol. Dial. Transplant.* **32**, 248–253 (2017).
27. Liapi, M. et al. Venous thromboembolism in ANCA-associated vasculitis: a population-based cohort study. *Rheumatology* **60**, 4616–4623 (2021).
28. Tabakovic, D. et al. High risk of stroke in ANCA-associated vasculitis-a population-based study. *Rheumatology* **62**, 2806–2812 (2023).
29. Armbruster, A. L. et al. The role of inflammation in the pathogenesis and treatment of arrhythmias. *Pharmacotherapy* **42**, 250–262 (2022).
30. Vegting, Y. et al. Traditional and disease-related cardiovascular risk factors in ANCA-associated vasculitis: a prospective, two-centre cohort study. *Joint Bone Spine* **90**, 105540 (2023).
31. Moiseev, S. et al. Association of venous thromboembolic events with skin, pulmonary and kidney involvement in ANCA-associated vasculitis: a multinational study. *Rheumatology* **60**, 4654–4661 (2021).
32. Busch, M. H. et al. The intrinsic coagulation pathway plays a dominant role in driving hypercoagulability in ANCA-associated vasculitis. *Blood Adv.* **8**, 1295–1304 (2024).
33. Go, E. et al. Von Willebrand factor antigen as a marker of disease activity in childhood-onset antineutrophil cytoplasmic antibody–associated vasculitis. *Rheumatology* **63** (SI2), SI228–SI232 (2024).
34. van Zaane, B. et al. Systematic review on the effect of glucocorticoid use on procoagulant, anti-coagulant and fibrinolytic factors. *J. Thromb. Haemost.* **8**, 2483–2493 (2010).
35. Sartorelli, S. et al. Revisiting characteristics, treatment and outcome of cardiomyopathy in eosinophilic granulomatosis with polyangiitis (formerly Churg–Strauss). *Rheumatology* **61**, 1175–1184 (2022).
36. Zampieri, M. et al. Cardiac involvement in eosinophilic granulomatosis with polyangiitis (formerly Churg–Strauss syndrome): prospective evaluation at a tertiary referral centre. *Eur. J. Intern. Med.* **85**, 68–79 (2021).
37. Bond, M. et al. At the heart of eosinophilic granulomatosis with polyangiitis: into cardiac and vascular involvement. *Curr. Rheumatol. Rep.* **24**, 337–351 (2022).
38. Watanabe, R. et al. Pathogenesis of giant cell arteritis and Takayasu arteritis — similarities and differences. *Curr. Rheumatol. Rep.* **22**, 1–11 (2020).
39. Pugh, D. et al. Large-vessel vasculitis. *Nat. Rev. Dis. Prim.* **7**, 93 (2022).
40. Hunder, G. G. et al. The American College of Rheumatology 1990 criteria for the classification of giant cell arteritis. *Arthritis Rheum.* **33**, 1122–1128 (1990).
41. De Souza, A. W. S. & de Carvalho, J. F. Diagnostic and classification criteria of Takayasu arteritis. *J. Autoimmun.* **48–49**, 79–83 (2014).
42. Salvarani, C. et al. Risk factors for visual loss in an Italian population-based cohort of patients with giant cell arteritis. *Arthritis Rheum.* **53**, 293–297 (2005).
43. Gonzalez-Gay, M. A. et al. Strokes at time of disease diagnosis in a series of 287 patients with biopsy-proven giant cell arteritis. *Medicine* **88**, 227–235 (2009).
44. Samson, M. et al. Stroke associated with giant cell arteritis: a population-based study. *J. Neurol. Neurosurg. Psychiatry* **86**, 216–221 (2015).
45. Salvarani, C. et al. Risk factors for severe cranial ischaemic events in an Italian population-based cohort of patients with giant cell arteritis. *Rheumatology* **48**, 250–253 (2009).
46. Penet, T. et al. Giant cell arteritis-related cerebrovascular ischemic events: a French retrospective study of 271 patients, systematic review of the literature and meta-analysis. *Arthritis Res. Ther.* **25**, 1–13 (2023).
47. Gonzalez-Gay, M. A. et al. Influence of traditional risk factors of atherosclerosis in the development of severe ischemic complications in giant cell arteritis. *Medicine* **83**, 342–347 (2004).
48. Dejaco, C. et al. The spectrum of giant cell arteritis and polymyalgia rheumatica: revisiting the concept of the disease. *Rheumatology* **56**, 506–515 (2017).
49. Czihal, M. et al. Sonographic and clinical pattern of extracranial and cranial giant cell arteritis. *Scand. J. Rheumatol.* **41**, 231–236 (2012).
50. Robson, J. C. et al. The relative risk of aortic aneurysm in patients with giant cell arteritis compared with the general population of the UK. *Ann. Rheum. Dis.* **74**, 129–135 (2015).
51. Evans, J. M., O'Fallon, W. M. & Hunder, G. G. Increased incidence of aortic aneurysm and dissection in giant cell (temporal) arteritis. A population-based study. *Ann. Intern. Med.* **122**, 502–507 (1995).
52. Hata, A. et al. Angiographic findings of Takayasu arteritis: new classification. *Int. J. Cardiol.* **54** (Suppl(S2)), S155–S163 (1996).
53. Yang, L. et al. Clinical features and outcomes of Takayasu arteritis with neurological symptoms in China: a retrospective study. *J. Rheumatol.* **42**, 1846–1852 (2015).
54. Mwipatayi, B. P. et al. Takayasu arteritis: clinical features and management: report of 272 cases. *ANZ J. Surg.* **75**, 110–117 (2005).
55. Comarmond, C. et al. Long-term outcomes and prognostic factors of complications in Takayasu arteritis: a multicenter study of 318 patients. *Circulation* **136**, 1114–1122 (2017).
56. Kim, H. & Barra, L. Ischemic complications in Takayasu's arteritis: a meta-analysis. *Semin. Arthritis Rheum.* **47**, 900–906 (2018).
57. Serra, R. et al. Updates in pathophysiology, diagnosis and management of Takayasu arteritis. *Ann. Vasc. Surg.* **35**, 210–225 (2016).
58. Chen, Z. et al. The renal artery is involved in Chinese Takayasu's arteritis patients. *Kidney Int.* **93**, 245–251 (2018).
59. Uysal, S. et al. Carotid artery ultrasonography and shear wave elastography in Takayasu's arteritis: a comparative analysis with diabetes mellitus. *Clin. Exp. Rheumatol.* **43**, 636–646 (2025).
60. Ucar, A. K. et al. Increased arterial stiffness and accelerated atherosclerosis in Takayasu arteritis. *Semin. Arthritis Rheum.* **60**, 152199 (2023).
61. Seyahi, E. et al. Aortic and coronary calcifications in Takayasu arteritis. *Semin. Arthritis Rheum.* **43**, 96–104 (2013).

62. Mirouse, A. et al. Cerebrovascular ischemic events in patients with Takayasu arteritis. *Stroke* **53**, 1550–1557 (2022).
63. Duarte, M. M. et al. Stroke and transient ischemic attack in Takayasu's arteritis: a systematic review and meta-analysis. *J. Stroke Cerebrovasc. Dis.* **25**, 781–791 (2016).
64. Hwang, J. et al. Ischemic stroke in Takayasu's arteritis: lesion patterns and possible mechanisms. *J. Clin. Neurol.* **8**, 109–115 (2012).
65. Aviña-Zubieta, J. A. et al. The risk of deep venous thrombosis and pulmonary embolism in giant cell arteritis: a general population-based study. *Ann. Rheum. Dis.* **75**, 148–154 (2016).
66. Michailidou, D. et al. Risk of venous and arterial thromboembolism in patients with giant cell arteritis and/or polymyalgia rheumatica: a Veterans Health Administration population-based study in the United States. *J. Intern. Med.* **291**, 665–675 (2022).
67. Ly, K. H. et al. Venous thrombosis in patients with giant cell arteritis: features and outcomes in a cohort study. *Joint Bone Spine* **84**, 323–326 (2017).
68. Unizony, S. et al. Temporal trends of venous thromboembolism risk before and after diagnosis of giant cell arteritis. *Arthritis Rheumatol.* **69**, 176–184 (2017).
69. Michailidou, D. et al. Predictive models for thromboembolic events in giant cell arteritis: a US veterans health administration population-based study. *Front. Immunol.* **13**, 997347 (2022).
70. Yazici, Y. et al. Behçet syndrome. *Nat. Rev. Dis. Prim.* **7**, 1–14 (2021).
71. Emmi, G. et al. Behçet's syndrome. *Lancet* **403**, 1093–1108 (2024).
72. Torgutalp, M. et al. Patient characteristics in Behçet's Syndrome and their associations with major organ involvement: a single-centre experience of 2118 cases. *Scand. J. Rheumatol.* **51**, 50–58 (2022).
73. Ideguchi, H. et al. Characteristics of vascular involvement in Behçet's disease in Japan: a retrospective cohort study. *Clin. Exp. Rheumatol.* **29** (4 Suppl. 67), S47–S53 (2011).
74. Saadoun, D. et al. Long-term outcome of arterial lesions in Behçet disease: a series of 101 patients. *Medicine* **91**, 18–24 (2012).
75. Tascilar, K. et al. Vascular involvement in Behçet's syndrome: a retrospective analysis of associations and the time course. *Rheumatology* **53**, 2018–2021 (2014).
76. Ozguler, Y. et al. Clinical course of acute deep vein thrombosis of the legs in Behçet's syndrome. *Rheumatology* **59**, 799–806 (2020).
77. Fei, Y. et al. Major vascular involvement in Behçet's disease: a retrospective study of 796 patients. *Clin. Rheumatol.* **32**, 845–852 (2013).
78. Bettiol, A. et al. Vascular Behçet syndrome: from pathogenesis to treatment. *Nat. Rev. Rheumatol.* **19**, 111–126 (2022).
79. Seyahi, E. et al. Pulmonary artery involvement and associated lung disease in Behçet disease: a series of 47 patients. *Medicine* **91**, 35–48 (2012).
80. Sanduleanu, S. & Jansen, T. L. T. A. Hughes–Stovin syndrome (HSS): current status and future perspectives. *Clin. Rheumatol.* **40**, 4787–4789 (2021).
81. Yazici, H. et al. Behçet syndrome: a contemporary view. *Nat. Rev. Rheumatol.* **14**, 107–119 (2018).
82. Ordu, B. et al. Pulmonary artery involvement due to Behçet's syndrome and Hughes Stovin syndrome: a comparative study. *Clin. Exp. Rheumatol.* **42**, 2021–2031 (2024).
83. Yıldırım, R. et al. Scoping beyond pulmonary artery involvement; pulmonary involvement in Behcet's disease; a retrospective analysis of 28 patients. *Clin. Rheumatol.* **42**, 849–853 (2023).
84. Tuzun, H. et al. Surgical treatment of pulmonary complications in Behçet's syndrome. *Semin. Thorac. Cardiovasc. Surg.* **30**, 369–378 (2018).
85. Ylldlzeli, Ş. O. et al. Outcomes of patients with Behçet's syndrome after pulmonary endarterectomy. *Thorac. Cardiovasc. Surg.* **66**, 187–192 (2018).
86. Tuzun, H. et al. Management and prognosis of nonpulmonary large arterial disease in patients with Behçet disease. *J. Vasc. Surg.* **55**, 157–163 (2012).
87. Geri, G. et al. Spectrum of cardiac lesions in Behçet disease: a series of 52 patients and review of the literature. *Medicine* **91**, 25–34 (2012).
88. Alibaz-Oner, F. et al. Behçet disease with vascular involvement: effects of different therapeutic regimens on the incidence of new relapses. *Medicine* **94**, e494 (2015).
89. Aksoy, A. et al. Predictors for the risk and severity of post-thrombotic syndrome in vascular Behçet's disease. *J. Vasc. Surg. Venous Lymphat. Disord.* **9**, 1451–1459 (2021).
90. Seyahi, E. Behçet's disease: how to diagnose and treat vascular involvement. *Best Pract. Res. Clin. Rheumatol.* **30**, 279–295 (2016).
91. Seyahi, E. et al. An outcome survey of 43 patients with Budd-Chiari syndrome due to Behçet's syndrome followed up at a single, dedicated center. *Semin. Arthritis Rheum.* **44**, 602–609 (2015).
92. Sakr, M. A. et al. Characteristics and outcome of primary Budd–Chiari syndrome due to Behçet's syndrome. *Clin. Res. Hepatol. Gastroenterol.* **44**, 503–512 (2020).
93. Uluduz, D. et al. Clinical characteristics of pediatric-onset neuro-Behçet disease. *Neurology* **77**, 1900–1905 (2011).
94. Yesilot, N. et al. Cerebral venous thrombosis in Behçet's disease compared to those associated with other etiologies. *J. Neurol.* **256**, 1134–1142 (2009).
95. Seyahi, E. et al. Increased vein wall thickness in Behçet disease. *J. Vasc. Surg. Venous Lymphat. Disord.* **7**, 677–684.e2 (2019).
96. Alibaz-Oner, F. et al. Femoral vein wall thickness measurement: a new diagnostic tool for Behçet's disease. *Rheumatology* **60**, 288–296 (2021).
97. Karadeniz, H. et al. Diffuse generalized venulitis as the primary pathology of Behçet's disease: a comprehensive magnetic resonance venography study. *Semin. Arthritis Rheum.* **62**, 152246 (2023).
98. Neaime, S. A. C. et al. Evaluation of common femoral vein thickness as a diagnostic tool for Behçet's disease in a non-endemic area. *Clin. Exp. Rheumatol.* **42**, 2032–2039 (2024).
99. Buckley, C. D. et al. Endothelial cells, fibroblasts and vasculitis. *Rheumatology* **44**, 860 (2005).
100. Halbwachs, L. & Lesavre, P. Endothelium-neutrophil interactions in ANCA-associated diseases. *J. Am. Soc. Nephrol.* **23**, 1449–1461 (2012).
101. Huugen, D. et al. Aggravation of anti-myeloperoxidase antibody-induced glomerulonephritis by bacterial lipopolysaccharide: role of tumor necrosis factor-α. *Am. J. Pathol.* **167**, 47 (2005).
102. Nakazawa, D. et al. Pathogenesis and therapeutic interventions for ANCA-associated vasculitis. *Nat. Rev. Rheumatol.* **15**, 91–101 (2018).
103. Hess, C., Sadallah, S. & Schifferli, J. A. Induction of neutrophil responsiveness to myeloperoxidase antibodies by their exposure to supernatant of degranulated autologous neutrophils. *Blood* **96**, 2822–2827 (2000).
104. Kraaij, T. et al. Excessive neutrophil extracellular trap formation in ANCA-associated vasculitis is independent of ANCA. *Kidney Int.* **94**, 139–149 (2018).
105. Moiseev, S. et al. The alternative complement pathway in ANCA-associated vasculitis: further evidence and a meta-analysis. *Clin. Exp. Immunol.* **202**, 394–402 (2020).
106. Réau, V. et al. Venous thrombosis and predictors of relapse in eosinophil-related diseases. *Sci. Rep.* **11**, 1–10 (2021).
107. Slungaard, A. et al. Eosinophil cationic granule proteins impair thrombomodulin function. A potential mechanism for thromboembolism in hypereosinophilic heart disease. *J. Clin. Invest.* **91**, 1721–1730 (1993).
108. Uderhardt, S. et al. Enzymatic lipid oxidation by eosinophils propagates coagulation, hemostasis, and thrombotic disease. *J. Exp. Med.* **214**, 2121–2138 (2017).
109. Ciccia, F. et al. New insights into the pathogenesis of giant cell arteritis: are they relevant for precision medicine? *Lancet Rheumatol.* **3**, e874–e885 (2021).
110. Watanabe, R. et al. Immune checkpoint dysfunction in large and medium vessel vasculitis. *Am. J. Physiol. Heart Circ. Physiol.* **312**, H1052–H1059 (2017).
111. Wen, Z. et al. The microvascular niche instructs T cells in large vessel vasculitis via the VEGF-Jagged1-Notch pathway. *Sci. Transl. Med.* **9**, eaal3322 (2017).
112. Samson, M. et al. Th1 and Th17 lymphocytes expressing CD161 are implicated in giant cell arteritis and polymyalgia rheumatica pathogenesis. *Arthritis Rheum.* **64**, 3788–3798 (2012).
113. Shirai, T. et al. Macrophages in vascular inflammation — from atherosclerosis to vasculitis. *Autoimmunity* **48**, 139 (2015).
114. Watanabe, R. et al. MMP (matrix metalloprotease)-9-producing monocytes enable T cells to invade the vessel wall and cause vasculitis. *Circ. Res.* **123**, 700–715 (2018).
115. Weyand, C. M. & Goronzy, J. J. Immune mechanisms in medium and large-vessel vasculitis. *Nat. Rev. Rheumatol.* **9**, 731–740 (2013).
116. Salvarani, C. et al. PlA1/A2 polymorphism of the platelet glycoprotein receptor IIIA and risk of cranial ischemic complications in giant cell arteritis. *Arthritis Rheum.* **56**, 3502–3508 (2007).
117. Tong, B. et al. Immunopathogenesis of Behcet's disease. *Front. Immunol.* **10**, 435349 (2019).
118. Emmi, G. et al. Behçet's syndrome as a model of thrombo-inflammation: the role of neutrophils. *Front. Immunol.* **10**, 446757 (2019).
119. Le Joncour, A. et al. Critical role of neutrophil extracellular traps (NETs) in patients with Behcet's disease. *Ann. Rheum. Dis.* **78**, 1274–1282 (2019).
120. Becatti, M. et al. Neutrophil activation promotes fibrinogen oxidation and thrombus formation in Behçet disease. *Circulation* **133**, 302–311 (2016).
121. Acikgoz, N. et al. Elevated oxidative stress markers and its relationship with endothelial dysfunction in Behçet disease. *Angiology* **62**, 296–300 (2011).
122. Kose, K. et al. Lipid peroxidation and erythrocyte antioxidant enzymes in patients with Behçet's disease. *Tohoku J. Exp. Med.* **197**, 9–16 (2002).
123. Yazici, C. et al. Increased advanced oxidation protein products in Behçet's disease: a new activity marker? *Br. J. Dermatol.* **151**, 105–111 (2004).
124. Buldanlioglu, S. et al. Nitric oxide, lipid peroxidation and antioxidant defence system in patients with active or inactive Behçet's disease. *Br. J. Dermatol.* **153**, 526–530 (2005).
125. Fuchs, T. A., Brill, A. & Wagner, D. D. Neutrophil extracellular trap (NET) impact on deep vein thrombosis. *Arterioscler. Thromb. Vasc. Biol.* **32**, 1777–1783 (2012).
126. Houben, E. et al. Prevalence and management of cardiovascular risk factors in ANCA-associated vasculitis. *Rheumatology* **58**, 2333–2335 (2019).
127. Choi, M. et al. Extracellular signal-regulated kinase inhibition by statins inhibits neutrophil activation by ANCA. *Kidney Int.* **63**, 96–106 (2003).
128. Yamaguchi, M. et al. Association between statin use and incidence of relapse in anti-neutrophil cytoplasmic antibody-associated vasculitis: a single-center retrospective cohort study. *Rheumatol. Int.* **40**, 1291–1299 (2020).
129. Floyd, L. et al. Cardiovascular disease and ANCA-associated vasculitis: are we missing a beat? *Clin. Kidney J.* **15**, 618–623 (2022).
130. Fussner, L. A. et al. Alveolar hemorrhage in antineutrophil cytoplasmic antibody-associated vasculitis: results of an international randomized controlled trial (PEXIVAS). *Am. J. Respir. Crit. Care Med.* **209**, 1141–1151 (2024).
131. Jayne, D. R. W. et al. Avacopan for the treatment of ANCA-associated vasculitis. *N. Engl. J. Med.* **384**, 599–609 (2021).
132. Zheng, S. L. & Roddick, A. J. Association of aspirin use for primary prevention with cardiovascular events and bleeding events: a systematic review and meta-analysis. *JAMA* **321**, 277–287 (2019).
133. Simon, H. U. et al. Refining the definition of hypereosinophilic syndrome. *J. Allergy Clin. Immunol.* **126**, 45–49 (2010).
134. Aukstuolis, K. et al. Hypereosinophilic syndrome presenting as coagulopathy. *Allergy Asthma Clin. Immunol.* **18**, 25 (2022).

135. Ames, P. R. J. et al. Eosinophilia and thrombophilia in Churg Strauss syndrome: a clinical and pathogenetic overview. *Clin. Appl. Thromb. Hemost.* **16**, 628–636 (2009).
136. Simon, H. U. & Klion, A. Therapeutic approaches to patients with hypereosinophilic syndromes. *Semin. Hematol.* **49**, 160–170 (2012).
137. Pan, L. et al. Takayasu arteritis with dyslipidemia increases risk of aneurysm. *Sci. Rep.* **9**, 1–9 (2019).
138. Tomasson, G. et al. Risk for cardiovascular disease early and late after a diagnosis of giant-cell arteritis: a cohort study. *Ann. Intern. Med.* **160**, 73 (2014).
139. Amiri, N. et al. Increased risk of cardiovascular disease in giant cell arteritis: a general population-based study. *Rheumatology* **55**, 33–40 (2016).
140. Uddhammar, A. et al. Increased mortality due to cardiovascular disease in patients with giant cell arteritis in northern Sweden. *J. Rheumatol.* **29**, 737–742 (2002).
141. Lee, M. S. et al. Antiplatelet and anticoagulant therapy in patients with giant cell arteritis. *Arthritis Rheum.* **54**, 3306–3309 (2006).
142. Nesher, G. et al. Low-dose aspirin and prevention of cranial ischemic complications in giant cell arteritis. *Arthritis Rheum.* **50**, 1332–1337 (2004).
143. Martínez-Taboada, V. M. et al. Effect of antiplatelet/anticoagulant therapy on severe ischemic complications in patients with giant cell arteritis: a cumulative meta-analysis. *Autoimmun. Rev.* **13**, 788–794 (2014).
144. Mollan, S. P. et al. Aspirin as adjunctive treatment for giant cell arteritis. *Cochrane Database Syst. Rev.* **2014**, CD0104532014 (2014).
145. Hellmich, B. et al. 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. *Ann. Rheum. Dis.* **79**, 19–30 (2020).
146. Misra, D. P. et al. Management of Takayasu arteritis. *Best Pract. Res. Clin. Rheumatol.* **37**, 101826 (2023).
147. Zeiser, R. Immune modulatory effects of statins. *Immunology* **154**, 69–75 (2018).
148. Narváez, J. et al. Statin therapy does not seem to benefit giant cell arteritis. *Semin. Arthritis Rheum.* **36**, 322–327 (2007).
149. Merkel, P. et al. Efficacy and safety of upadacitinib in patients with giant cell arteritis (SELECT-GCA): a double-blind, randomized controlled phase 3 trial [abstract]. *Arthritis Rheumatol.* **76**, 232–233 (2024).
150. Hatemi, G. et al. 2018 update of the EULAR recommendations for the management of Behçet's syndrome. *Ann. Rheum. Dis.* **77**, 808–818 (2018).
151. Emmi, G. et al. Adalimumab-based treatment versus disease-modifying antirheumatic drugs for venous thrombosis in Behçet's Syndrome: a retrospective study of seventy patients with vascular involvement. *Arthritis Rheumatol.* **70**, 1500–1507 (2018).
152. Hatemi, G. et al. Infliximab for vascular involvement in Behçet's syndrome. *Clin. Immunol.* **253**, 109682 (2023).
153. Saadoun, D. et al. Infliximab versus cyclophosphamide for severe Behçet's syndrome. *NEJM Evid.* **33**, EVIDoa2300354 (2024).
154. Kötter, I. et al. The use of interferon α in Behçet disease: review of the literature. *Semin. Arthritis Rheum.* **33**, 320–335 (2004).
155. Wang, Z. et al. Baricitinib for the treatment of refractory vascular Behçet's disease. *Clin. Immunol.* **250**, 109298 (2023).
156. Liu, J. et al. Baricitinib for the treatment of intestinal Behçet's disease: a pilot study. *Clin. Immunol.* **247**, 109241 (2023).
157. Liu, J. et al. A pilot study of tofacitinib for refractory Behçet's syndrome. *Ann. Rheum. Dis.* **79**, 1517–1520 (2020).
158. Desbois, A. C. et al. Immunosuppressants reduce venous thrombosis relapse in Behçet's disease. *Arthritis Rheum.* **64**, 2753–2760 (2012).
159. Ahn, J. K. et al. Treatment of venous thrombosis associated with Behcet's disease: immunosuppressive therapy alone versus immunosuppressive therapy plus anticoagulation. *Clin. Rheumatol.* **27**, 201–205 (2008).
160. Erol, S. et al. Does anticoagulation in combination with immunosuppressive therapy prevent recurrent thrombosis in Behçet's disease? *J. Invest. Med.* **72**, 387–391 (2024).
161. Liu, Q. et al. Outcomes of vascular intervention and use of perioperative medications for nonpulmonary aneurysms in Behçet disease. *Surgery* **159**, 1422–1429 (2016).
162. Koo, B. K. et al. Endovascular therapy combined with immunosuppressive treatment for pseudoaneurysms in patients with Behçet's disease. *J. Endovasc. Ther.* **10**, 75–80 (2003).
163. Ding, Y. et al. Tocilizumab in the treatment of severe and/or refractory vasculo-Behçet's disease: a single-centre experience in China. *Rheumatology* **57**, 2057–2059 (2018).
164. Zhong, H. et al. Efficacy and safety of tocilizumab in Behçet's syndrome with refractory arterial lesions: a single-centre observational cohort study in China. *Rheumatology* **61**, 2923–2930 (2022).
165. Zhou, Q. et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. *N. Engl. J. Med.* **370**, 911–920 (2014).
166. Geraldo, A. F. et al. Widening the neuroimaging features of adenosine deaminase 2 deficiency. *Am. J. Neuroradiol.* **42**, 975–979 (2021).
167. Barron, K. S. et al. The spectrum of the deficiency of adenosine deaminase 2: an observational analysis of a 60 patient cohort. *Front. Immunol.* **12**, 811473 (2022).
168. Elbracht, M. et al. Stroke as initial manifestation of adenosine deaminase 2 deficiency. *Neuropediatrics* **48**, 111–114 (2017).
169. Verschoof, M. A. et al. Neurological phenotype of adenosine deaminase 2 deficient patients: a cohort study. *Eur. J. Neurol.* **31**, e16043 (2024).
170. Signa, S. et al. Adenosine deaminase 2 deficiency (DADA2): a crosstalk between innate and adaptive immunity. *Front. Immunol.* **13**, 935957 (2022).
171. Fayand, A. et al. [Multiple facets of ADA2 deficiency: vasculitis, auto-inflammatory disease and immunodeficiency: a literature review of 135 cases from literature]. *Rev. Med. Interne* **39**, 297–306 (2018).
172. Bulut, E. et al. Deficiency of adenosine deaminase 2; special focus on central nervous system imaging. *J. Neuroradiol.* **46**, 193–198 (2019).
173. Belot, A. et al. Mutations in *CECR1* associated with a neutrophil signature in peripheral blood. *Pediatr. Rheumatol. Online J.* **12**, 44 (2014).
174. Carmona-Rivera, C. et al. Deficiency of adenosine deaminase 2 triggers adenosine-mediated NETosis and TNF production in patients with DADA2. *Blood* **134**, 395–406 (2019).
175. Meyts, I. & Aksentijevich, I. Deficiency of adenosine deaminase 2 (DADA2): updates on the phenotype, genetics, pathogenesis, and treatment. *J. Clin. Immunol.* **38**, 569–578 (2018).
176. Cooray, S. et al. Anti-tumour necrosis factor treatment for the prevention of ischaemic events in patients with deficiency of adenosine deaminase 2 (DADA2). *Rheumatology* **60**, 4373–4378 (2021).
177. Ombrello, A. K. et al. Treatment strategies for deficiency of adenosine deaminase 2. *N. Engl. J. Med.* **380**, 1582–1584 (2019).
178. Lee, P. Y. et al. Evaluation and management of deficiency of adenosine deaminase 2: an international consensus statement. *JAMA Netw. Open* **6**, e2315894 (2023).
179. Beck, D. B. et al. Somatic mutations in *UBA1* and severe adult-onset autoinflammatory disease. *N. Engl. J. Med.* **383**, 2628–2638 (2020).
180. Khitri, M. Y. et al. Comparison between idiopathic and VEXAS-relapsing polychondritis: analysis of a French case series of 95 patients. *RMD Open* **8**, e002255 (2022).
181. Kusne, Y. et al. Venous and arterial thrombosis in patients with VEXAS syndrome. *Blood* **143**, 2190–2200 (2024).
182. Obiorah, I. E. et al. Benign and malignant hematologic manifestations in patients with VEXAS syndrome due to somatic mutations in *UBA1*. *Blood Adv.* **5**, 3203–3215 (2021).
183. Georgin-Lavialle, S. et al. Further characterization of clinical and laboratory features in VEXAS syndrome: large-scale analysis of a multicentre case series of 116 French patients. *Br. J. Dermatol.* **186**, 564–574 (2022).
184. Oo, T. M. et al. Thrombosis in VEXAS syndrome. *J. Thromb. Thrombolysis* **53**, 965–970 (2022).
185. Butta, N. V. et al. Endothelial dysfunction and altered coagulation as mediators of thromboembolism in Behçet disease. *Semin. Thromb. Hemost.* **41**, 621–628 (2015).
186. Khider, L. et al. Systematic search for the *UBA1* mutation in men after a first episode of venous thromboembolism: a monocentric study. *J. Thromb. Haemost.* **20**, 2697–2699 (2022).
187. Grayson, P. C., Patel, B. A. & Young, N. S. VEXAS syndrome. *Blood* **137**, 3591–3594 (2021).
188. Bindoli, S. et al. JAK inhibitors for the treatment of VEXAS syndrome. *Exp. Biol. Med.* **248**, 394–398 (2023).
189. Koster, M. J. et al. VEXAS syndrome: clinical, hematologic features and a practical approach to diagnosis and management. *Am. J. Hematol.* **99**, 284–299 (2024).
190. Mourguet, M. et al. Increased ischemic stroke, acute coronary artery disease and mortality in patients with granulomatosis with polyangiitis and microscopic polyangiitis. *J. Autoimmun.* **96**, 134–141 (2019).
191. Al-Ani, B. Simvastatin inhibits neutrophil degranulation induced by anti-neutrophil cytoplasm auto-antibodies and N-formyl-methionine-leucine-phenylalanine (fMLP) peptide. *Saudi Med. J.* **34**, 477–483 (2013).
192. Sen, D., Rosenstein E. D., Kramer N. ANCA-positive vasculitis associated with simvastatin/ezetimibe: expanding the spectrum of statin-induced autoimmunity? *Int. J. Rheum. Dis.* **13**, e29–e31 (2010).
193. Haroon, M. & Devlin, J. A case of ANCA-associated systemic vasculitis induced by atorvastatin. *Clin. Rheumatol.* **27**, 75–77 (2008).
194. Yamashita, Y., Morimoto, T. & Kimura, T. Optimal duration of anticoagulation therapy for venous thromboembolism in autoimmune diseases. *Eur. J. Intern. Med.* **95**, 102–103 (2022).
195. Laurent, C. et al. Prevalence of cardiovascular risk factors, the use of statins and of aspirin in Takayasu Arteritis. *Sci. Rep.* **11**, 14404 (2021).
196. Kwon, O. C. et al. Statins reduce relapse rate in Takayasu arteritis. *Int. J. Cardiol.* **287**, 111–115 (2019).
197. Salvo, F. & Franchini, S. Statin therapy for Takayasu arteritis. *Int. J. Cardiol.* **287**, 118–119 (2019).
198. Inanc, M. T. et al. Effects of atorvastatin and lisinopril on endothelial dysfunction in patients with Behçet's disease. *Echocardiography* **27**, 997–1003 (2010).
199. Jennette, G. C. et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum.* **65**, 1–11 (2013).

Acknowledgements
The authors thank R. Padoan (Department of Internal Medicine and Rheumatology, University Hospital of Padua, Italy) for providing Fig. 2h.

Author contributions
G.E., F.B. and F.F. researched data for the article. All authors contributed substantially to discussion of the content, wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Rheumatology* thanks Melek Kechida, Durga Misra and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Review criteria A search for original articles was performed across multiple databases, including PubMed, Scopus, and Embase. Search terms included disease-specific terms (for example, "ANCA vasculitis", "large vessel vasculitis", "Behçet syndrome", "DADA2", "VEXAS syndrome") in combination with thrombosis-related keywords ("arterial thrombosis", "venous thrombosis", "vascular complications", "prothrombotic state"). The search was restricted to peer-reviewed publications available in English, with a focus on studies published from 1990 to 2024 to ensure relevance to contemporary clinical practice. Inclusion criteria included original research articles, systematic reviews, meta-analyses and case series that provided data on vasculitis-related thrombotic risk, pathophysiology of thromboses or management of these conditions. Exclusion criteria included non-relevant studies, single case reports (unless of substantial clinical interest) and articles lacking sufficient data. Additional sources were identified through manual screening of the references of key articles to ensure comprehensive coverage of the topic.

[1]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy. [2]Department of Internal Medicine 3, Friedrich-Alexander University Erlangen-Nuremberg and Universitätsklinikum Erlangen, Erlangen, Germany. [3]Deutsches Zentrum für Immuntherapie, Friedrich-Alexander University Erlangen-Nuremberg and Universitätsklinikum Erlangen, Erlangen, Germany. [4]Department of Clinical and Biological Sciences, University of Torino, Torino, Italy. [5]Rheumatology Unit, The Queen Elizabeth Hospital, Woodville, South Australia, Australia. [6]Department of Medicine, University of Adelaide, Adelaide, South Australia, Australia. [7]Department of Clinical Sciences, Rheumatology, Lund University, Lund, Sweden. [8]Department of Medicine, University of Cambridge, Cambridge, UK. [9]Tareev Clinic of Internal Disease, Sechenov First Moscow State Medical University, Moscow, Russia. [10]Meyer Children's Hospital IRCCS, Florence, Italy. [11]Division of Rheumatology, Department of Internal Medicine and Behçet's Disease Research Centre, Istanbul University-Cerrahpasa, School of Medicine, Istanbul, Turkey. [12]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy. [13]Clinical Medicine and Rheumatology Unit, Cattinara University Hospital, Trieste, Italy. [14]Centre for Inflammatory Diseases, Monash University Department of Medicine, Monash Medical Centre, Melbourne, Victoria, Australia. [15]These authors contributed equally: Federica Bello, Filippo Fagni.