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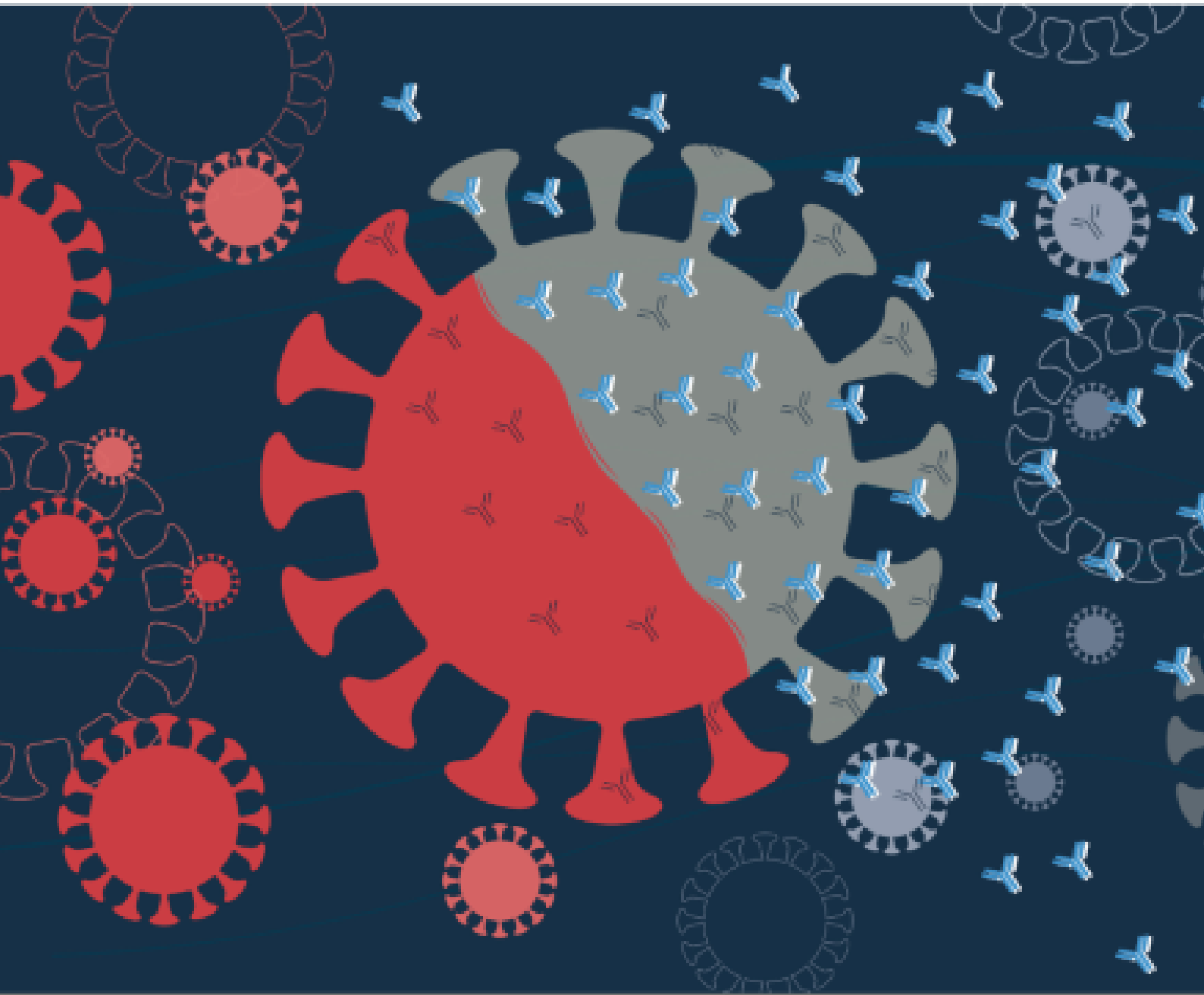


COVID-19
Risks, outcomes and clinical concerns for people with rheumatic disease

Treatment of axial spondyloarthritis
Reviewing the latest developments

AUTOIMMUNITY

Freeing PDL1 alleviates autoimmunity

Credit: Alex Whitworth/Springer Nature Limited

Immunotherapy with targeted blockade of the inhibitory co-receptor PD1 is now a valuable tool for treating cancer; conversely, targeted potentiation of PD1 is expected to ameliorate autoimmune disease, but achieving this effect has proved elusive. New research published in *Nature Immunology* demonstrates that PD1 function can be elicited by freeing up its ligand, PDL1, from binding to CD80, thereby improving symptoms of autoimmune disease in animal models.

In previous work, the researchers determined that CD80 binds to PDL1 on antigen-presenting cells (APCs) to form a *cis*-PDL1–CD80 duplex, thus restricting PD1 function, and that this mechanism is required for optimal induction of T cell responses. In the present study, they sought to remove this restriction and suppress autoreactive T cells by using anti-CD80 antibodies that detach CD80 from the duplex and enable PDL1 to engage PD1.

The researchers generated monoclonal antibodies against mouse and human CD80 (termed TKMG48 and TKMF5, respectively), which physically dissociated the *cis*-PDL1–CD80 duplex and enabled PD1 to associate with PDL1 on activated dendritic cells (DCs) in the presence of CD80. The PD1–PDL1 association induced by the antibodies enabled PD1 signalling that inhibited T cell activation. In C57BL/6N mice, TKMG48 was able to increase the PD1-binding capacity of DCs and weaken antigen-specific T cell responses.

TKMG48 alleviated symptoms of arthritis in two different mouse models of the disease, even when administered to mice with established arthritis. The anti-CD80 treatment was also effective in animal models of other autoimmune diseases, namely Sjögren syndrome and multiple sclerosis.

> “PD1 function can be elicited by freeing up its ligand, PDL1, from binding to CD80”

The investigators plan to develop a humanized antibody to test the efficacy of this approach in human autoimmune diseases.

Sarah Onuora

ORIGINAL ARTICLE Sugiura, D. et al. PD-1 agonism by anti-CD80 inhibits T cell activation and alleviates autoimmunity. *Nat. Immunol.* https://doi.org/10.1038/s41590-021-01125-7 (2022)

SYSTEMIC LUPUS ERYTHEMATOSUS

proBDNF blockade modulates SLE

New research shows that antibody-secreting cells that express brain-derived neurotrophic factor precursor (proBDNF) and its high-affinity pan-75 neurotrophin receptor ($p75^{NTR}$) are associated with clinical features and autoantibody titres in patients with systemic lupus erythematosus (SLE). The proBDNF pathway could provide a biomarker for SLE disease activity and a therapeutic target.

proBDNF has roles outside the central nervous system. It is an inflammatory mediator that is thought to contribute to autoimmune diseases by affecting the function of immune cells. However, the affected types of immune cells have not previously been identified.

New research has determined the immune-cell expression of proBDNF and $p75^{NTR}$ in relation to SLE. “proBDNF and $p75^{NTR}$ were highly expressed in the antibody-secreting cells (ASCs) in patients with SLE,” explains corresponding author Ru-Ping Dai. proBDNF expression was detected in 15% of $CD19^{+}CD27^{hi}CD38^{hi}$ ASCs from 52 healthy individuals and 28% of ASCs from 67 patients with SLE, and expression correlated with clinical symptoms of SLE (such as SLE disease activity index scores) and with titres of autoantibodies.

proBDNF signalling was also investigated in two mouse models of lupus. In MRL/MpJ-Fas^{lpr}/J mice, expansion of $proBDNF^{+}$ ASCs occurred in the spleen compared with non-lupus controls. In mice with pristane-induced splenomegaly, expression of proBDNF and $p75^{NTR}$ in splenic ASCs was higher than in untreated mice. Treatment with a monoclonal anti-proBDNF antibody reduced splenomegaly and immune-cell expansion, inhibited autoantibody production and ameliorated nephritis and proteinuria compared with IgG-treated controls. Knockout of $p75^{NTR}$ expression in $CD19^{+}$ B cells also reduced pristane-induced splenic B cell expansion, autoantibody production and renal pathology compared with $p75^{NTR+}$ mice. “Blocking $proBDNF^{+}$/$p75^{NTR}$ signalling attenuated disease progress in SLE mouse models by ameliorating the dysfunction of ASCs,” says Dai. Similarly, in mouse and human ASCs in vitro, anti-proBDNF antibody treatment blocked Toll-like receptor agonist-induced ASC proliferation and antibody secretion.

Credit: Rebecca Manns/500px

> “Treatment with a monoclonal anti-proBDNF antibody reduced splenomegaly and immune-cell expansion”

The potential of proBDNF as a biomarker of SLE activity, and of anti-proBDNF treatment to modify this activity, can now be determined.

Robert Phillips

ORIGINAL ARTICLE Shen, W. Y. et al. Up-regulation of $proBDNF/p75^{NTR}$ signaling in antibody-secreting cells drives systemic lupus erythematosus. *Sci. Adv.* **8**, eabj2797 (2022)

RHEUMATOID ARTHRITIS

TNF inhibition enhances depletion of synovial fibroblasts by ferroptosis

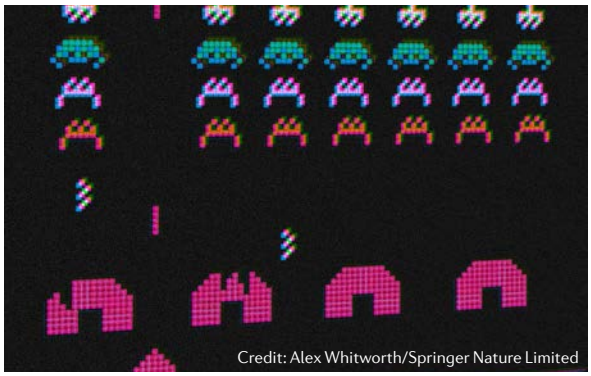
In a newly reported study, activation of the ferroptosis pathway of necrosis reduced numbers of synovial fibroblasts in the mouse collagen-induced arthritis (CIA) model, thereby limiting damage to articular cartilage and bone and attenuating progression of arthritis. Ferroptotic targeting of activated synovial fibroblasts could provide a new therapeutic strategy for rheumatoid arthritis (RA).

In progressive RA, synovial fibroblasts proliferate in the presence of reactive oxygen species and lipid oxidation, contributing to inflammation, angiogenesis and matrix degradation. RA can be controlled, but not cured, by treatment with DMARDs. "If we could develop a new therapeutic approach targeting activated synovial fibroblasts, that would be of great importance," explain the study's corresponding author Ping Zhu and first author Jiao Wu.

Mesenchymal cells such as fibroblasts are known to be sensitive to ferroptosis, and the researchers found that, in the CIA mouse model, the ferroptosis inducer imidazole ketone erastin (IKE) reduced numbers of synovial fibroblasts and attenuated synovial inflammation. Some fibroblasts were resistant to IKE-induced ferroptosis, and in these cells the TNF transcriptional pathway was enriched.

Pro-inflammatory TNF promotes fibroblast activation, and in synovial fibroblasts from patients with RA, TNF administration in vitro conferred dose-dependent resistance to IKE-induced ferroptosis via activation of NF-κB and biosynthesis of glutathione. By contrast, IKE treatment depleted glutathione.

> these results suggest that ferroptosis is a therapeutic target for RA



Credit: Alex Whitworth/Springer Nature Limited

In mice with CIA, a low dose of IKE (20 mg/kg twice per week) combined with a low dose of the TNF inhibitor etanercept (2 mg/kg twice per week) overcame the TNF-induced resistance to ferroptosis. The combination of IKE and etanercept also increased the sensitivity of human fibroblasts from individuals with RA to ferroptosis.

Although these results suggest that ferroptosis is a therapeutic target for RA, "long-term administration of ferroptosis inducers may increase the risk of lethal inflammation and tissue damage," caution Zhu and Wu. "Thus, fibroblast-directed ferroptosis strategies are necessary, and future studies will aim to identify surface proteins that are specific to fibroblasts."

Robert Phillips

ORIGINAL ARTICLE Wu, J. et al. TNF antagonist sensitizes synovial fibroblasts to ferroptotic cell death in collagen-induced arthritis mouse models. *Nat. Commun.* **13**, 676 (2022)

SYSTEMIC SCLEROSIS

PLG nanoparticles target inflammatory monocytes in SSc

A novel nanoparticle made of carboxylated poly(lactic-co-glycolic acid) (PLG), a biodegradable polymer approved for clinical use in resorbable sutures, could provide a new therapeutic option for systemic sclerosis (SSc). "PLG nanoparticles simultaneously modify aberrantly activated myofibroblasts and disease-driven deleterious immune responses," reports corresponding author Stephen Miller. "Our study also challenges the widely accepted notion that myofibroblasts cannot be reverted to quiescent fibroblasts as seen in steady state tissues," he adds.

The PLG nanoparticles selectively recognize and bind a subset of inflammatory monocytes–macrophages via the scavenger receptor MARCO (macrophage receptor with collagenous structure), the expression of which is upregulated on activated tissue-specific macrophages and is associated with increased phagocytic activity and production of pro-inflammatory cytokines. In the present work, the researchers showed that MARCO$^+$ monocytes and macrophages are enriched in the lesional skin and lungs of patients with SSc and in mice with bleomycin-induced fibrosis, a model of SSc.

In mice, treatment with PLG nanoparticles at the same time as bleomycin administration attenuated skin and lung fibrosis. The treatment restricted the accumulation of activated immune cells in the tissues at both sites. Notably, when administered to mice on day 14 after bleomycin treatment, the PLG nanoparticles were able to reverse established fibrosis.

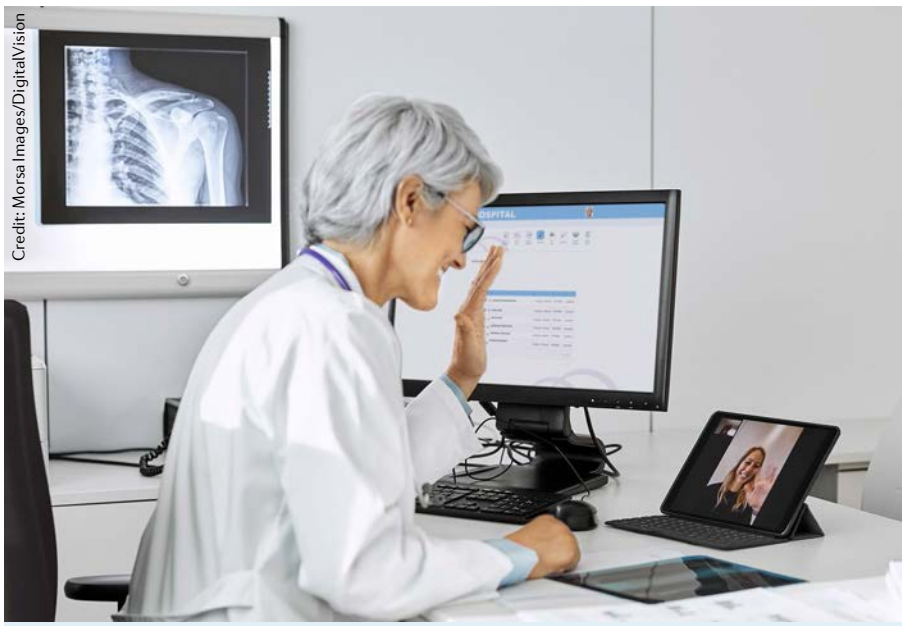
In vitro investigations demonstrated that the PLG nanoparticles directly affect myofibroblast differentiation and regulate transforming growth factor-β via pSmad and pSTAT. "Abnormally transformed myofibroblasts [can return] to base line levels comparable to healthy control animals," says Miller. "We anticipate this novel treatment will continue gaining momentum to be translated as an innovative therapy for the treatment of SSc or other forms of fibrotic diseases."

Credit: Alex Whitworth/Springer Nature Limited

> the PLG nanoparticles were able to reverse established fibrosis

Sarah Onuora

ORIGINAL ARTICLE Xu, D. et al. PLG nanoparticles target fibroblasts and MARCO$^+$ monocytes to reverse multi-organ fibrosis. *JCI Insight* https://doi.org/10.1172/jci.insight.151037 (2022)



CLINICAL PRACTICE

Telemedicine: a solution for everyone?

Martin Krusche

The COVID-19 pandemic has caused a rapid transition towards telemedicine, raising concerns about assessment accuracy, medical-relationship building and potential inequalities between patient groups. For some rheumatology patients, telemedicine is convenient and acceptable, but careful selection and choice are important.

Refers to Sloan, M. et al. Telemedicine in rheumatology: a mixed methods study exploring acceptability, preferences and experiences among patients and clinicians. *Rheumatology* https://doi.org/10.1093/rheumatology/keab796 (2021).

The COVID-19 pandemic has led to a considerable increase in the numbers of phone and video consultations in rheumatology. However, the extent to which this new healthcare approach is useful for the diagnosis and assessment of disease activity is not yet known. Studies of the benefits of telemedicine in rheumatology (tele-rheumatology) are still limited, but tend to exhibit a high risk of bias[1]. Furthermore, it is not clear to what extent the use of tele-rheumatology will influence medical-relationship building. In a multi-stage, mixed-methods study, Sloan and colleagues have investigated the acceptability of tele-rheumatology and the associated preferences and experiences among patients and clinicians, and identified limitations that could inform the development of this increasingly important approach[2].

The study involved 1,340 adult patients and 111 clinicians who participated in an online survey that was conducted between April and July 2021 in the UK[2]. Additionally, in-depth interviews were performed with 31 of the patients and 29 of the clinicians. Patients with diagnoses of an autoimmune inflammatory rheumatological condition who received at least one telemedicine appointment (phone or video) were included.

Patient–clinician partnership was one major issue that was assessed in the study[2]. A majority of clinicians (90%) and of patients (69%) reported that telemedicine consultations were worse (or much worse) than face-to-face consultations for relationship building. Clinicians' listening was rated worse (or much worse) for telemedicine than for face-to-face by around 50% of both clinicians and patients. Furthermore, for telemedicine, many patients reported feeling more rushed and stated that consultations followed a 'tick list'. These results suggest that, because of the physical separation, and the lack of non-verbal communication and physical contact, the patient–physician interaction is compromised in telemedicine consultations. Another important aspect of tele-rheumatology examined in the study was the perception of the accuracy of assessment. Notably, a majority of patients (86%) and of clinicians (93%) perceived that the accuracy of diagnosis was worse than in face-to-face consultation. The authors also noted that misdiagnosis was often attributed to the absence of examinations and tests. These findings highlight another key argument against the use of telemedicine, as current technologies are not able to capture all physical and non-verbal information (they lack the 'therapeutic touch')[3] for an accurate diagnosis. In addition, diagnostic tests (laboratory or imaging) generally cannot be performed remotely.

Notably, Sloan et al. found that >60% of patients and clinicians considered telemedicine to be more convenient than face-to-face consultation, even though remote appointments did not always save the clinicians' time[2]. These findings are consistent with those from other studies, and reflect one major advantage of telemedicine, which is that it is location independent. Telemedicine enables remote working for clinicians, and can save travel time and costs for patients, which might be particularly beneficial for those in rural areas[4].

An intriguing aspect of this study is its findings in relation to the barriers to care associated with the use of telemedicine[2]. The study participants reported concerns about triage by telemedicine and responsiveness

> Telemedicine is not a 'one size fits all' solution

from their care centres, with only 55% of patients being confident that their rheumatology department would respond within 48 h, highlighting problems for emergency access. The results indicated that telemedicine might disadvantage certain patient groups, such as those with complex multisystem diseases, or elderly or socio-economically disadvantaged patients. It is important that inequalities between patient groups are not increased by developments such as the use of telemedicine, and this issue should be taken very seriously.

Acceptance of telemedicine is influenced by the context in which it is used. Sloan et al. found that among patient preferences for routine and emergency appointments and clinician preferences for emergency appointments, the majority favoured a mostly or entirely face-to-face approach[2]. Notably, physicians preferred face-to-face meetings for emergency appointments, which is consistent with results from previous studies[5], and might be explained by the additional benefit resulting from physical examination and the ability to order laboratory tests for disease assessment. However, an important concern is that telemedicine might be employed as a measure to save costs and time. Although these are desirable aims in health-care provision[6], the best interests of patients are paramount.

Sloan et al. highlighted important concerns relating to the use of tele-rheumatology[2]. However, because of the unique nature of the UK NHS, it is important to note that the study results are not entirely applicable to other health-care systems that are already more digitized or in which private health-care providers have already implemented remote care systems. Furthermore, the study methodology did not differentiate between phone and video consultations. A large proportion of telemedicine in the UK is carried out by phone, and a trend towards a preference for phone consultation was identified, which is surprising, as results from other studies have highlighted the use and preference of video consultation[7]. It is possible that the preponderance of phone consultations contributed to the fact that this approach was preferred and that the acceptance of a video consultation was therefore reduced. Greater and more widespread use of video consultation might help to improve its acceptance.

The observations of Sloan et al.[2] need to be interpreted with respect to the time frame of the study, which took place during the first COVID-19 phase in 2021, when telemedicine was rapidly adopted to mitigate the risk of SARS-CoV-2 transmission. Many clinics and patients did not have adequate experience and/or equipment to get the best from telemedicine. This approach was an emergency reaction rather than a carefully planned transformation of care provision. To what extent the results will be transferrable to a post-pandemic world is not yet known. With the increasing digitization of all aspects of life and the widespread adoption of digital tools, the use and acceptance of telemedicine services is likely to continue to increase. In addition, it is important to differentiate which aspects of telemedicine are being included in an analysis, particularly as other technologies such as digital health-care applications and wearables (such as smart watches) become available. These technologies can be used for active and for passive monitoring of disease activity and measurement of disease flares[8]. Potentially, these tools can close the information gap between telemedicine and face-to-face consultation.

Telemedicine is not a 'one size fits all' solution, and its use requires careful consideration. Nevertheless, similar results were reported for telemedicine and for face-to-face visits in terms of acceptance in patients with systemic lupus erythematosus[9] and in medication prescription in patients with connective tissue diseases[10]. Going forwards, clinical trials are needed, focusing on specific disease entities and features (such as duration, disease activity and severity), to enable comparison with routine care and to provide a precise assessment of any benefits of telemedicine. A key contributor to the applicability and adoption of telemedicine will be digital literacy and access to appropriate technology for patients and medical caregivers. Special attention should be directed to the support of vulnerable patient groups to prevent the occurrence of further inequality and widening of the 'digital divide'.

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Competing interests
The author declares no competing interests.

UNDIFFERENTIATED ARTHRITIS

The undifferentiated arthritis dilemma: the story continues

Daniel Aletaha

Undifferentiated arthritis (UA) was redefined by the introduction of the 2010 rheumatoid arthritis (RA) classification criteria. But UA is more than just not having RA — it is about selecting the right patients for DMARD treatment even before diagnosis, and about protecting those with self-limiting disease from potential drug toxicity.

Refers to Verstappen, M., Matthijssen, X. M. E. & van der Helm-van Mil, A. H. M. Undifferentiated arthritis; a changing population who did not benefit from enhanced DMARD-strategies-results from a 25-years longitudinal inception cohort. *Rheumatology* https://doi.org/10.1093/rheumatology/keab880 (2021).

Undifferentiated arthritis (UA). We have heard that term before. A concept that has come a long way in ≥30 years is now put on the spot again by the findings of a study by Verstappen and colleagues[1]. Put on the spot not by questioning UA as an entity, but by challenging the diagnostic and therapeutic efforts in this patient group. Briefly, in a nicely conducted retrospective study the authors investigated how UA presented, was treated and evolved clinically and diagnostically over the past 25 years.

But let us go back one step. Why is it that UA is so notoriously difficult to define, that we still have so much uncertainty about when to treat arthritis that is not yet classifiable (or 'differentiated'), and that we are still definitely in need of studies like the one from Verstappen and colleagues[1]? In fact, the concept of UA is exemplary for a classic diagnosis by exclusion. Over the years, several different workup algorithms have been defined (and revised)[2]: the inherent idea of labelling a presentation as 'undifferentiated' is that there is some reasonable (but usually not clearly defined) workup that does not result in the diagnosis of any known disease. In some ways it is the rheumatologist's 'fever of unknown origin', a condition that invokes a similar concept, being defined by an unsuccessful workup for underlying conditions plus a minimum time-frame of symptom duration. Or in other words: if you do not look hard enough, or if you do not give the presentation sufficient time to resolve by self-limitation, then you cannot claim it is a fever of unknown origin. Both criteria similarly apply to UA, but the time-frame required to ensure that the presentation is not self-limiting is not clearly defined, or rather it is a moving target, which might lie somewhere between 3 months and 4 months[3,4]. Although Verstappen et al. did not focus on the duration of symptoms, their data showed that — after exclusion of those who would have fulfilled the American Rheumatism Association (ARA) 1987 or the ACR–EULAR 2010 rheumatoid arthritis (RA) classification criteria — symptom duration of remaining individuals with 'UA' was >4 months in 75%, and >11 months in 50%[1].

Discussion of UA also requires discussion of disease-modifying treatments and their indications. Considering treatment of a patient who has had chronic arthritis for several months, we have to briefly go back to 2017, when a EULAR Task Force on early arthritis made the following recommendation: "Patients at risk of persistent arthritis should be started on DMARDs as early as possible (ideally within 3 months), even if they do not fulfil classification criteria for an inflammatory rheumatologic disease"[5]. In lay terms, this approximates to 'if arthritis is present for >3 months and no diagnosis can be established, then treatment with DMARDs is indicated (regardless of the potential cause)', which in fact is an approach that is commonly taken in clinical practice. Verstappen et al. presented overall DMARD treatment rates, but it is not clear how many of the patients with UA with symptoms for >3 months were treated with DMARDs[1]. However, it is likely that many were untreated, despite persistent arthritis.

Despite the increase in use of DMARDs (mostly methotrexate) over the 25 years covered by their analysis, Verstappen et al. found a disappointing lack of improvement of outcomes: aside from relatively small average improvements in disease activity, neither long-term functional outcomes nor frequency of transitions to full classifiable RA had changed[1]. The partial interpretation of these findings was that they were an indication of potential overtreatment. Notably, in the debate around early treatment, claimed concerns range from careless undertreatment to harmful overtreatment.

Undertreatment can be prevented by identifying the appropriate (high-risk) patients for methotrexate therapy, as was done in the PROMPT study[6], where at least in the exploratory subgroup of anti-citrullinated protein antibody-positive patients, RA developed at a faster rate and more frequently overall. Initiating treatment in such subgroups clearly reduces the risk of undertreatment for those at greatest risk for diagnosable RA. Considerations relating to overtreatment require further, fundamental debate. For example, should methotrexate be considered a harmful or dangerous drug, or not?

Credit: fotosipsak/E+

> these latest findings demonstrate that higher treatment rates are not reflected in improved outcomes

One argument is that methotrexate is among the most widely used and best-known treatments in our field, and the therapeutic window is large enough to sufficiently balance effectiveness against adverse reactions. Nevertheless, as these latest findings demonstrate that higher treatment rates are not reflected in improved outcomes[1], a fair conclusion might be that the most important consideration is the appropriate selection of candidates for treatment in this population, in other words, who to treat and who not to treat. Whether treatment given to a patient who turns out not to need it is an overtreatment, even if no harm was incurred, is debatable from both clinical and ethical viewpoints: where there is no harm, there can also be no imbalance between benefit and harm, even in the absence of benefit.

What is clear is that rheumatologists have strived for earlier and earlier institution of disease-modifying treatment of RA. In fact, the 2010 RA classification criteria were developed to facilitate earlier classification[7], in the hope that it would lead to earlier treatment, thereby, perhaps, changing the disease status of some patients from UA to RA. To confirm this, it would have been interesting to see the results of the retrospective analysis separately applied to patients who missed only the ARA 1987 classification criteria, and to those who missed only the ACR/EULAR 2010 criteria. More importantly, in the clinical discussion, caution is advised about not mixing (at least not too much) classification with diagnosis. Although this issue is well known, and full Reviews have been published on the difference between these two concepts[8], it is nevertheless important to say that in a patient with (undifferentiated) arthritis, a clinical diagnosis of RA can be established without fulfillment of classification criteria (for example, a strong family history can trigger a clinical diagnosis even in absence of classifiable disease). In addition, progressively earlier treatment initiation in UA can make self-limiting disease indistinguishable from effectively treated disease, creating another dilemma that is not yet completely resolved.

The results presented by Verstappen et al. demonstrate that timely prospective intervention studies are needed to study true UA in 2022, and relevant guidance on this issue was published in 2021 (REF.[9]). Studies should include as UA those patients who do not fulfill the 2010 classification criteria for RA (the outdated 1987 criteria need not be considered) or classification criteria for other arthritic conditions. In addition to the new, more sensitive classification criteria, additional markers supporting further risk stratification in the remaining 'undifferentiated' group will certainly be warranted.

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Competing interests

The author declares no competing interests.

COVID-19 in people with rheumatic diseases: risks, outcomes, treatment considerations

Rebecca Grainger[1], Alfred H. J. Kim[2], Richard Conway[3], Jinoos Yazdany[4] and Philip C. Robinson[5,6]✉

Abstract | The COVID-19 pandemic has brought challenges for people with rheumatic disease in addition to those faced by the general population, including concerns about higher risks of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and poor outcomes of COVID-19. The data that are now available suggest that rheumatic disease is associated with a small additional risk of SARS-CoV-2 infection, and that outcomes of COVID-19 are primarily influenced by comorbidities and particular disease states or treatments. Despite considerable advances in our knowledge of which therapeutic agents provide benefits in COVID-19, and of what constitutes effective vaccination strategies, the specific considerations that apply to people with rheumatic disease are yet to be definitively addressed. An overview of the most important COVID-19 studies to date that relate to people with rheumatic disease can contribute to our understanding of the clinical-care requirements of this population.

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Cases of what would later be named COVID-19 were identified in December 2019. The sequence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was available shortly afterwards, and the health-system and public-health responses have been a global focus for the past 2 years. Although several effective therapeutics have been identified, vaccines hold the greatest promise for effective management of the pandemic. The rapid development and deployment of highly effective vaccines against SARS-CoV-2 has been one of the greatest scientific achievements of our time[1].

At the beginning of the COVID-19 pandemic, it was not known whether people with rheumatic disease (autoimmune and/or inflammatory disease affecting joints and/or muscles) would be at a higher risk of poor outcomes than the general population. Immunological alteration as a direct result of rheumatic disease or an indirect effect of treatment has the potential to contribute to poor COVID-19 outcomes[2]. Although vaccination against SARS-CoV-2 reduces symptomatic COVID-19 infection rates and poor outcomes in the general population, vaccination has additional considerations for people with rheumatic disease, including sub-optimal vaccine responses that reduce seroconversion rates, and rheumatic-disease flare[3,4]. Notably, clinical development and phase III efficacy trials for SARS-CoV-2 vaccines did not include people with rheumatic disease[5]. Therefore, initial vaccination recommendations were based on first principles or extrapolated from experience with vaccinations against diseases such as influenza. In the second year of the pandemic, multiple research efforts have begun to fill the data deficit. In this article, we provide an overview of the COVID-19 research findings that have the greatest relevance to people with rheumatic disease. We describe the risks of SARS-CoV-2 infection, the outcomes of COVID-19 (including factors associated with poor outcomes) and the management of COVID-19, and provide an overview of relevant vaccination strategies and considerations.

SARS-CoV-2 infection

Upon SARS-CoV-2 infection, initial COVID-19 disease is caused by intracellular viral replication and virus-mediated cell death with associated immunological host responses. Infected individuals are generally either asymptomatic or have mild symptoms, such as headache, fever, fatigue and sore throat. A small proportion of those who are infected progress to severe illness resulting from a pathogenic host response with a hyperinflammatory state and multi-organ damage[6,7].

SARS-CoV-2 is transmitted from person to person via the aerosol and droplet routes[8]. Maternal-to-fetal in utero transmission and fomite-based transmission might also occur, but are probably rare[8]. Susceptibility to SARS-CoV-2 infection is influenced by viral, host and environmental factors[8]. Age seems to be the most important determinant of host infection risk, possibly reflecting age-related variation in respiratory tract expression

Key points

- People with immune or inflammatory rheumatic disease might have a higher risk of infection with SARS-CoV-2 after exposure than the general population, although the additional risk is probably small.
- Risk of poor COVID-19 outcomes in patients with rheumatic disease seems to be mediated by the presence of comorbidities, treatment with glucocorticoids or rituximab, and high disease activity.
- People with immune or inflammatory rheumatic disease who experience mild COVID-19 symptoms should stop taking immunomodulating medications for 1–3 weeks from the onset of disease.
- People with rheumatic disease with positive SARS-CoV-2 test results or mild COVID-19 symptoms and risk factors for poor outcomes should stop taking immunomodulating medications and consider treatment with antiviral medications.
- Most patients with treated rheumatic disease generate antibody responses to SARS-CoV-2 vaccines, but medications such as B cell-depleting therapies and mycophenolate confer a high risk of poor responses.
- People with immune or inflammatory rheumatic disease are strongly recommended to receive SARS-CoV-2 vaccination, including booster doses if recommended, despite some evidence of a diminished response in particular groups.

of angiotensin-converting enzyme 2 receptor, a cellular receptor for SARS-CoV-2 binding[9,10]. Environmental conditions, including poor ventilation and overcrowding, are risks for high viral infection rates[11,12], and their possible contribution to superspreading events is a particular concern[11,13]. Practical steps that can reduce infection risk include the avoidance of locations where such conditions occur, maintenance of social and/or physical distancing, appropriate hand hygiene and the use of face masks[14]. These measures are also key elements for people with rheumatic disease to reduce their risk of infection. In addition to such general considerations, it is important to understand whether any additional risk of SARS-CoV-2 infection is specifically conferred by rheumatic disease or its treatments.

Several studies have produced comparative SARS-CoV-2 infection rates for individuals with rheumatic disease and for the general population. In an early study from Wuhan in China, a worryingly high relative risk of infection was observed for people with rheumatic disease compared with the general population (OR 10.90; 95% CI 5.43–21.89)[15]. Notably, however, this analysis was based on a low number of people with rheumatic disease and COVID-19 diagnoses, resulting in a wide confidence interval. In a multicentre study of individuals in Hubei province in China, in households with a confirmed diagnosis of COVID-19, the rate of infection among family members with rheumatic disease was higher than that among family members without rheumatic disease (OR 2.68; 95% CI 1.14–6.27)[16]. In a large, retrospective, hospital-based, multicentre study conducted in Spain during the first wave of SARS-CoV-2 infection in 2020, people with rheumatic disease had a 30% higher risk of infection than the wider population[17]. Similarly, in a cohort study from South Korea, the risk of SARS-CoV-2 infection in patients with rheumatic disease was higher than in matched individuals without rheumatic disease (adjusted OR 1.19; 95% CI 1.03–1.40)[18]. By contrast, in population-based cohort studies from Korea and Italy, no definite increase was observed in the risk of SARS-CoV-2 infection for people with rheumatic disease[19,20]. The interpretation of these data is complicated by potential confounding resulting from the effects of comorbidities, medication, rheumatic-disease activity and health behaviours in this group.

The risks of SARS-CoV-2 infection have been examined in specific rheumatic diseases. In a case–control study from Italy, the estimated risk of infection was 64% higher for people with rheumatoid arthritis (RA) than for the general population, but there was no increase in the risk for people with connective tissue disease (CTD)[21]. Analysis of the UK Biobank dataset of nearly half a million people found that individuals with gout had a risk of infection that was no higher than those without gout, whereas those with RA had a point estimate of 34% higher risk than those without RA[22]. Similarly, in a study of 33,886 people with RA in the US Veterans Affairs system, the risk of COVID-19 diagnosis was 25% higher than in 33,886 people without RA[23]. The heterogeneity in these results might reflect differences in study design or in SARS-CoV-2 testing rates, which are influenced by multiple personal and health-system factors. Further data on risk in rheumatic-disease subsets is becoming available, and more information of this nature will, over time, help to provide a more granular picture[24,25].

In summary, the available evidence (mostly from retrospective analyses) indicates that there might be a small elevation of the risk of SARS-CoV-2 infection in people with rheumatic disease, with the caveat that these data have inherent limitations. Notably, in a meta-analysis of the 23 studies published up until mid-February 2021 that reported SARS-CoV-2 infection rates in people with rheumatic disease, the relative risk compared with the general population was 1.52 (95% CI 1.16–2.00)[26]. Regardless of the risk of infection, it is also important to know whether people with rheumatic disease have worse outcomes from COVID-19.

COVID-19 outcomes in rheumatic disease

For many people, COVID-19 is a self-limiting viral illness. However, severe COVID-19 can cause pneumonitis, acute respiratory distress syndrome, renal failure, thrombotic complications, cytokine storm[27], multi-organ failure and death[28]. Overall, infection fatality rates range from 0.5 to 2.7%, and they are influenced by the presence of risk factors for poor outcomes. Non-rheumatic disease factors can affect the risk of death from COVID-19 in people with rheumatic disease[29,30]. Therefore, studies in which the methods adjust for confounding factors that might influence COVID-19 outcomes are the most informative. The limitations of many studies in this rapidly moving field also mean that we must interpret data cautiously.

Many of the large population-based or health-system-based studies conducted to date have reported point estimates suggesting elevation of the risk of COVID-19 hospitalization or death in people with rheumatic disease (TABLE 1). In a large Danish population-based study of 11,122 individuals with SARS-CoV-2 infection confirmed by PCR in early 2020, people with RA or CTD had higher odds of hospitalization or death than those without these conditions, using an unadjusted model[31].

Seroconversion
Changing from seronegativity to anti-SARS-CoV-2 antibodies to seropositivity to anti-SARS-CoV-2 antibodies.

Superspreading events
Events where a disease is spread more than usual.

However, with adjustment for age, sex and comorbidities, there were no higher odds of these poor outcomes[31]. In a subsequent Danish data-linking study for a 6-month period from March 2020, the risk of hospitalization with COVID-19 was 46% higher for people with rheumatic disease than for the general population, but, using a fully adjusted model, only people with RA still had an elevated risk of a severe outcome (HR 1.72; 95% CI 1.29–2.30)[32]. In a study of 17 million adults in the UK primary care database OpenSAFELY, the risks of COVID-19-associated death for the combined group of people with RA, systemic lupus erythematosus (SLE) or psoriasis were slightly higher than for the general population[33]. Similarly, in the UK Biobank study, compared with unaffected individuals, the risk of COVID-19-associated death was elevated for people with RA, but not for those with gout[22]. In an analysis of >31,000 adults in a US electronic medical-record database (TriNetX), elevation of the risk for COVID-19 death was not significant (OR 1.17; 95% CI 0.85–1.60) for people with rheumatic disease[34]. Among >30,000 patients with RA and the same number of matched comparators from the US Veterans Affairs health-care system, the risk of hospitalization or death was higher for patients with RA (HR 1.35; 95% CI 1.10–1.66)[23]. In other, smaller, comparative cohort studies, estimates of the risks of poor outcomes have varied. A small, but well-conducted US-based cohort study of 143 people with rheumatic disease and 688 matched comparators reported hazard ratios of 0.87 (95% CI 0.68–1.11) for hospitalization, 1.27 (95% CI 0.86–1.86) for intensive-care unit (ICU) admission, 1.51 (95% CI 0.93–2.44) for mechanical ventilation and 1.02 (95% CI 0.53–1.95) for death[35]. By contrast, in a US-based multicentre comparative cohort study that also used the TriNetX dataset, researchers reported an increased risk of hospitalization (relative risk (RR) 1.14; 95% CI 1.03–1.26) and ICU admission (RR 1.32; 95% CI 1.03–1.68), but not mechanical ventilation (RR 1.05; 95% CI 0.77–1.44) or death (RR 1.08; 95% CI 0.81–1.44) for people with rheumatic disease compared with matched comparators[36]. When the model was expanded to incorporate comorbidities and health-care utilization, the risks were attenuated. In a South Korean study involving 8,297 patients with autoimmune inflammatory rheumatic diseases, the risk of COVID-19-related death was greater than in a matched cohort without rheumatic disease (adjusted OR 1.69; 95% CI 1.01–2.84)[18]. A similar risk of death (OR 1.74; 95% CI 1.08–2.79) was identified in a meta-analysis of 13 studies published up to mid-February 2021, whereas the risks of hospitalization (OR 1.25; 95% CI 0.68–2.31), ICU admission (OR 1.16; 95% CI 0.62–2.18) and mechanical ventilation (OR 1.58; 95% CI 0.88–2.84) were not significantly different in individuals with and those without rheumatic and musculoskeletal diseases (RMDs), although point estimates all showed the same direction of effect[26]. Overall, the results of the individual studies, supported by the meta-analysis, indicate that compared with the general population, people with rheumatic disease are at an increased risk of hospitalization, and potentially of other severe outcomes of COVID-19, with some of the risk being attributable to comorbidities. The high incidence among people with rheumatic disease of comorbidities that are known to be associated with poor outcomes of COVID-19 indicates that a detailed focus on the influence of these factors is essential. The COVID-19 Global Rheumatology Alliance (C19-GRA) physician-reported registry of people with rheumatic disease and COVID-19 was launched at the beginning of the pandemic, and has provided further insights into COVID-19 outcomes for people with rheumatic disease[37–39].

Table 1 | **Reports of COVID-19 hospitalization or death risks in people with rheumatic disease**

Study location	Rheumatic disease population (*n*)	Comparator population (*n*)	Hospitalization[a]; OR/HR/RR (95% CI)	Death[a]; OR/HR (95% CI)	Ref.
Denmark	RA, CTD with PCR test (348 SARS-CoV-2 positive, 13,498 negative)	General population with PCR test (11,122 positive, 410,697 negative)[b]	OR 1.5 (1.1–1.9)	OR 1.1 (0.8–1.6)	31
Denmark	RA, spondyloarthritis, CTD, vasculitis (58,052)	General population (~4.5 million)[b]	HR 1.46 (1.15–1.86)	NR	32
South Korea	Inflammatory arthritis, CTD with PCR test (8,297)	General population with PCR test (133,609)[b]	NR	OR 1.69 (1.01–2.84)	18
UK	RA, systemic lupus erythematosus, psoriasis (878,475)	General population (17,278,392)[b]	NR	HR 1.19 (1.11–1.27)	33
UK	RA (5,409), gout (13,105)	General population (473,139)[b]	NR	RA OR 1.9 (1.2–3.0), gout OR 1.2 (0.8–1.7)	22
USA	Rheumatic disease (681)	COVID-19-positive general population (31,461)[b]	NR	OR 1.17 (0.85–1.60)	34
USA	Rheumatic disease (143)	Patients from same hospital without rheumatic disease (688)[c]	OR 0.87 (0.68–1.11)	OR 1.02 (0.53–1.95)	35
USA	Autoimmune rheumatic disease and COVID-19 (2,379)	Matched individuals with COVID-19 without autoimmune rheumatic disease (2,379)[c]	RR 1.14 (1.03–1.26)	RR 1.08 (0.81–1.44)	36
USA	RA (33,886)	Individuals without RA (33,886)[c]	HR 1.35 (1.10–1.66) for hospitalization or death		23

CTD, connective tissue disease; NR, not reported; RA, rheumatoid arthritis. [a]HR and OR reported are from models including adjustment for the largest number or type of possible confounders. [b]Comparator population included the rheumatic disease population. [c]Comparator population was separate from the rheumatic disease population.

Table 2 | **Studies from the COVID-19 Global Rheumatology Alliance reporting outcomes in patients with rheumatic disease**

Conditions included	Date of data accumulation	*n*	Main outcome of interest	Key findings	Ref.
Any rheumatic disease	20 April 2020	600	COVID-19 hospitalization	Age, comorbidities and glucocorticoid dose associated with hospitalization; no clear increased risk from anti-rheumatic treatment	40
Any rheumatic disease	1 July 2020	3,729	COVID-19 related death	Age, comorbidities, rituximab, sulfasalazine, glucocorticoid use and disease activity associated with COVID-19-related death	41
Any rheumatic disease	26 August 2020	1,324	COVID-19 hospitalization, ventilation or death	African American patients, Latinx patients and Asian patients had higher odds of hospitalization and ventilatory support than white patients	45
Rheumatoid arthritis	12 April 2021	2,869	COVID-19 outcome assessed on WHO ordinal scale	Use of Janus kinase inhibitors or rituximab more likely to be associated with poor outcomes than TNF inhibitors	24
Pregnant women with any rheumatic disease	14 January 2021	39	COVID-19 outcomes and pregnancy outcomes	Two women were hospitalized and required supplemental oxygen, with no maternal deaths; 19 of 21 with recorded pregnancy outcomes had live births	51
Any rheumatic disease, inflammatory bowel disease, skin psoriasis	1 February 2021	6,077	COVID-19 hospitalization and death	TNF inhibition plus azathioprine or 6-mercaptopurine, azathioprine or 6-mercaptopurine monotherapy, methotrexate monotherapy or Janus kinase inhibition associated with higher risk of poor outcomes than TNF inhibition alone	52

The C19-GRA registry now has >20,000 records from individuals in 81 countries, each of which has detailed demographic and clinical data about the rheumatic disease, its treatments and the COVID-19 disease course[37,38]. Among the first 600 records (up to April 2020), 277 individuals (46%) were hospitalized[40], and among 3,729 reports to the end of June 2020, there were 390 deaths (10.5%)[41]. Although voluntary reporting registries are not appropriate for estimation of rates of poor outcomes, because of selection and other biases, the strength of this dataset is the large number of reports, compiled from many countries, providing an opportunity to evaluate risk of poor outcomes in people with rheumatic disease. This broad reach of the C19-GRA is a strength in terms of external validity, but also a potential limitation in application of the results to any individual country or setting; the C19-GRA collects data from countries that vary greatly in terms of ethnicity, socioeconomic conditions and health-care systems.

In the analysis of the cohort of 3,729 patients with rheumatic disease recorded in the C19-GRA registry up to June 2020 (1,105 (29.6%) from North America and 2,315 (62%) from Europe) in which COVID-19-related death was the outcome, the risk of death was associated with age, comorbidity and glucocorticoid use (≥10 mg prednisone-equivalent daily)[41] (TABLE 2). Similar factors were identified as being associated with risk of hospitalization in the analysis of the first 600 records[40]. Rheumatic-disease factors that were associated with the risk of death included disease activity, rituximab use and sulfasalazine use[41]. The results of a post hoc analysis suggested that the association of glucocorticoid use with the risk of death might be the result of confounding by indication, and that underlying disease activity was influencing the risk of COVID-19-related death[42]. An alternative explanation is that use of glucocorticoids during the initial viral-replication stage of COVID-19 might be harmful, and this idea is supported by the observation in the RECOVERY trial of a trend towards poor outcomes in people who did not require oxygen and were treated with dexamethasone[43,44].

Four further studies from the C19-GRA have provided important additional information. The first was an examination of outcomes by ethnicity in patients with rheumatic disease in the USA up to August 2020 (REF.[45]). African American, Latinx and Asian patients all had higher odds of requiring ventilatory support and of hospitalization for COVID-19 than white patients. Similarly, data from the general population have identified poor outcomes in many non-white groups[46,47], and these findings reinforce the need for rheumatologists to advocate for access to care for patient groups that experience inequitable health outcomes. In the second study, C19-GRA researchers examined the risk of poor outcomes and the influence of the use of targeted synthetic DMARDs and biologic DMARDs (bDMARDs) in a cohort of individuals with RA in the C19-GRA registry[24]. Patients with RA who were receiving a Janus kinase (JAK) inhibitor or rituximab prior to the onset of COVID-19 had higher odds of poor outcomes than those who were receiving TNF-inhibitor therapy. One possible explanation for this result is that, like glucocorticoids, JAK inhibitors in COVID-19 might have divergent effects, depending on the underlying disease, although this idea has not yet been confirmed[44,48,49]. The association of the use of rituximab with poor outcomes was not unexpected, as it was similar to the findings in the main cohort study[41]. Here, however, the magnitude of the risk elevation was better defined, and relative to the use of TNF inhibitors, the risk of poor COVID-19 outcomes (combined end point of hospitalization, ventilatory support or death) with rituximab treatment was clear (OR 4.15; 95% CI 3.16–5.44)[24]. Rituximab is a B cell-depleting therapy (BCDT), and BCDTs are also associated with poor outcomes for COVID-19 in people with conditions other than rheumatic diseases[50]. In the third study, researchers reported generally favourable pregnancy outcomes in 39 women with rheumatic

disease who had COVID-19 whilst pregnant[51]. The fourth study contained an analysis of pooled data from the C19-GRA registry and from international registries for people with COVID-19 and inflammatory bowel disease or skin psoriasis, to examine outcomes for people receiving treatment with TNF inhibitors[52]. In >6,000 patients with COVID-19 from 74 countries, the risks of hospitalization or death were higher for those who received TNF inhibitors in combination with azathioprine or 6-mercaptopurine, or who received azathioprine or 6-mercaptopurine monotherapy, methotrexate monotherapy or JAK inhibitor monotherapy than for the patients who received only TNF inhibitors. The implication of these results is that in people with a range of immune-mediated inflammatory diseases, TNF inhibitor monotherapy might confer some benefit with respect to COVID-19 outcomes, and this possibility is currently being further explored in intervention studies of the use of TNF inhibitors to treat COVID-19 (REF.[53]). These examples demonstrate that the C19-GRA registry has provided useful insights to inform people with rheumatic disease and their health-care providers during the COVID-19 pandemic.

Overall, the available data suggest that the use of many conventional synthetic DMARDs and bDMARDs does not confer increased risk of poor outcomes in COVID-19, which is consistent with the recommendations from the ACR and EULAR to continue current treatment with these agents in the absence of known exposure to SARS-CoV-2 in order to maintain good disease control[54,55]. However, there are some notable exceptions such as rituximab[56,57], for which the risk of poor outcomes is becoming increasingly apparent. The issue of rituximab use is challenging, because the risk of poor outcomes from COVID-19 must be considered against the severity of the rheumatic disease that is being treated. Rituximab is most commonly used for treatment of diseases such as vasculitis, where the alternative (which is often cyclophosphamide) is likely to carry a similar risk of poor outcomes, although because of the relatively infrequent use of cyclophosphamide this possibility has not been well examined.

The practical application of data relating to COVID-19 in the care of people with rheumatic disease is summarized in clinical guidance from ACR and EULAR[54,55]. People with rheumatic disease should follow recommendations for the general population for reduction of exposure to SARS-CoV-2, and should consult with their rheumatologists to make individualized decisions about rheumatic-disease treatment. Although people with rheumatic disease seem to have some increased risk of poor outcomes from COVID-19 compared with the general population, much of this risk might be the result of the unmodifiable burden of comorbid conditions, with additional risk from some treatments and from active rheumatic disease. It would seem prudent to minimize glucocorticoid use where possible, whilst also maintaining the lowest possible disease activity. If a person with rheumatic disease develops COVID-19, active management of the COVID-19 and the rheumatic disease will need to be addressed, with the approaches summarized in the following section.

Management of COVID-19

The management of COVID-19 in people with rheumatic disease should be predicated on whether the patient is in the early, viral replication phase or the late, hyperinflammatory phase (FIG. 1 and TABLE 3). Treatments that target viral binding or replication are generally expected to have the greatest effect early in the disease, whereas immunomodulating therapies have a role in later disease. The NIH COVID-19 Guidelines have described the clinical spectrum of SARS-CoV-2 infection severity that guides treatment (BOX 1). In addition to illness severity, the NIH COVID-19 Treatment Guidelines Panel recommends tailoring therapeutic management to the patient's physical location (ambulatory or hospitalized)[58]. Notably, in people who are substantially immunosuppressed, the viral-replication phase can be prolonged, and instances of variant evolution have been described[59,60]. Therefore, treatment recommendations outlined for the general population often need to be customized for high-risk patients with rheumatic diseases. The management of COVID-19 in people with rheumatic disease should therefore be based on the known risk factors for poor outcomes, such as age >65 years and the presence of comorbidities, the degree of immunosuppression, clinical severity of COVID-19 and location of care provision. Rheumatology services will need to proactively ensure that health-care systems have monitoring in place for patients with rheumatic disease who have one or more of these characteristics, and that patients know to seek further medical advice early with any deterioration, particularly increased dyspnoea.

Management of outpatients. During the early, viral-replication phase, most people with rheumatic disease and mild symptoms can be observed with appropriate simple self-care, particularly if they are on minimal immunosuppression and do not have major comorbidities. The ACR recommends temporarily stopping most immunosuppressive medications in people with COVID-19 for 7–14 days after symptom resolution or 10–17 days after a positive SARS-CoV-2 test[61,62]. EULAR recommends an individualized approach to rheumatic-disease medication use in infected people[55]. In the general population, 100% of infected people have produced anti-SARS-CoV-2 antibodies within 19 days of infection[63]; thus, prolonged holding of medication in people who are recovering does not seem justified.

In patients with rheumatic disease who are at a risk of clinical deterioration, monoclonal antibodies targeting virus receptor-binding domains or viral replication can be considered early in the disease, usually in the first 10 days after the onset of symptoms. Neutralizing monoclonal-antibody therapy directed at the spike binding-protein components of SARS-CoV-2 can reduce COVID-19 progression in outpatients[64–67]. Although clinical trials to date have not included large numbers of people with rheumatic disease or who were receiving immunocompromising medication, this antiviral strategy would theoretically remain effective in these clinical settings. Several oral antiviral therapies are available for use, including molnupiravir and ritonavir-boosted

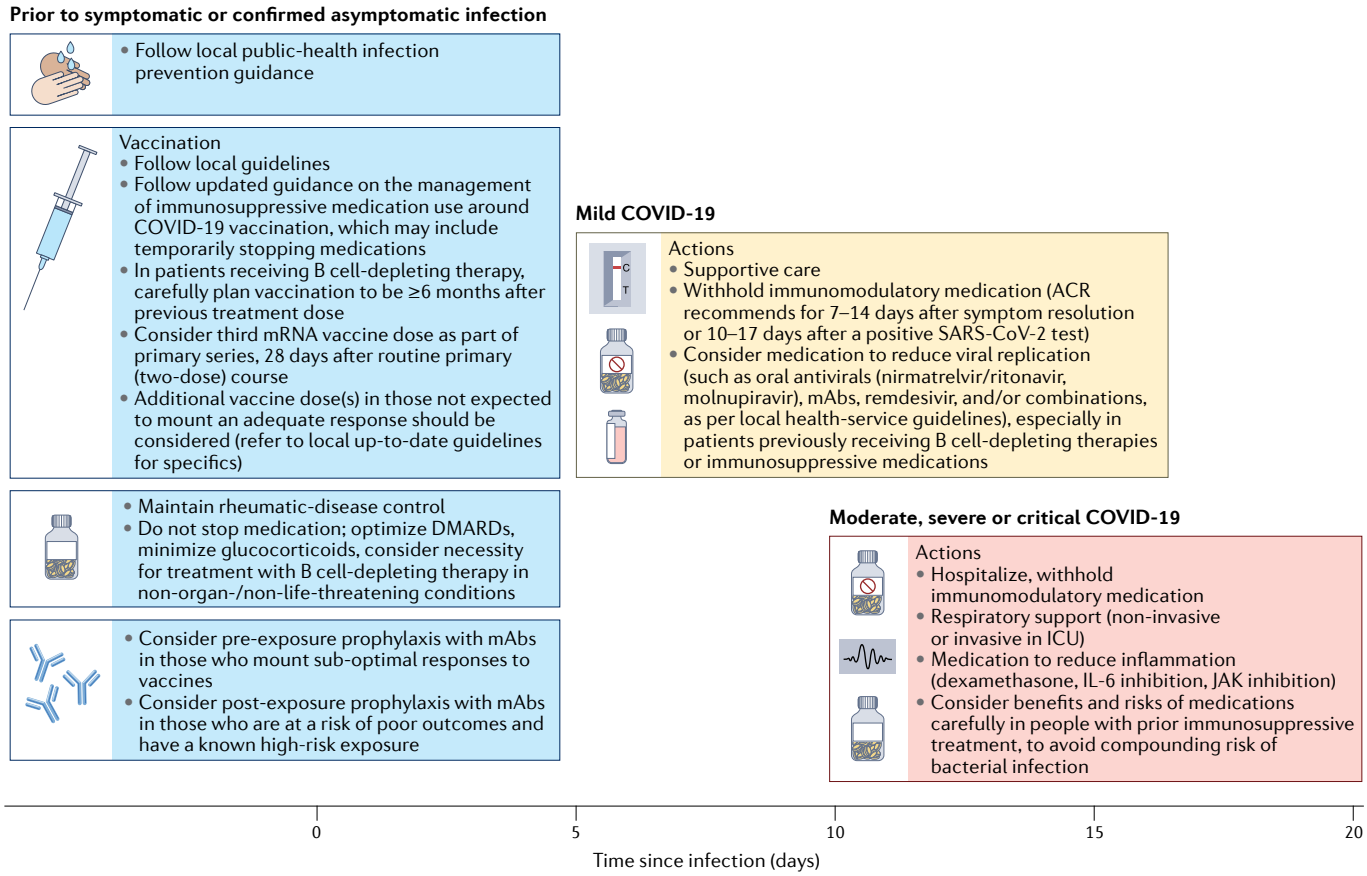

Fig. 1 | **SARS-CoV-2 pre-infection and post-infection considerations for people with rheumatic disease.** Actions for people with rheumatic disease to take while living in an area with community transmission of SARS-CoV-2, dependent on whether they are uninfected, or have confirmed asymptomatic or symptomatic (mild, moderate, severe or critical) COVID-19.

nirmatrelvir (Paxlovid). Specific data in immunocompromised individuals are lacking, but use of these drugs in patients who are at a high risk is recommended[58,68].

The US Food and Drug Administration has expanded the Emergency Use Authorization for anti-SARS-CoV-2 monoclonal-antibody therapy as post-exposure prophylaxis (PEP) for high-risk patients on the basis of the results of two randomized controlled trials[69]. Individuals with rheumatic diseases who are not fully vaccinated or who are at a risk of an inadequate immune response to vaccination should be considered for PEP. Specific choices of drug combinations (such as sotrovimab, bamlanivimab plus etesevimab or casirivimab plus imdevimab) should be made on the basis of regional SARS-CoV-2 susceptibility patterns, which change over time. PEP should be administered within 7 days of high-risk exposure. In regions where monoclonal-antibody therapy is available, patients should be strongly counselled to inform their health-care teams about high-risk exposures as soon as possible, so that appropriate treatment can be arranged.

Studies evaluating the use of pre-exposure prophylaxis with monoclonal antibodies against SARS-CoV-2 in immunocompromised individuals are ongoing and are of particular interest in relation to those with an inadequate vaccine response (for example, NCT04625725).

Management of hospitalized patients. The management of individuals who are hospitalized with COVID-19 is currently based on the severity of illness, particularly with regard to oxygen requirements and ventilatory status[70]. For example, those who are hospitalized but who do not require supplemental oxygen might not need any specific therapy, whereas those who have progressed to requiring low-flow supplemental oxygen should receive remdesivir, dexamethasone or a combination of these two medications[70]. Those with more-severe disease should receive dexamethasone and, in some instances, additional immunomodulation with IL-6 inhibitors or JAK inhibitors, as described below. Although treatment guidelines such as those put forth by the NIH[58] should generally be applied to individuals with rheumatic diseases, customization may be required, for example, with the use of anti-SARS-CoV-2 monoclonal antibodies in hospitalized patients with persistent viral replication, or avoidance of IL-6 inhibitors or JAK inhibitors in those who are already heavily immunosuppressed.

Remdesivir, an inhibitor of viral RNA-dependent RNA polymerases, reduces disease progression in hospitalized patients with COVID-19 who require supplemental oxygen, according to results from the ACTT-1 trial, but not the SOLIDARITY trial[71,72]. Although remdesivir is not recommended for those requiring invasive

mechanical ventilation, as viral replication is not considered to be the main factor in this later phase of illness, for hospitalized patients with rheumatic disease who require oxygen therapy, remdesivir should be considered[73].

For patients who have progressed to the more severe, inflammatory phase of COVID-19, specific management focuses on dampening the pathological immune response. Currently, dexamethasone treatment is associated with survival benefits in hospitalized patients who require supplemental oxygen, particularly those with severe disease who have progressed to invasive mechanical ventilation[43]. Results suggest that combination immunosuppressive therapy lessens disease progression and might reduce mortality in certain subsets of patients. For example, compared with glucocorticoid therapy alone, addition of IL-6 inhibitors such as tocilizumab or sarilumab reduced mortality among patients who were experiencing rapid respiratory decompensation in the REMAP-CAP study[69] and those with high concentrations of C-reactive protein and oxygen requirements in the RECOVERY study[74,75]. Similarly, data suggest that addition of a JAK inhibitor, such as baricitinib or tofacitinib, to corticosteroid therapy improves outcomes for hospitalized patients with COVID-19 requiring oxygen support[48,49]. IL-1 inhibition might also have a role in some hypoxaemic hospitalized patients, as results from one randomized controlled trial of anakinra demonstrated reduction of disease progression and mortality among patients with plasma concentrations of the inflammatory marker soluble urokinase plasminogen activator receptor ≥6 ng ml^{-1} (REF.[76]).

Monoclonal antibodies against SARS-CoV-2 might have a role in the treatment of hospitalized patients with COVID-19. A preliminary report from the RECOVERY study identified that among individuals who were seronegative at baseline for SARS-CoV-2, hospital mortality was

Table 3 | **Therapeutics for COVID-19 currently licensed or with emergency use authorization**

Drug	Action	Indications for use in people with rheumatic disease	Considerations in people with rheumatic disease
Direct antivirals			
Casirivimab plus imdevimab	Anti-SARS-CoV-2 mAbs that bind to non-overlapping epitopes of the spike protein receptor binding domain	Treatment of non-hospitalized patients with mild or moderate COVID-19 who are at a risk of progression	Treatment of high-risk hospitalized patients with COVID-19 who have a poor humoral response to SARS-CoV-2 should be considered when mAbs are available (compassionate use) Patients with rheumatic disease should be counselled to alert their clinicians about SARS-CoV-2 infection or exposure as early as possible, so that mAb therapy can be arranged
		Post-exposure prophylaxis in non-hospitalized patients who are unvaccinated or vaccinated but not expected to mount an adequate immune response	
Sotrovimab	Anti-SARS-CoV-2 mAb, targets an epitope in the receptor binding domain of the spike protein that is conserved between SARS-CoV and SARS-CoV-2	Treatment of non-hospitalized patients with mild or moderate COVID-19 who are at a risk of progression	
Bamlanivimab plus etesevimab	Anti-SARS-CoV-2 mAbs that bind to different but overlapping epitopes in the spike protein receptor binding domain	Treatment of non-hospitalized patients with mild or moderate COVID-19 who are at a risk of progression	
		Post-exposure prophylaxis in non-hospitalized patients who are unvaccinated or vaccinated, but not expected to mount an adequate immune response	
Tixagevimab plus cilgavimab	Anti-SARS-CoV-2 mAbs that bind to epitopes in the spike protein receptor binding domain	Pre-exposure prophylaxis of COVID-19 in adults and children ≥12 years old	This treatment should be considered in those who have sub-optimal responses to vaccines (such as patients treated with B cell-depleting therapies)
Remdesivir	RNA-polymerase inhibitor	Hospitalized with moderate disease only	Consider for patients who require low-flow supplemental oxygen, but not for those with more severe disease requiring invasive mechanical ventilation
Anti-inflammatories			
Dexamethasone	Glucocorticoid	Hospitalized, requiring supplemental oxygen	Dexamethasone can be used in most people with rheumatic disease and COVID-19, even those on other immunosuppressive therapies Addition of a second immunomodulator to dexamethasone for COVID-19 treatment should be considered on a case-by-case basis, especially in patients who are immunosuppressed with other drugs for their rheumatic disease
Baricitinib	Janus kinase inhibitor	Hospitalized, with rapidly increasing oxygen needs and systemic inflammation	
Tofacitinib	Janus kinase inhibitor	Hospitalized, with rapidly increasing oxygen needs and systemic inflammation	
Tocilizumab	IL-6 receptor inhibitor	Hospitalized, with rapidly increasing oxygen needs and systemic inflammation	
Sarilumab	IL-6 receptor inhibitor	Hospitalized, with rapidly increasing oxygen needs and systemic inflammation	

mAb, monoclonal antibody.

Box 1 | **NIH clinical spectrum of SARS-CoV-2 infection**[142]

Asymptomatic or pre-symptomatic infection

- Individuals who test positive for SARS-CoV-2 using a virological test (that is, a nucleic-acid amplification test or an antigen test) but who have no symptoms that are consistent with COVID-19.

Mild illness

- Individuals who have any of the various signs and symptoms of COVID-19 (such as fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhoea, loss of taste and smell) but who do not have shortness of breath, dyspnoea, or abnormal chest imaging.

Moderate illness

- Individuals who show evidence of lower-respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO_2) ≥94% when breathing room air.

Severe illness

- Individuals who have SpO_2 <94% when breathing room air, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mmHg, a respiratory rate >30 breaths per min, or lung infiltrates >50%.

Critical illness

- Individuals who have respiratory failure, septic shock and/or multiple-organ dysfunction.

lower in those who received casirivimab plus imdevimab than in those who received the usual standard of care alone[77]. Hospitalized patients with rheumatic disease who have received BCDTs or other immunosuppression that severely impairs their humoral immune responses are expected to benefit from this therapy.

In summary, management of COVID-19 in people with rheumatic disease should generally follow guidance for the wider local population. Individualized treatment decisions, guided by rheumatological consultation, might be desirable for patients who already receive immunosuppressive treatment, as combining immunosuppressive drugs could increase the risk of adverse events (particularly infections)[55]. For example, for a patient receiving high-dose glucocorticoids and cyclophosphamide at the time of COVID-19 hospitalization, further immunosuppression with IL-6 inhibition, JAK inhibition or IL-1 inhibition could increase the risk of serious hospital-acquired infections to a level that exceeded any likely COVID-19-related benefits. By contrast, a patient using long-term, low-dose methotrexate who develops severe COVID-19 might be advised to temporarily discontinue the methotrexate, and to receive treatment with dexamethasone combined with IL-6 inhibition or JAK inhibition, which have demonstrated benefits for serious COVID-19. In addition, because the viral replication and inflammatory stages of COVID-19 can overlap in patients with an altered or compromised immune system, concomitant antiviral therapies, such as monoclonal antibodies directed against SARS-CoV-2, should be considered when the patient has not produced neutralizing antibodies to the virus, even as the risks of increasing immunosuppression are being evaluated.

COVID-19 vaccination considerations

As mentioned in the Introduction, the rapid development of vaccines for COVID-19 is one of the greatest achievements of modern medical science. There are six COVID-19 vaccines in widespread use, including mRNA vaccines (BNT162b2 (Pfizer–BioNTech) and mRNA-1273 (Moderna)), viral-vector vaccines (ChAdOx1 (AstraZeneca), Ad26.COV2.S (Johnson & Johnson) and Gam-COVID-Vac (also known as Sputnik V)), killed-virus vaccines (CoronaVac (Sinovac) and Sinopharm). The protein-subunit vaccine NVX-CoV2373 (Novavax) is also likely to have widespread use, but its approval has lagged behind that of the others. The construction of these vaccines is discussed elsewhere[1], and the immune response that provides protection is outlined in FIG. 2. All of these vaccines lead to the production of neutralizing antibodies and have efficacy against symptomatic SARS-CoV-2 infection in the general population[78]. In published real-world effectiveness studies of vaccination programmes, viral-vector vaccines and mRNA vaccines provide similar (very good) levels of protection[79]. Waning immunity following an initial series of vaccinations is an issue that is being addressed with additional doses of vaccine[80,81]. Data on vaccine efficacy against SARS-CoV-2 variants will lag behind the spread of virus variants. Despite these considerations, everyone should be strongly supported to receive COVID-19 vaccination.

Immunogenicity: antibody and neutralization titres. SARS-CoV-2 vaccination in immunocompetent hosts generates high titres of antibodies against the viral spike protein, with virtually all vaccinated individuals achieving seropositivity[82–85]. Most people with immune or inflammatory rheumatic disease also generate antibody responses after vaccination, although lower antibody titres might be produced than in the wider population[4,86–98]. In a study conducted in Israel, rates of spike-protein seropositivity following mRNA vaccination in people with rheumatic disease were 86%, compared with 100% in a healthy population[88]. Another large Israeli cohort of people with rheumatic disease also had an 86% seroconversion rate after mRNA vaccination[95]. In a German cohort, 94% of the participants with rheumatic disease were seropositive post-vaccination, including 90.5% with neutralizing responses[90]. These data provide reassurance that the majority of people with rheumatic disease respond to COVID-19 vaccination with an antibody response. However, the data on post-vaccination antibody titres in people with rheumatic disease compared with the general population warrant further scrutiny. In a small study conducted in Germany, antibody titres were 24% lower in people with rheumatic disease than in healthy individuals[86]. In the US COVaRiPAD study, people with rheumatic disease had, on average, post-vaccination antibody titres that were one-third of those in immunocompetent participants, and approximately 85% of those in the rheumatic-disease cohort generated antibody responses[4]. Neutralization titres of antibodies to the common variant (D614G) of the spike protein generally mirror total anti-spike antibody titres closely; thus, neutralization titres also tend to be lower in people with rheumatic disease than in the general population[4,86,90]. Overall, these data suggest that most people with rheumatic disease will have antibody responses to SARS-CoV-2 vaccination that are similar to those of

Vaccine efficacy
A measure of proportional reduction in cases using a specific outcome (infection or hospitalization) within well-defined conditions among vaccinated individuals, such as those within clinical trials.

Seropositivity
Assessment of whether anti-spike antibodies are present or not, versus the levels of antibodies (see antibody titres/levels).

Antibody titre
All antibodies that bind to the target antigen (for SARS-CoV-2, the whole spike protein or the receptor binding domain of the spike protein) expressed as a titre (the dilution of plasma or sera where anti-spike antibodies become undetectable).

Neutralization titres
Ability of antibodies to block in vitro binding of receptor binding domain of spike protein to angiotensin-converting enzyme 2, measured using target cells that form plaques after virus infection, which neutralizing antibodies block, and usually expressed as half-maximal neutralization titres.

the wider population, with antibody titres that are protective, at least in the short term. However, people with rheumatic disease can differ from each other in terms of their intrinsic alterations of immune functions and use of medications that might affect vaccination responses.

Evidence suggests that some treatments for rheumatic disease can reduce, or even prevent, antibody response to SARS-CoV-2 vaccination. The therapies of most concern involve BCDTs, glucocorticoids, mycophenolate and JAK inhibitors (FIG. 2). In a study conducted in Israel, 22 of the 47 people receiving the BCDT rituximab did not mount an antibody response, which represented 59% of all non-responders in the cohort[95]. In another study from Israel, the lowest seropositivity rates occurred in people who had received BCDT, with 39% seropositivity overall in this group. Of the 39% who became seropositive, 20% of this group who received BCDT within 6 months prior to vaccination achieved seropositivity. At 1 year following receipt of BCDT, nearly 50% of this group were seropositive[88]. The association of BCDT with reduction in antibody response has been observed in patients with rheumatic disease[4,88,92,95–97,99–102] and kidney transplantation[103]. The effect of BCDT on vaccine-induced humoral responses is not surprising given the central role in these responses of B cell activation and differentiation to antibody-secreting cells. Notably, BCDT also affects immune responses to influenza vaccination and pneumococcal vaccination

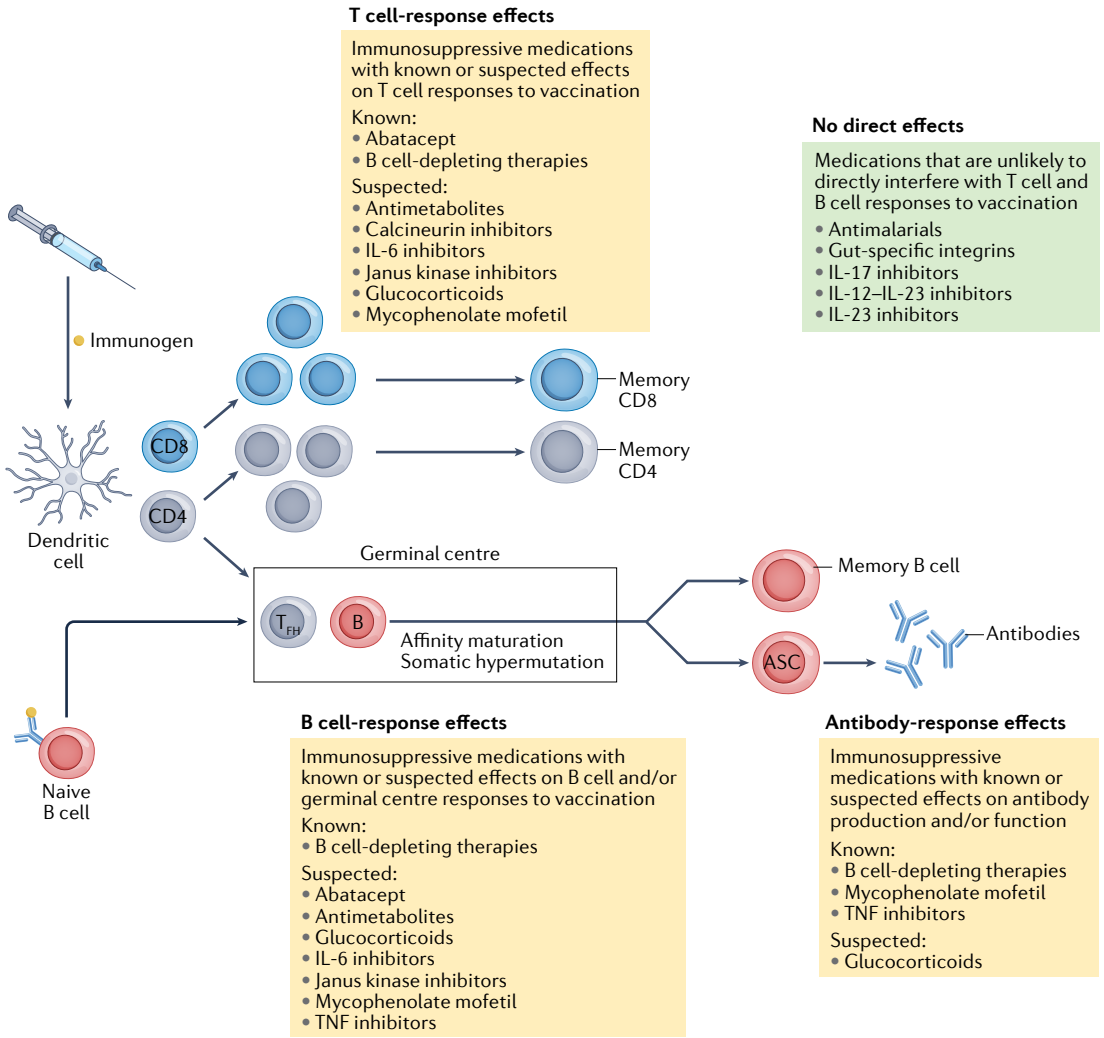

Fig. 2 | **Vaccine-induced immune responses and potential effects of immunosuppression.** Protective responses generated by vaccination require sequential activation of several immune cells. Following delivery of the immunogen by vaccination, dendritic cells activate $CD4^+$ T cells, which polarize into a variety of helper T cell subsets, including T follicular helper (T_{FH}) cells. Soluble immunogens also activate immunogen-specific naive B cells, which encounter T_{FH} cells. This interaction is a critical step in the induction of T cell-dependent B cell responses to initiate the germinal centre response, which generates a pool of mature B cells harbouring a diverse array of B cell receptors with high affinity for the immunogen. These mature B cells can further differentiate into memory B cells or antibody-secreting cells (ASCs). The diversification of the B cell receptor repertoire (and thus antibody secretion) is critical for broad coverage of the numerous epitopes the immunogen contains, and for neutralization of virus variants. Immunosuppressive medications influence T and B cell function, some more specifically than others. Immunosuppressives with known or suspected effects on T cell and B cell responses to SARS-CoV-2 vaccination are shown. Medications that affect the immune system but are unlikely to directly interfere with T cell and B cell responses to vaccination because of their mechanisms of action are also shown.

in patients with RA[104,105]. The presence of peripheral B cells at >10 cells per microlitre around 6 months after treatment with BCDT seems to be a reliable indication that seroconversion will occur following SARS-CoV-2 vaccination in people with RA or ANCA-associated vasculitis[4,88,106–109].

The use of mycophenolate (including mycophenolate mofetil and mycophenolic acid) is associated with substantial impairment of humoral immunity following SARS-CoV-2 vaccination. Mycophenolate inhibits inosine monophosphate dehydrogenase, which impairs proliferation of lymphocytes and consequently inhibits cell-mediated and humoral immune responses. In the Johns Hopkins cohort, the seroconversion rate following two vaccine doses in patients with RMDs who were treated with mycophenolate was 73%, and median antibody titres were lower than in patients treated with TNF inhibitors (8 U ml^{-1} versus >250 U ml^{-1})[97]. Similarly, the seroconversion rate was 64% in individuals treated with mycophenolate mofetil in an Israeli cohort[88]. Mycophenolate is also associated with detrimental effects on antibody titres in kidney-transplant recipients, among whom seroconversion rates are as low as 29%[110]. In recipients of solid-organ transplant with immunosuppression, a third SARS-CoV-2 mRNA vaccine dose can achieve 55% seroconversion, compared with 18% after two vaccine doses[111]. Results suggest that withholding mycophenolate before and/or after SARS-CoV-2 vaccination has beneficial effects on the probability of antibody response, and on antibody titres[112]. Although specific data on the outcomes of this strategy in people with rheumatic disease are not yet available, the ACR has already recommended that a third mRNA vaccine dose should be administered to all patients with autoimmune and inflammatory rheumatic disease ≥28 days after completing the second dose of the primary vaccination course, unless they are on hydroxychloroquine monotherapy[113]. Vaccination strategies are likely to continue to be updated as new data become available.

Data consistently indicate that immunogenicity following SARS-CoV-2 vaccination is affected in people who are treated with glucocorticoids[4,88,91,97] (FIG. 2). In US (Johns Hopkins) and Dutch cohorts, approximately 80% of people with rheumatic disease who received glucocorticoids seroconverted[91,97], and this value was 66% in an Israeli cohort[88]. In the US COVaRiPAD study, antibody titres in people with rheumatic disease were lower than those in people who were considered immunocompetent (only 65% of people with rheumatic disease had titres consistent with seropositivity), and titres were lower still in people who received low-dose prednisone (<7.5 mg per day)[4]. Notably, confounding by additional immunosuppression (especially BCDT and mycophenolate use) was observed in the Johns Hopkins cohort, as prednisone users with reduced antibody titres were generally also receiving these medications[97]. Studies with larger cohorts will be required to further explore the impact of glucocorticoid use and use of combined immunosuppressive therapies on immunogenicity following SARS-CoV-2 vaccination.

Several classes of medication seem to result in modest reductions in antibody titres, including TNF inhibitors, antimetabolites (such as methotrexate, sulfasalazine and leflunomide) and JAK inhibitors[4,87,88,90,91,95,97,98] (FIG. 2). In the SAGA cohort, seroconversion rates were lower (62%) in people with rheumatic disease who received methotrexate than in immunocompetent controls (98% seroconversion) or in people with rheumatic disease who were treated with other agents (92% seroconversion)[87]. Methotrexate was associated with a similar effect in a UK cohort of patients with psoriasis[98]. Despite relatively good seroconversion rates in patients receiving TNF inhibitors, preliminary data from the COVaRiPAD group showed that TNF inhibitor monotherapy is associated with lower neutralization titres against the B.1.617.2 (delta) variant than those in immunocompetent and otherwise immunosuppressed participants[114]. For some classes of immunosuppression, such as T cell co-stimulation blockers, IL-12–IL-23 inhibitors, IL-17 inhibitors and IL-1 inhibitors, little is known about the effects on immunogenicity. Some preliminary data from studies with small sample sizes suggest that treatment with IL-12–IL-23 inhibitors[4], IL-17 inhibitors[88] or IL-1 inhibitors[115] does not have an appreciable effect on antibody titres.

To date, a handful of studies have examined immunogenicity following a first dose of mRNA-based SARS-CoV-2 vaccine[86,90,91,116]. The results indicate that seroconversion is delayed in individuals with autoimmune diseases who receive immunosuppressive therapies[90,91], and in recipients of solid-organ transplants[117]. In patients with RMDs, the lowest antibody titres after one vaccine dose are found in those who receive BCDT or mycophenolate[116], as well as in those treated with methotrexate (who can achieve reasonable antibody levels following a second vaccine dose)[91]. Delaying administration of the second dose of vaccine might be riskier for those with rheumatic disease receiving immunosuppressive therapy than for those who receive their second dose as recommended, as this strategy could result in delay of seroconversion with no demonstrated benefit to the eventual rate of seroconversion.

Although most studies have focused on the effects that immunosuppression in people with rheumatic disease has on vaccine immunogenicity, few data exist in relation to whether having a rheumatic disease is in itself a risk factor for reduced immunogenicity. Analyses using a German cohort suggested that having a rheumatic disease was independently associated with reduced antibody responses, as anti-spike antibody titres were lower in participants with untreated immune or inflammatory rheumatic disease than in people who were considered to be immunocompetent, even after controlling for age, sex and time from first vaccination date[90]. Nevertheless, greater reductions in antibody titres occurred in this cohort in people who were treated with bDMARDs, targeted synthetic DMARDs and conventional synthetic DMARDs than in those receiving other therapies, supporting the association of immunosuppression with antibody responses induced by SARS-CoV-2 vaccination[90]. Overall, it seems clear that the development of specific vaccination strategies for patients with rheumatic disease, taking therapy use into consideration, should be a key focus for future research,

Antibody levels
All antibodies that bind to target antigen (for SARS-CoV-2, the whole spike protein or the receptor binding domain of the spike protein) expressed as a level (for a given dilution of plasma or sera, an absolute quantification of antibodies binding spike protein).

to ensure that data-informed vaccination approaches are available.

Immunogenicity: cell-mediated immunity. The data relating to cell-mediated responses to SARS-CoV-2 vaccination are limited. Results from a study involving 82 individuals with immune-mediated inflammatory disease (and 208 healthy individuals) showed that methotrexate use did not affect increases in spike-specific B cells, $CD4^+$ T cells and most $CD8^+$ T cell subsets after vaccination with the BNT162b2 mRNA vaccine[87]. This finding suggests that methotrexate monotherapy has minimal impact on most cellular responses following vaccination; however, total numbers of activated $CD8^+$ T cells did not increase in methotrexate-treated individuals with rheumatic disease in response to vaccination.

Several studies have produced data on T cell responses in relation to BCDT[92,101,118]. In a small study that included people with RA or ANCA-associated vasculitis treated with rituximab, concentrations of spike-specific B cells, total T follicular helper cells and $CD4^+$ and $CD8^+$ T cells were lower in individuals without seroconversion than in those with seroconversion[118] (FIG. 2). Additionally, spike-specific $CD4^+$ T cells secreted less IFNγ upon peptide stimulation in individuals without seroconversion, suggesting that the absence of B cells attenuates T cell activation, possibly through inadequate co-stimulation. Results from two larger studies demonstrated that although individuals who do not generate humoral responses in the context of BCDT can generate spike-specific $CD4^+$ T cells, these cells have impaired function, as assessed by IFNγ release[92,101]. The clinical import of these findings is not known, but they highlight the differential effects that immunosuppressive medications can have on cellular responses.

Breakthrough infections in individuals with rheumatic disease. Although numerous groups have published immunogenicity data assessing surrogates of protection, ultimately vaccine effectiveness data will provide the final verdict on the effect of immunosuppression on SARS-CoV-2 vaccination. Results from a study conducted in Israel revealed that vaccine effectiveness for prevention of symptomatic COVID-19 in those on immunosuppression was 71% compared with 94% in immunocompetent individuals[119]. Similarly, vaccine effectiveness for avoiding hospitalization was lower in immunosuppressed than in immunocompetent individuals (62.9% versus 91.3%)[120]. Indeed, individuals with immunosuppression represented almost half of the hospitalized breakthrough infections in studies in Israel and the USA[120,121] and four out of 14 patients with breakthrough severe or critical illness in another US study[122]. Among 16 individuals with breakthrough COVID-19 in yet another US study, only one was not receiving any DMARD or glucocorticoid at the time of vaccination[123]. Nevertheless, data from the EULAR COVID-19 registry and COVAX registry suggest that breakthrough rates are low (<1%) in fully vaccinated individuals with inflammatory RMDs[124]. Notably, the observations described herein were largely made prior to the outbreak of the delta and omicron variants of SARS-CoV-2, which might result in alteration of the association of particular immunosuppressive medications with breakthrough infections.

Adverse events after vaccination and flares of rheumatic disease. Although the data relating to immunogenicity in people with rheumatic disease are important for understanding which patients might have a suboptimal vaccine response, all patients are interested in the risk of adverse reactions. Comparative data on the rate of adverse events following vaccination in people with rheumatic disease are scarce, but results from studies conducted pre-pandemic suggest similar rates of local and systemic reactions in people with SLE who receive placebo or live herpes zoster vaccine (although the vaccine was associated with higher rates of injection-site reactions)[125–127].

mRNA vaccination is associated with a theoretical risk of disease flares, particularly in patients with SLE. mRNA is a trigger of interferon responses via activation of pattern-recognition receptors such as Toll-like receptors or the intracellular sensors stimulator of interferon genes (STING) and retinoic acid-inducible gene I (RIG-I). Systemic concentrations of type I interferon increase in response to viral infections (including SARS-CoV-2 infection)[128], and are associated with mRNA vaccination[129]. Because concentrations of type I interferon are elevated in diseases such as SLE[130] (in which inhibition of type I interferon is a therapeutic target)[131,132], there is a risk that vaccination-induced elevation of type I interferon could cause flares of rheumatic disease. Notably, however, the introduction of modified nucleosides (such as pseudouridine) into current mRNA vaccines considerably reduces recognition by pattern-recognition receptors[133]. Specifically in relation to vaccination against SARS-CoV-2, self-reported flare rates among 1,101 patients with rheumatic disease who received mRNA-based vaccines were high (17%), with 23% of the flares occurring only after the first dose, 43% only after the second dose and 33% after both doses[134]. Similarly, among 1,377 patients with RMDs who received two doses of mRNA-based SARS-CoV-2 vaccines, 11% reported flares that required treatment; factors associated with these flares included prior SARS-CoV-2 infection, previous flare (within 6 months of vaccination) and use of combination immunomodulatory therapy[135]. In SLE, type I interferon responses strongly associate with disease activity[136], and in the vaccination against COVID in systemic lupus study[137], only 21 of 696 individuals (3%) with SLE who received any SARS-CoV-2 vaccine had a medically confirmed flare, and SLE disease-activity scores were unchanged following vaccination[94]. No notable flares of rheumatic disease were identified in four other studies that between them included over 6,000 people with rheumatic disease who received SARS-CoV-2 vaccines[86,88,95,138].

Vaccinations: practical considerations. Data relating to vaccination and rheumatic disease are being generated at a considerable rate, and recommendations for clinical practice will need to be regularly updated. The ACR has collated comprehensive, data-informed

Vaccine effectiveness
A measure of proportional reduction in cases using a specific outcome (infection or hospitalization) among vaccinated individuals, measured within a real-world setting.

vaccination recommendations that are revised regularly[139]. Practitioners must also review country-specific recommendations regarding vaccination.

Conclusions

The rapid response of the international rheumatology community to the COVID-19 pandemic has provided some answers to pressing clinical questions for people with rheumatic disease. Available evidence suggests that this group has a slightly higher risk of infection with SARS-CoV-2 and of poor medical outcomes from COVID-19 than the general population. Some of the risk of poor outcomes is associated with the burden of comorbidities in this group, but there also seems to be risk associated with active disease, and with the use of glucocorticoids and DMARDs. Although guidance is now available in relation to effective therapies for COVID-19 in the general population, specific data for people with rheumatic disease are required. Further work is also needed to inform vaccination strategies for people receiving medications that are associated with reduced vaccine responses, such as rituximab, and to provide clarification about the need to withhold common medications post-vaccination[113]. Numerous other knowledge gaps also remain, including the effects of specific rheumatic diseases on vaccine effectiveness, the usefulness of additional doses of vaccine and the relative importance of humoral and cell-mediated immunity in the prevention of severe outcomes. Alteration of infection dynamics and COVID-19 severity as variants emerge also creates uncertainty, as variants that increase transmissibility, pathogenicity and immune evasion might be of particular concern to people with rheumatic disease, especially those undergoing immunosuppressive therapy[140]. Despite these concerns, the research success in promptly addressing many key concerns relating to SARS-CoV-2 and rheumatic disease is impressive[141]. The rheumatology research community will need to maintain this momentum, to provide timely and informative data that can help to optimize the care of people with rheumatic disease in this evolving pandemic.

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Treatment of axial spondyloarthritis: an update

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Abstract | Diagnosis and management of axial spondyloarthritis (axSpA) has vastly improved over the past two decades. With advances in the discernment of immunopathogenesis of this disease, new therapies have become available, which are associated with substantial improvement in symptoms, signs and quality of life. The four broad categories of approved treatment options are physical therapy and exercise (which have been known to be beneficial for millennia), NSAIDs (since the 1950s), TNF inhibitors (first FDA approval in 2003) and IL-17 inhibitors (first FDA approval in 2016). In addition, there have been a host of new developments in the axSpA field, including new treatment guidelines, the FDA approval of three biologic DMARDs to treat non-radiographic axSpA, the FDA and EMA approval of Janus kinase (JAK) inhibitors for ankylosing spondylitis, new data on the effect of biologic DMARDs on structural progression in ankylosing spondylitis, strategy trials on tapering or stopping TNF inhibitors in patients in remission, trials of treat-to-target strategy in axSpA, and several new molecules in phase III studies. This Review explores the developments in the management of axSpA.

Axial spondyloarthritis (axSpA) is an immune-mediated inflammatory disease predominantly affecting the axial skeleton, but also the peripheral joints, entheses and extra-musculoskeletal organs, such as the eyes, skin and gut (FIG. 1). AxSpA mostly affects young adults during their work-productive age and is associated with chronic symptoms of pain, stiffness and fatigue, leading to impaired quality of life and frequent disability[1]. In the past two decades substantial progress in the diagnosis and management of axSpA has been witnessed. With the advent of the Assessment of Spondyloarthritis International Society (ASAS) classification criteria for axSpA[2], clinicians are able to identify patients at an earlier stage of the disease (non-radiographic axSpA, nr-axSpA), as well as those with radiographic sacroiliitis (termed ankylosing spondylitis (AS) or radiographic axSpA (r-axSpA)). The goals of treatment in axSpA are to improve the signs and symptoms; control inflammation and retard radiographic progression; prevent complications; maintain physical function, work and social participation; and ultimately improve health-related quality of life.

Although there are no laboratory biomarkers that are helpful in the diagnosis other than C-reactive protein (CRP) and HLA-B27, improved recognition and interpretation of abnormal lesions on MRI of the sacroiliac joints and spine have helped with the early diagnosis of axSpA. Regarding the pharmacotherapy, in addition to NSAIDs and TNF inhibitors, IL-17 inhibitors have been approved for AS and nr-axSpA by the FDA and EMA. Janus kinase (JAK) inhibitors have shown promise in phase III studies for AS, which led to EMA and FDA approval for tofacitinib in AS and EMA approval for upadacitinib in AS. Three biologic DMARDs (bDMARDs), certolizumab pegol (a TNF inhibitor), secukinumab and ixekizumab (both IL-17 inhibitors), have been approved for nr-axSpA by the FDA, whereas all TNF inhibitors (except for infliximab) and secukinumab and ixekizumab have been approved for nr-axSpA by the EMA. New evidence from strategy studies is now available to help clinicians to decide whether to switch bDMARDs, treat to target (T2T) or taper bDMARDs in patients with inactive disease. Finally, in 2019, the ACR, the Spondylitis Association of America (SAA) and the Spondyloarthritis Research and Treatment Network (SPARTAN) updated the treatment guidelines for axSpA[3], subsequent to the previous guidelines published in 2016 (REF.[4]) and the ASAS–EULAR guidelines published in 2017 (REF.[5]).

The availability of novel therapies, evolving data on drug safety and treatment strategy trials are making us rethink the place and timing of these drugs in the management of axSpA. This timely Review focuses on updates in the non-pharmacological and pharmacological (phase III trials and beyond) treatment of axSpA. Data on the effect of bDMARDs on structural progression in AS, strategy trials on tapering or stopping bDMARDs in patients in remission, and T2T strategy in axSpA, as well as promising future therapies are discussed. The treatment of axial psoriatic arthritis and

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Key points

- The therapeutic armamentarium for axial spondyloarthritis is expanding after a gap of several years since TNF inhibitors were approved.
- Two new classes of drugs (IL-17A and JAK inhibitors) with distinct mechanisms of action have now been approved, with more being studied.
- Long-term suppression of inflammation could lead to retardation of radiographic progression.
- Evidence-based guidelines from ACR–SAA–SPARTAN and ASAS–EULAR have many commonalities and few differences. They provide practical approaches towards the management of axial spondyloarthritis.
- Important unmet needs in the management of this disease include new biomarkers for assessing disease activity, understanding the true impact of 'treat-to-target' strategy on long-term outcomes, personalized medicine to determine predictors of response, and comparative effectiveness between different classes of medications.

axial involvement in inflammatory bowel disease (IBD) lie outside the scope of this Review.

Non-pharmacological management of axSpA

Arguably, in axSpA, physical therapy and regular exercise can be as important as the pharmacotherapy in improving the symptoms and function by maintaining posture and spinal flexibility. Consistent with the ASAS–EULAR recommendations[5], the 2019 ACR–SAA–SPARTAN treatment guidelines strongly recommend physical therapy in patients with active as well as stable axSpA[3]. A meta-analysis of 11 clinical trials showed that a home exercise programme is more effective than no programme at all, and supervised group physical therapy is better than home exercise[6]. Combined inpatient therapy followed by supervised weekly group physical therapy was found to be the most effective programme[6]. A subsequent systematic review of 24 studies found moderate evidence for improvement in physical function and disease activity with regular exercises, and low evidence for improvement in pain, stiffness and spinal mobility [7].

There is still uncertainty about the most effective exercise programme for axSpA; however, supervised group exercise provides more benefits than unsupervised home exercise[7]. Various exercise regimens should be individualized as per the patient's needs and ability. In a prospective study of patients with axSpA, those in the exercise group had improved disease activity (as measured by the Ankylosing Spondylitis Disease Activity Score (ASDAS)), mobility (as measured by the Bath Ankylosing Spondylitis Metrology Index (BASMI)) and reduced serum calprotectin levels compared with patients with axSpA in the control group, who did not receive any physical therapy or exercise programme, suggesting that exercise could have anti-inflammatory properties in patients with axSpA[8]. Despite the available evidence, the adherence to exercise is suboptimal among patients with axSpA[9], and only about half of these patients perform the recommended exercises[10]. A 2020 study from the UK showed that patients with AS who have sedentary behaviour have worse exercise capacity and quality of life than those with an active lifestyle[11]. Overall, patients with axSpA should be strongly encouraged to do regular strengthening exercises. Prospective studies have also confirmed the effectiveness of structured education intervention in patients with AS and have recommended these education programmes to be part of the comprehensive management of patients with AS[12–14].

Pharmacological management of axSpA

NSAIDs

NSAIDs are the drug of choice for the initial treatment of axSpA. They work by inhibiting cyclooxygenase 1 (COX1, also known as prostaglandin G/H synthase 1) and COX2 (also known as prostaglandin G/H synthase 2) enzymes, thereby leading to decreased levels of prostaglandin E_2, which is linked to inflammation as well as new bone formation in AS[15]. NSAIDs can improve spinal symptoms[16], enthesitis, peripheral arthritis and uveitis[17] in axSpA. A Cochrane review of 39 studies, including 29 randomized controlled trials (RCTs), suggested that both the conventional (that is, non-selective) NSAIDs and selective COX2 inhibitors are effective for treating axSpA[18]. In a German cross-sectional study on 1,080 patients with AS on continuous NSAIDs, 20% had a complete response (defined as complete pain relief), 35% had a 50% response, 25% had a 25% response and 20% had a minimal response, suggesting that about 50% of patients with axSpA can achieve disease control with NSAIDs alone[19]. Various NSAIDs are equally effective, and there is no preferred NSAID for the treatment of axSpA[3]. In patients with active disease, continuous NSAIDs are preferred over 'on demand' NSAIDs[3]. Before switching to bDMARDs, two different NSAIDs should be tried over 2–4 weeks[3].

About 25% of patients can experience intolerable adverse effects from NSAIDs[19]. Caution should be exercised while giving long-term NSAIDs, as they are associated with several comorbidities of axSpA, including hypertension[20], peptic ulcer disease, worsening of underlying IBD, chronic renal insufficiency and cardiovascular disease[21]. Although NSAIDs are associated with an increased risk of vascular diseases[22], ironically, they could offer some protection from cardiovascular mortality in AS, perhaps owing to the reduction in systemic inflammation[23].

Whether NSAIDs reduce the radiographic progression in AS is controversial[18,24]. In an RCT of 215 patients, individuals on continuous celecoxib were found to have reduced radiographic progression compared with those in the on-demand group at 2 years[24]. In the post hoc analysis of this study, the authors found that this difference in radiographic progression was explained by the underlying disease activity — when patients were stratified according to their baseline CRP levels, those with high CRP levels receiving continuous celecoxib showed a statistically significant reduction in radiographic progression compared with the group receiving 'on-demand' celecoxib[25]. However, a subsequent RCT showed that continuous diclofenac (a non-selective NSAID) was not associated with reduced radiographic progression in AS compared with on-demand therapy[26]. This result raises the issue whether COX2 inhibitors have a differential effect on structural progression compared with non-selective NSAIDs. A 2020 meta-analysis involving patients with axSpA (both AS and nr-axSpA)

included 8 studies and did not find differences in radiographic progression between patients in the NSAID group and the control group at 2 years[27].

Conventional synthetic DMARDs

Trials of conventional synthetic DMARDs (csDMARDs) such as methotrexate and sulfasalazine[28] in AS have shown disappointing results regarding axial symptoms, especially spinal pain. Methotrexate did not improve axial manifestations of AS in a 16-week open label study, although there was improvement in signs and symptoms of peripheral arthritis[29]; thus, csDMARDs can have a role in controlling peripheral inflammatory arthritis. Evidence favours using sulfasalazine for the treatment of peripheral arthritis in axSpA compared with methotrexate[30]. Retrospective cohort studies on the combination of a csDMARD (especially methotrexate) with a TNF inhibitor to increase drug survival (that is, treatment continuation rate) in patients with AS have produced contradictory results[31–34].

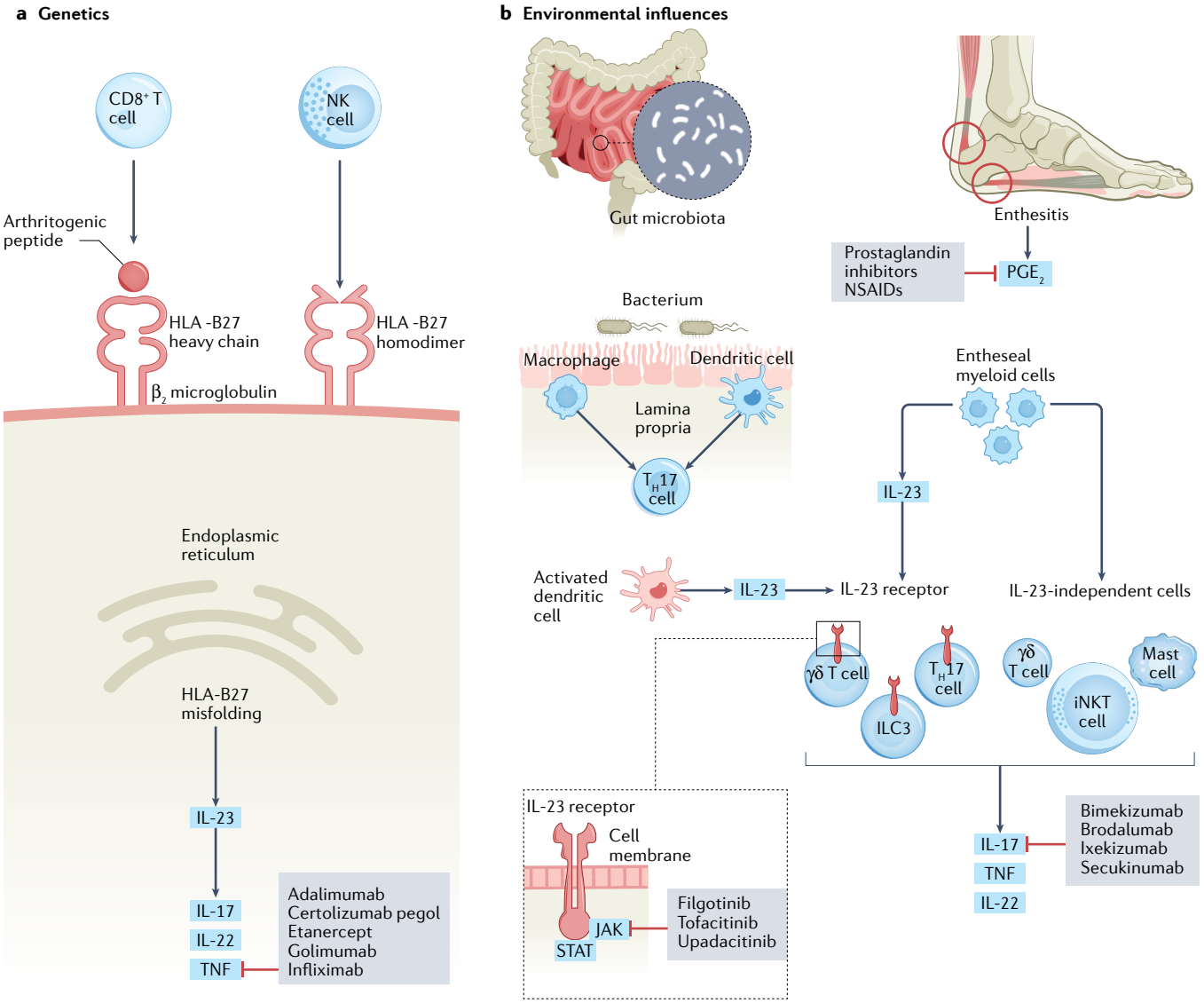

Fig. 1 | **Pathogenesis of axial spondyloarthritis.** The pathogenesis of axial spondyloarthritis (axSpA) is influenced by genetic and environmental factors. Important environmental influences include gut microbial dysbiosis and entheseal stress or trauma. **a** | It is hypothesized that HLA-B27, a MHC class 1 molecule, may initiate the inflammatory cascade by presenting an arthritogenic peptide to $CD8^+$ T cells, or by a natural killer (NK) cell recognizing an abnormally expressed heavy chain homodimer of HLA-B27 molecule on the antigen-presenting cell, or via endoplasmic reticulum stress produced by the misfolding of the HLA-B27 molecules. All these events lead to IL-23 production, and downstream production of IL-17, IL-22 and tumour necrosis factor (TNF). **b** | Macrophages and dendritic cells reach through the gut mucosal lining cells, sample the microbiota and then present microbial antigens to T helper (T_H)-17 cells. IL-23 released from the antigen-presenting cells acts on IL-23 receptor-bearing cells, leading to secretion of IL-17, IL-22 and other cytokines and/or chemokines. Phosphorylation and activation of JAK enzymes at the intracellular portion of a cytokine receptor leads to downstream phosphorylation of signal transduction and activation of transcription (STAT) molecules, which translocate to the nucleus and initiate gene transcription. In a genetically susceptible host, persistent entheseal trauma can lead to release of prostaglandin E_2 (PGE_2), and IL-23 secretion by entheseal innate lymphoid cells type 3 (ILC3), and γδ T cells. IL-17, IL-22 and TNF are secreted by IL-23 receptor-bearing and also by IL-23-independent cells. iNKT, invariant natural killer T.

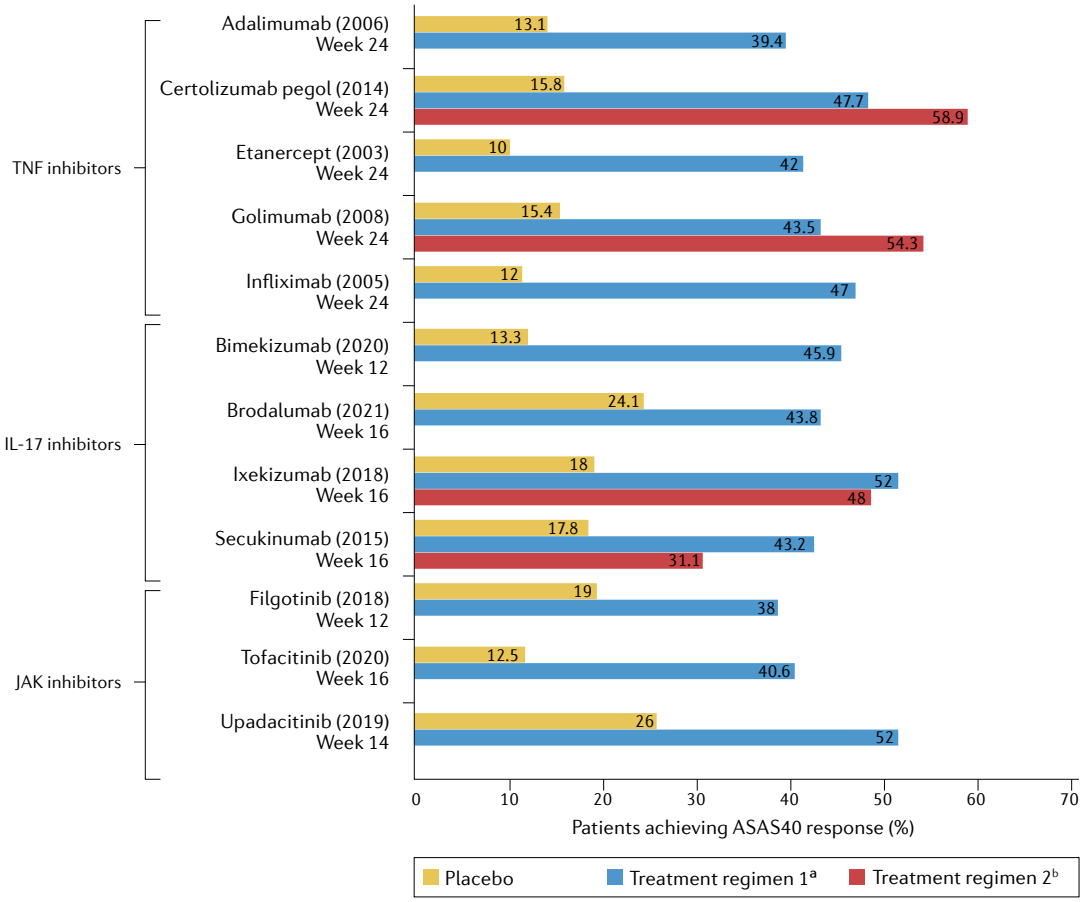

Fig. 2 | **ASAS40 responses from clinical trials in AS.** ASAS40 responses to TNF, IL-17 and JAK inhibitors in pivotal phase III studies. The results presented are not head-to-head studies and are presented for illustration purposes, not for direct comparison. The results are similar both within each class of medications and across the different classes. [a]Treatment regimen 1 as follows: adalimumab 40 mg subcutaneously (SQ) every other week[39]; certolizumab pegol 200 mg SQ every 2 weeks[41]; etanercept 25 mg SQ twice weekly[37]; golimumab 50 mg SQ every 4 weeks[40]; infliximab 5 mg/kg intravenously at weeks 0, 2, 6, 12 and 18[38]; bimekizumab 16, 64, 160 or 320 mg SQ every 4 weeks[99]; brodalumab 80 mg SQ at weeks 1, 2 and every 2 weeks thereafter[64]; ixekizumab 80 mg SQ every 2 weeks[60]; secukinumab 150 mg SQ at baseline, weeks 1, 2 and 3, and then Q4W[57]; filgotinib 200 mg by mouth once daily[101]; tofacitinib 5 mg twice daily[68]; upadacitinib 15 mg by mouth once daily[67]. [b]Treatment regimen 2 as follows: certolizumab pegol 400 mg SQ every 4 weeks[41]; golimumab 100 mg SQ every 4 weeks[40]; ixekizumab 80 mg SQ every 4 weeks[60]; secukinumab 75 mg SQ at baseline, weeks 1, 2 and 3, and then Q4W[57].

The 2019 ACR–SAA–SPARTAN guidelines recommend against using methotrexate as co-medication with TNF inhibitors[3].

Biologic DMARDs

TNF inhibitors. TNF is a pleotropic cytokine that promotes inflammation by activating leukocytes, triggering downstream production of pro-inflammatory cytokines by immune cells, stimulating migration of inflammatory cells into the intercellular matrix and inducing fibroblast proliferation[35]. Early translational studies reported increased amounts of TNF mRNA and protein in biopsy specimens of the sacroiliac joint from patients with AS[36]. On the basis of the results of subsequent clinical trials, TNF inhibitors have become the mainstay of the management of patients with axSpA who have an inadequate response or intolerance to NSAIDs[37–41].

Initial RCTs of the TNF inhibitors etanercept, infliximab, adalimumab and golimumab included patients with AS and showed that ~60% of patients receiving a TNF inhibitor achieved an ASAS20 response and 40% patients achieved an ASAS40 response, whereas only 20% and 14% of patients receiving placebo achieved ASAS20 and ASAS40 responses, respectively[37–40] (FIG. 2 and TABLE 1). A RCT of certolizumab pegol in patients with the full spectrum of axSpA also demonstrated similar response rates for AS and nr-axSpA[41]. All TNF inhibitors have been shown to improve spinal and peripheral musculoskeletal manifestations, such as enthesitis and dactylitis, as well as CRP levels and MRI-detectable inflammation in the sacroiliac joints and spine[42]. All TNF inhibitor monoclonal antibodies, but not the TNF soluble receptor (etanercept), are effective for uveitis and inflammatory bowel disease[3]. TNF inhibitors are associated with ASAS partial remission in 16–62% of patients and ASDAS-Inactive Disease (<1.3) in ~40% of patients with axSpA at varying time intervals from 12–28 weeks[43].

In a RCT in patients with nr-axSpA, certolizumab pegol was superior to placebo in achieving major

Table 1 | **Completed phase III RCTs for the treatment of AS and nr-axSpA**

Study	Study design; number of patients (*n*)	Treatment	Primary outcome	Results	Ref.
TNF inhibitors in AS					
Davis JC, et al. 2003	Multi-centre double-blind RCT (277)	Etanercept (25 mg subcutaneously) or placebo twice weekly for 24 weeks	ASAS20 response at weeks 12 and 24	At week 12, achieved by 59% of patients in the etanercept group compared with 28% of patients in the placebo group At week 24, achieved by 57% in etanercept group compared with 22% in placebo group	37
ASSERT	Multi-centre double-blind RCT (279)	Infliximab (5 mg/kg intravenously) or placebo at weeks 0, 2, 6, 12 and 18	ASAS20 response at week 24	Achieved by 61.2% of the infliximab group compared with 19.2% of the placebo group	38
ATLAS	Multi-centre double-blind RCT (315)	Adalimumab 40 mg subcutaneously every other week or placebo subcutaneously	ASAS20 response at week 12	Achieved by 58.2% of the adalimumab group compared with 20.6% of the placebo group	39
GO-RAISE	Multi-centre double-blind RCT (356)	Golimumab (50 mg or 100 mg subcutaneously) or placebo subcutaneously every 4 weeks	ASAS20 response at week 14	Achieved by 59.4% of the golimumab 50-mg group, 60.0% of the golimumab 100-mg group and 21.8% of the placebo group	40
RAPID-axSpA	Multi-centre double-blind RCT (325)	Certolizumab pegol (200 mg subcutaneously every 2 weeks or 400 mg subcutaneously every 4 weeks) or placebo subcutaneously	ASAS20 response at week 12	Achieved by 57.7% of the certolizumab pegol 200-mg group, 63.6% of the 400-mg group and 38.3% of the placebo group	41
TNF inhibitors in nr-axSpA					
C-axSpAnd	Multi-centre double-blind RCT (317)	Certolizumab pegol (400 mg subcutaneously at weeks 0, 2 and 4, followed by 200 mg every 2 weeks) plus non-bDMARD background medication, or placebo plus non-bDMARD background medication	ASDAS-MI at week 52	Achieved by 47.2% of patients in the certolizumab pegol group, compared with 7.0% in the placebo group	43
IL-17 inhibitors in AS					
MEASURE 1	Multi-centre double-blind RCT (371)	Secukinumab 10 mg/kg intravenous or intravenous placebo at weeks 0, 2 and 4 followed by subcutaneous secukinumab (150 mg or 75 mg) or subcutaneous placebo every 4 weeks starting at week 8	ASAS20 response at week 16	Achieved by 61% of the secukinumab 150-mg group, 60% of the secukinumab 75-mg group and 29% of the placebo group	57
MEASURE 2	Multi-centre double-blind RCT (219)	Secukinumab (150 mg or 75 mg) subcutaneous or subcutaneous placebo at weeks 0, 1, 2 and 3; and every 4 weeks starting at week 4; at week 16, patients in the placebo group were randomized to 150 mg or 75 mg subcutaneous secukinumab	ASAS20 response at week 16	Achieved by 61% of the secukinumab 150-mg group, 41% of the secukinumab 75-mg group and 28% of the placebo group	57
COAST-V	Multi-centre double-blind RCT (341)	Ixekizumab 80 mg subcutaneously every 2 weeks (Q2W) or 4 weeks (Q4W), adalimumab 40 mg subcutaneously every 2 weeks (active comparator) or placebo	ASAS40 response at week 16	Achieved by 52% of the ixekizumab Q2W group, 48% of the ixekizumab Q4W group, 36% of the adalimumab group and 18% of the placebo group	60
COAST-W	Multi-centre double-blind RCT (316)	Ixekizumab 80 mg subcutaneous or subcutaneous placebo every 2 weeks (IXEQ2W) or 4 weeks (IXEQ4W), with an 80 mg or 160 mg starting dose	ASAS40 response at week 16	Achieved by 30.6% of the IXEQ2W group, 25.4% of the IXEQ4W group and 12.5% of the placebo group	61

Table 1 (cont.) | **Completed phase III RCTs for the treatment of AS and nr-axSpA**

Study	Study design; number of patients (*n*)	Treatment	Primary outcome	Results	Ref.
IL-17 inhibitors in nr-axSpA					
COAST-X	Multi-centre double-blind RCT (303)	Ixekizumab (80 mg every 4 weeks (IXEQ4W) or every 2 weeks (IXEQ2W) or placebo	ASAS40 response at weeks 16 and 52	At week 16, achieved by 35% of the IXEQ4W group, 40% of the IXEQ2W group and 19% of the placebo group At week 52, achieved by 30% of the IXEQ4W group, 31% of the IXEQ2W group and 13% of the placebo group	63
PREVENT	Multi-centre double-blind RCT (555)	Secukinumab (150 mg subcutaneously with (LD) or without (NL) a loading dose) or placebo weekly for 4 weeks then every 4 weeks	ASAS40 response at weeks 16 and 52	At week 16, achieved by 41.5% of the LD group, 42.2% in the NL group and 29.2% of the placebo group At week 52, achieved by 35.4% in the LD group, 39.8% of the NL group and 19.9% of the placebo group	62
JAK inhibitors in AS[a]					
SELECT AXIS 1	Multi-centre double-blind RCT (178)	Upadacitinib 15 mg daily or placebo for 14 weeks	ASAS40 response at week 40	Achieved by 52% in the upadacitinib group and 26% in the placebo group	67
Tofacitinib	Multi-centre double-blind RCT (133)	Tofacitinib 5 mg twice daily or placebo for 16 weeks	ASAS20 response at week 16	Achieved by 56.4% in the tofacitinib group and 29.4% in the placebo group ASAS40 response was 40% and 12.5%, respectively	68

AS, ankylosing spondylitis; ASDAS-MI, Ankylosing Spondylitis Disease Activity Score major improvement; axSpA, axial spondyloarthritis; bDMARD, biologic DMARD; nr-axSpA, non-radiographic axSpA; RCT, randomized controlled trial. [a]Filgotinib trial was not included as it was a phase II study.

improvement in ASDAS at weeks 12 and 52 (47% patients compared with 7% in the placebo group at week 52)[44]. On the basis of the results of this trial, certolizumab pegol became the only FDA-approved TNF inhibitor for the treatment of nr-axSpA in the USA. All TNF inhibitors except infliximab have been approved for nr-axSpA in the EU since 2012 (FIG. 3 and TABLE 1).

Predictors of response to TNF inhibitors in AS include young age, male sex, high baseline disease activity (measured using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and CRP levels), low physical function score (measured with the Bath Ankylosing Spondylitis Functional Index (BASFI)), an absence of enthesitis and the presence of HLA-B27 (REFS[45,46]). Patients with short disease duration respond better than those who have a disease duration of >2 years[47]. A 2020 British registry study reported that low education level, obesity and poor mental health were associated with lack of response to TNF inhibitors in axSpA[48].

About 60–75% of patients with AS respond well to the first TNF inhibitor that they receive, based on the ASAS20 responses in various trials. However, 15–25% of patients do not respond to the treatment (primary non-response), and a substantial proportion of patients experience diminished efficacy after an initial period of response (secondary non-response, in 13–68%), intolerance or adverse reactions (13–57%) and might need to switch to another bDMARD[49,50]. One-year drug retention rate of the first prescribed TNF inhibitor has been reported to be about 77%[51]. As the different TNF inhibitors vary in their structure, half-life and immunogenicity, it is reasonable to switch to another TNF inhibitor in the case of secondary non-response or adverse effects (the major adverse effect of all TNF inhibitors includes increased susceptibility to viral, bacterial, fungal and opportunistic infections[52]). Patients are more likely to respond to a second TNF inhibitor if they had a secondary non-response to the first TNF inhibitor rather than primary non-response[49,53]. Although the ACR–SAA–SPARTAN guidelines recommend switching to alternative bDMARDs after 12 weeks[3], real-world data show that there could be a delay of almost 1 year before patients with failure of the first TNF inhibitor switch to an alternative TNF inhibitor, leading to poor disease control and quality of life[50].

IL-17 inhibitors. Evidence from genetic, animal and translational studies has confirmed the role of the IL-23–IL-17 axis in the pathogenesis of AS (FIG. 1). Genetic polymorphisms of the gene encoding IL-23 receptor and their associations with AS were the earliest links found between IL-23 and AS[54]. IL-17 levels are elevated in the serum and synovial fluid of patients with active AS[55]. Studies have demonstrated strong expression of IL-17 by neutrophils and mononuclear cells in facet joint specimens from patients with AS[56].

Secukinumab and ixekizumab are IL-17A inhibiting monoclonal antibodies, and both were found to improve clinical signs and symptoms of AS in multiple phase III studies[57–60] (FIG. 2 and TABLE 1). Both were associated with improved spinal symptoms, peripheral arthritis, dactylitis, enthesitis and psoriasis, as well as improved spinal mobility, physical function, health-related quality of life and work productivity. Data on their effect on uveitis are still evolving. The ASAS20 response rates

for both these IL-17 inhibitors have varied between 60–65%, compared with response rates of 28–40% with placebo[57,60]. In a phase III study, ixekizumab was associated with superior (25–30%) ASAS40 response compared with placebo (12.5%) at week 16 in patients with AS with prior intolerance or inadequate response to TNF inhibitors[61]. Both agents have completed studies in patients with nr-axSpA and have shown similar efficacy (ASAS40 responses of 40% compared with 20–30% with placebo)[62,63]. (FIG. 3 and TABLE 1), leading to FDA and EMA approval for the treatment of nr-axSpA. A systematic review reported that the remission rate (defined as ASAS partial remission or ASDAS inactive disease) among patients on IL-17 inhibitor (secukinumab or ixekizumab) was 15–20% at week 16[43].

Brodalumab is an anti-IL-17 receptor A (IL-17RA) monoclonal antibody. It inhibits several cytokines of the IL-17 family, including IL-17A–IL17F heterodimer, IL-17C and IL-17E. In a phase III study involving patients with axSpA, brodalumab was associated with a 43% ASAS40 response compared with 24% in the placebo group[64]. These results are comparable with those of IL-17A inhibitors.

Dual inhibition of IL-17A and IL-17F using bimekizumab is being studied in AS and nr-axSpA (NCT04436640). The dual blockade has the potential to be associated with increased anti-inflammatory effect, albeit with increased risk of *Candida* spp. infection.

IL-17 is an important cytokine for maintaining the mucosal integrity in the gastrointestinal tract[65]. There have been reports of new onset IBD or exacerbation of underlying IBD in patients treated with secukinumab and ixekizumab[66]. The recommendation is that IL-17 inhibitors should be avoided in patients with IBD, and patients should be closely observed for signs and symptoms of incident IBD. Increased incidence of fungal infections, especially non-systemic candidiasis, is observed with the use of IL-17 inhibitors[66].

JAK inhibitors. Tofacitinib and upadacitinib have been studied in phase III RCTs in patients with AS. Both are associated with significantly better ASAS40 response compared with the placebo arms[67,68] (TABLE 1). Upadacitinib (a selective JAK1 inhibitor) showed sustained improvement in disease activity over 1 year in the open label extension of the SELECT-AXIS study[69]. The FDA recently approved tofacitinib for the treatment of AS, whereas both tofacitinib and upadacitinib have been approved for AS by the EMA. Of note, two phase III clinical trials of the selective JAK1 inhibitor filgotinib in AS and psoriatic arthritis were halted by the manufacturer because of FDA concerns about the risk–benefit profile of this drug (NCT04483687). In addition, in September 2021, on the basis of the review of data from an RCT, the FDA concluded that there is an increased risk of major cardiovascular events (such as heart attack or stroke), cancer, blood clots and death from tofacitinib use. As upadacitinib shares the same mechanism of action, the FDA is requiring the same boxed warning for all 3 JAK inhibitors and is advocating that clinicians consider using TNF inhibitors before JAK inhibitors[70].

Effect of bDMARDs on radiographic progression. There are no long-term (>6 months) placebo-controlled prospective trials in patients with AS to demonstrate the effect of bDMARDs on radiographic progression. All evidence is either based on comparisons with historical cohorts or on retrospective analyses of cohorts. In the Outcome in AS International Study (OASIS), individuals treated with etanercept, infliximab or adalimumab for

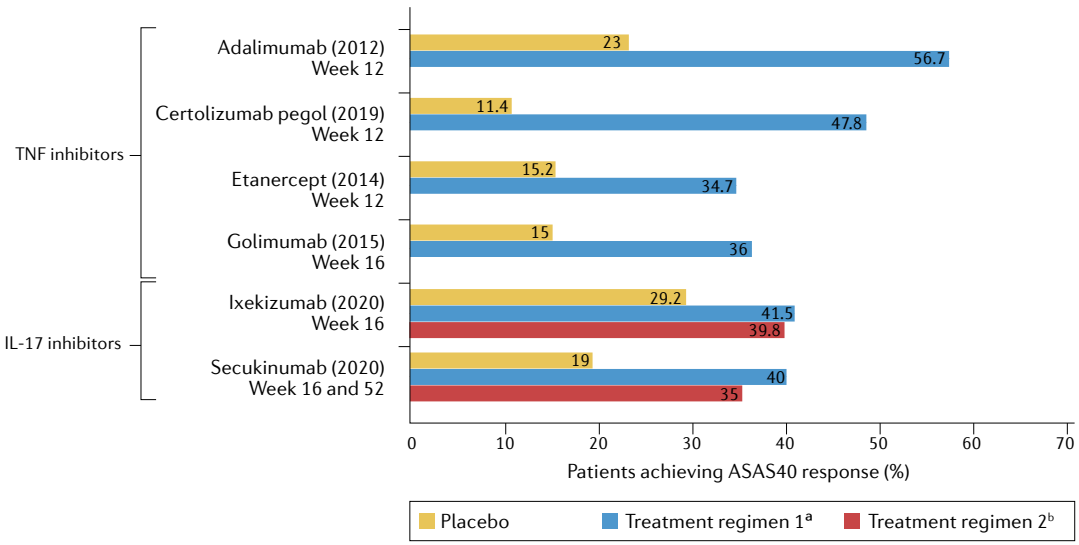

Fig. 3 | **ASAS40 responses from clinical trials in nr-axSpA.** ASAS40 responses in pivotal phase III studies in non-radiographic axial spondyloarthritis (nr-axSpA). The results presented here are not head-to-head studies and are presented for illustration purposes, not for direct comparison. [a]Treatment regimen 1 as follows: adalimumab 40 mg subcutaneously (SQ) every other week[102]; certolizumab pegol 400 mg SQ at weeks 0, 2 and 4, followed by 200 mg every 2 weeks[44]; etanercept 50 mg SQ every week[103]; golimumab 50 mg SQ every 4 weeks[104]; ixekizumab 80 mg SQ every 2 weeks[63]; secukinumab 150 mg SQ with a loading dose weekly, then every 4 weeks starting at week 4 (REF.[62]). [b]Treatment regimen 2 as follows: ixekizumab 80 mg SQ Q4W[63]; secukinumab 150 mg SQ without a loading dose weekly, then every 4 weeks starting at week 4 (REF.[62]).

2 years in pivotal RCTs did not show slowing of radiographic progression as measured by the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) when compared with a historical cohort of patients with AS who were not treated with a bDMARD[71–73]. However, retrospective observational studies on several cohorts suggested that TNF inhibitors could reduce the structural progression or osteoproliferation if used for prolonged periods (that is, >2 years)[74]. Retrospective analysis of a North American cohort revealed that patients with AS treated with TNF inhibitors had radiographic progression reduced by 50% compared with those not treated with TNF inhibitors[74]. The 50% reduction in radiographic progression in patients with AS treated with TNF inhibitors was confirmed in a Swiss cohort, and it was shown that this effect was mediated through control of disease activity (as measured by ASDAS)[75]. In a Dutch cohort study, the rate of osteoproliferation in patients with AS on TNF inhibitors progressively diminished at 6 and 8 years compared with at 4 years during the observation period[76]. A 4-year-extension of RCT of certolizumab pegol in axSpA showed limited progression in spinal and sacroiliac joints, both by radiography and by MRI[77]. Secukinumab was associated with a low rate of spinal radiographic progression in a 4-year-long phase III study[78]. Comparing 2-year data from a phase III trial of secukinumab with a historical cohort of bDMARD-naive patients treated with NSAIDs indicated a favourable but statistically non-significant effect of secukinumab on structural progression[79]. A 2020 systematic review and meta-analysis concluded that there could be favourable effects of long-term treatment with TNF inhibitors (>4 years) in patients with axSpA; however, there was no beneficial effect of NSAIDs and secukinumab on radiographic progression at 2 years[27].

As long-term reduction in inflammation reduces the structural damage in axSpA, it is conceivable that IL-17 inhibitors would also retard the radiographic progression over a long duration of therapy. An ongoing head-to-head clinical trial of secukinumab and adalimumab may provide more information in this regard[80].

Role of IL-23 inhibitor therapy. Tildrakizumab, risankizumab and guselkumab are IL-23p19 inhibitors, which have been effective in the treatment of psoriasis and psoriatic arthritis[81]. IL-23 and IL-12 share a common p40 subunit, and ustekinumab is an IL-23p40 inhibitor. The first RCT of ustekinumab in TNF inhibitor-naive patients with AS failed to achieve the primary end point of ASAS40 response; thus, two other phase III studies were prematurely discontinued[82].

In a phase II study, risankizumab failed to meet the primary end point of ASAS40 response in patients with active AS, suggesting that IL-23 inhibition has a limited role in the management of axial inflammation in AS[83]. Several reasons for the inefficacy of IL-23 inhibitors in axSpA have been postulated. One reason could be a reduced number of IL-23-secreting myeloid cells in the axial skeleton compared with the peripheral skeleton[84]. Other reasons could be tissue cytokine hierarchy — certain cytokines are more important in some tissues[85], the fact that IL-23 could be important in the initiation of the disease but not as important in the continuation of immune-mediated inflammation in the axial skeleton[86], or the presence of IL-23-independent sources of IL-17 (REF.[86]).

Overall approach to treatment of axSpA

Treatment guidelines

In 2016, the ASAS and the EULAR published the revision of their 2009 guidelines for the treatment of axSpA[5]. It contained 5 overarching principles and 13 recommendations. Salient recommendations included the use of the T2T strategy and flexibility around tapering bDMARDs in the case of sustained remission. These guidelines recognized axSpA as one disease spectrum encompassing both AS and nr-axSpA.

In 2019, the ACR–SAA–SPARTAN updated their previous (2015) guidelines, a revision made necessary by the advent of new medications and new treatment strategies[34]. These guidelines covering both AS and nr-axSpA used the Grading of Recommendations, Assessment, Development, and Evaluation methodology and were created to answer specific clinical questions (population, intervention, comparator and outcome questions) faced by clinicians in practice. The guidelines cover patients with active as well as stable axSpA separately. They recommend TNF inhibitors as the preferred bDMARD agents after failure of NSAIDs, and IL-17 inhibitors as the third-line therapy in patients with axSpA with a primary non-response to TNF inhibitors. They also recommend the JAK inhibitor tofacitinib as the fourth-line therapy. These guidelines conditionally recommend against the T2T strategy in axSpA, against tapering bDMARDs as a standard of care and also recommend using MRI sparingly in daily practice, only for investigating disease activity in patients who are not responding as expected[3].

The 2016 ASAS–EULAR treatment guidelines and the 2019 ACR–SAA–SPARTAN treatment guidelines have more similarities than differences[3,5]. They both recommend a very similar algorithm of physical therapy and NSAIDs first, followed by TNF inhibitors and then IL-17 inhibitors in non-responders for both AS and nr-axSpA. Both sets of guidelines suggest that the presence of extra-articular manifestations, such as uveitis, IBD or psoriasis, influence treatment decisions. Unlike the ACR–SAA–SPARTAN treatment guidelines, the ASAS–EULAR guidelines do not mention tofacitinib or biosimilars, as the phase II and III studies on tofacitinib and the biosimilar trials in AS were published afterwards[5]. The other differences between the two guidelines are related to bDMARD tapering in patients in remission and the use of the T2T strategy (ASAS–EULAR guidelines recommend both, whereas ACR–SAA–SPARTAN guidelines conditionally recommend against tapering as a standard of care, and conditionally recommend against T2T). Both treatment guidelines will undoubtedly need further revisions in the coming years, but as they take into account national and regional health-care provision realities, it is neither unusual nor improper that they have few differences.

With limited options available to treat patients with axSpA, it is not uncommon for a patient to cycle through all available bDMARDs and have multiple

pharmacological therapy failures. We suggest an approach to such a situation for clinicians (BOX 1).

Box 1 | **Approach to patients with axSpA, after multiple pharmacological therapy failures**

- Is the diagnosis correct?
- Is the disease still active (consider C-reactive protein level, erythrocyte sedimentation rate, sacroiliac joint or spine MRI)
- What am I treating? Inflammation or structural damage?
- Is the patient compliant with treatment?
- Is fibromyalgia, depression or sleep disturbance causing the symptoms?
- Have I set realistic expectations with the patient (and myself)?
- Should I try sacroiliac joint corticosteroid injections, nerve ablation (pain clinic), intravenous pamidronate (a bisphosphonate)[105], maximize NSAIDs, or conventional synthetic DMARDs?

axSpA, axial spondyloarthritis.

Tapering or discontinuation of bDMARDs

Several studies have been conducted in the past few years to assess the effect of tapering or discontinuation of bDMARDs in patients with axSpA (TABLE 2). In one such strategy trial, patients with nr-axSpA who had achieved sustained inactive disease (as measured by ASDAS of <1.3) with adalimumab for 28 weeks were randomized to receive continued therapy with adalimumab or placebo. In the adalimumab group, 70% of patients remained flare free, compared with 47% of patients on placebo at week 68 (REF.[87]). In an open label, phase IV study in patients with nr-axSpA who achieved inactive disease with etanercept at week 24, about 25% of patients remained in a state of inactive disease for 40 weeks after withdrawal of therapy, and among those who experienced flares, 65% re-achieved inactive disease by resuming etanercept[88]. In a two-part RCT, patients with early axSpA (AS or nr-axSpA with symptom duration of <5 years) received either the same dose or half-dose certolizumab pegol or placebo for 48 weeks after achieving inactive disease with open-label certolizumab pegol for the first 48-week period. Authors concluded that patients achieving remission could reduce the dose but not discontinue therapy to maintain remission and avoid flares[89]. In a long-term extension study of ixekizumab, 155 patients who participated in 3 RCTs of ixekizumab entered a randomized treatment withdrawal period and were given ixekizumab 80 mg every 2 weeks, 80 mg every 4 weeks or placebo for the next 40 weeks. Of the patients in the ixekizumab groups, 83% (85/102) remained flare free, compared with 54% (29/53) in the placebo group, suggesting that therapy withdrawal is associated with increased flares[90]. A 2021 meta-analysis looking at axSpA clinical trials showed no clinical benefit from the reduction of therapy with TNF inhibitors, and maintaining the standard dose improved the sustained effect on disease activity and prevented disease flares[91].

Treat-to-target strategy in axSpA

The T2T strategy is well established in rheumatoid arthritis and gout, and its adoption in these disorders is based on several high-quality studies. In the field of axSpA, the challenging issues to implementing T2T strategies include a lack of direct evidence of benefit for clinical and radiographic outcomes and, equally importantly, limited therapeutic options[92]. It is argued that T2T in axSpA can result in increased costs of care, possibly increased adverse effects and rapid cycling of the few available bDMARDs[92]. The 2016 ASAS–EULAR recommendations suggest using a treatment target mainly based on the evidence that high disease activity is associated with the formation of new syndesmophytes but without defining a preferred target[5]; however, the 2019 update of the ACR–SAA–SPARTAN guidelines in axSpA conditionally recommends against T2T, owing to a lack of robust evidence for T2T, concerns about burdening the patients and health-care providers and about rapid cycling of bDMARDs[3]. After these guidelines were published, a cluster randomized prospective study failed to show a clear benefit of a T2T study in axSpA[93]. In this study, 160 patients were randomized into usual care or tight control groups for 1 year. Patients in the tight control group were assessed every 4 weeks, and those in the usual care group were assessed every 12 weeks. At 1 year, tight control was not statistically superior to usual care in improving the ASAS health index, despite a greater number of bDMARD prescriptions. There were favourable trends towards improved societal health and economic considerations in the tight control group[94]. Further long-term studies and improved discriminatory outcome measures as targets are required to assess the effect of T2T in axSpA.

Management of comorbid conditions in axSpA

Anxiety and depression are common comorbidities of axSpA and can confound the disease activity measures[95]. Sleep apnoea is a common association among patients with AS[96]. Clinicians should address the depression and sleep issues to improve the overall quality of life and prevent inappropriate escalation of bDMARD therapy. Fibromyalgia is prevalent in 11%–34% patients with axSpA and can pose a challenge to accurate measurement of disease activity[97]. In a patient not responding to multiple bDMARD therapies, concurrent fibromyalgia should be considered, and, if present, should be appropriately managed. Frequently, patients have mechanical back pain related to degenerative disc disease, facet arthritis and spinal stenosis, which should be addressed and managed appropriately. Smoking cessation is an important consideration, as smoking is a risk factor for radiographic progression and possibly poor response to TNF inhibitors[98]. In addition, multidisciplinary management of the comorbidities associated with axSpA, such as hypertension, cardiovascular diseases and stroke, gastroduodenal ulcers and osteoporosis, should become an integral part of the treatment of axSpA.

Treatments on the horizon

In a 2020 phase IIb RCT (BE-AGILE), 29–46% of patients with AS treated with various doses of bimekizumab (a dual inhibitor of IL-17A and IL-17F) achieved an ASAS40 response at week 12, compared with 13%

Table 2 | **Tapering strategy trials for axSpA**

Study	Study design; number of patients	Strategy	Results	Ref.
ABILITY-3	Multi-centre, randomized, double-blind; 305	Adalimumab withdrawal. Patients who achieved inactive disease (ASDAS <1.3) with open-label adalimumab treatment were randomly assigned to treatment with adalimumab or placebo for 40 weeks	70% of patients continuing adalimumab did not experience flare, compared with 47% of those who received placebo	87
RE-EMBARK	Multi-centre, open-label, phase IV trial; 119 (in the withdrawal phase)	Etanercept withdrawal. Patients who achieved inactive disease after treatment with etanercept (50 mg subcutaneously weekly) for 24 weeks discontinued treatment	75% of patients experienced flare within 40 weeks; 50% experienced flare within 16 weeks. The probability of experiencing ≥1 flare after etanercept withdrawal increased from 22% at week 4 to 67% at week 40	88
C-OPTIMISE	Two-part multi-centre phase IIIb, open-label; 313 randomized at week 48	CZP dose reduction or withdrawal study. Patients with ASDAS <1.3 after open-label treatment with CZP for 48 weeks[a] were randomized to CZP 200 mg subcutaneously every 2 weeks (CZPQ2W), CZP 200 mg subcutaneously every 4 weeks (CZPQ4W) or placebo for a further 48 weeks	83.7% of patients in the CZPQ2W group and 79.0% in the CZPQ4W group remained flare free through weeks 48–96, compared with 20.2% of patients in the placebo group	89
COAST-Y	Double-blind RCT long-term extension; 155	IXE withdrawal Patients completing COAST-V, COAST-W and COAST-X trials (with ASDAS <1.3 at week 24[b]) were enrolled and treated with open label ixekizumab. Patients were randomized to IXE 80 mg Q4W, 80 mg Q2W or placebo for the next 40 weeks	83% of patients are free compared with 54% of those in the placebo group	90

ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; CZP, certolizumab pegol; IXE, ixekizumab; RCT, randomized controlled trial. [a]During 48 week open-label period all patients received CZP 200 mg every 2 weeks (CZPQ2W). [b]After being in one of the 3 RCTs for ixekizumab[60,61,63].

of patients in the placebo group. At week 48, 60% of patients on bimekizumab achieved an ASAS40 response. A significant dose–response was observed[99]. As mentioned earlier, bimekizumab is undergoing phase III studies in AS and nr-axSpA (NCT04436640).

JAK inhibitors add a new and attractive oral option for the treatment of axSpA. In a phase III double-blind RCT in AS, 56% of patients in the tofacitinib group achieved an ASAS20 response at week 16, compared with 29.4% in the placebo group[100]. No new safety risks were identified. Tofacitinib has been approved for AS by both the FDA and the EMA. In a phase III study involving the use of upadacitinib in patients with active AS, an ASAS40 response was achieved by 52% of patients in the upadacitinib group and 26% in the placebo group at week 14 (REF.[67]). Upadacitinib has now been approved for treatment of active AS by the EMA. In a phase II RCT in AS, filgotinib was associated with a significant reduction of ASDAS compared with placebo at week 12 (REF.[101]).

Conclusions

After a gap of several years since TNF inhibitors were approved for axSpA, the field is rapidly evolving. Although individualized treatment remains a major unmet need, substantial progress has been made in the management of axSpA in the past decade. In addition to TNF inhibitors, bDMARD agents blocking IL-17A and JAK inhibitors have been approved, and phase III trials on dual IL-17A and IL-17F inhibitors are underway. With the advent of many novel therapeutic agents, the treatment algorithms are also evolving, as reflected by several strategy trials showing that tapering is preferred over discontinuation in patients in remission. The T2T approach might not yet be ready for widespread use in axSpA management. Long-term suppression of inflammation seems to reduce radiographic progression in retrospective studies, and a head-to-head trial comparing a TNF inhibitor with an IL-17 inhibitor will be the first study to investigate this important question prospectively. We will need long-term data from large cohorts to assess whether aggressive control of inflammation in axSpA leads to changing the course of the disease by altering the morbidity and even mortality. It is safe to say that the field of spondyloarthritis treatment is going through the same excitement and expansion that the rheumatoid arthritis treatment went through 10 years ago.

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Joint-on-chip platforms: entering a new era of in vitro models for arthritis

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Abstract | Arthritis affects millions of people worldwide. With only a few disease-modifying drugs available for treatment of rheumatoid arthritis and none for osteoarthritis, a clear need exists for new treatment options. Current disease models used for drug screening and development suffer from several disadvantages and, most importantly, do not accurately emulate all facets of human joint diseases. A humanized joint-on-chip (JoC) model or platform could revolutionize research and drug development in rheumatic diseases. A JoC model is a multi-organ-on-chip platform that incorporates a range of engineered features to emulate essential aspects and functions of the human joint and faithfully recapitulates the joint's physiological responses. In this Review, we propose an architecture for such a JoC platform, discuss the status of the engineering of individual joint tissues and the efforts to combine them in a functional JoC model and identify unresolved issues and challenges in constructing an accurate, physiologically relevant system. The goal is to ultimately obtain a reliable and ready-to-use humanized model of the joint for studying the pathophysiology of rheumatic diseases and screening drugs for treatment of these conditions.

Some of the most prevalent rheumatic diseases are characterized by inflammation and/or degradation of joint tissues, resulting in loss of joint function and consequently a reduction in both mobility and quality of life of affected patients and, in severe cases, disability[1]. Rheumatic diseases comprise over 100 different disorders, of which osteoarthritis (OA) and rheumatoid arthritis (RA) are the most common[2,3].

Although both OA and RA are arthritic joint diseases, they have clearly distinct aetiologies. OA is a disease that affects the function of all tissues in the joint and can be triggered by multiple factors, such as improper mechanical loading, trauma, genetic factors, elevated body weight and metabolic syndrome. OA is generally confined to one or a few diarthrodial joints[4]. By contrast, RA is a systemic autoimmune disorder, in which the patient's own immune system attacks the joint tissue, leading to a strong inflammatory response that progressively affects almost all joints in the body[5]. Sex and age are additional risk factors for OA and RA, with higher predisposition in elderly women[2,3].

Despite decades of research, only a few disease-modifying drugs are available for RA, and none for OA, with current treatments being palliative at best. Multiple factors can explain this lack of disease-modifying osteoarthritic drugs, such as the substantial inter-patient heterogeneity in disease manifestation, which is linked to the wide range of disease triggers[6]. Although the mechanisms underlying disease onset and development are poorly understood, it has become increasingly clear that each disease aetiology requires a specific treatment regimen. Furthermore, it is commonly accepted that none of the currently available animal models truly reflect the complexity and multifaceted presentation of OA and RA, as is the case for many human diseases, and frequently used in vitro cellular models lack physiological relevance with respect to, for example, joint loading and inter-tissue communication (TABLE 1). This is particularly true for many 2D or 3D cell cultures, which are the most commonly used in vitro models, both in industry and academia, owing to their low cost, simplicity, robustness and experimental reproducibility. However, many of these common models lack crucial features found in vivo, which dramatically impacts cell phenotype, behaviour and response to tested drugs[7]. The advantages and disadvantages of these standard models[8–10], as well as how they compare with organ-on-chip (OoC) models[11], have been reviewed elsewhere. In particular, emulating inter-tissue communication in combination with mechanical loading is challenging in 2D and 3D cell cultures. Another example, which is particularly relevant for chondrocytes, is the progressive loss of chondrogenic features in primary chondrocytes cultured in 2D, a process that is also referred to as chondrocyte dedifferentiation and results in dramatic changes in the response of these cells

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Key points

- Current in vitro and in vivo models only partly recapitulate the complexity of human arthritic diseases and consequently lack translational power in the development of new disease-modifying treatments.
- Engineering a miniaturized version of the human joint as a joint-on-chip platform that faithfully emulates key aspects of a healthy joint and in which disease-specific triggers can be introduced could substantially advance research into arthritic diseases.
- The minimal functional joint-on-chip requires an osteochondral unit and a synovial membrane unit that emulate the composition of the extracellular matrix and appropriate cell types in the respective tissues and that are connected to each other using microfluidic coupling.
- The minimal joint-on-chip can be extended with additional tissue units, such as those emulating the meniscus, ligaments and Hoffa's fat pad; inter-organ communication could be achieved by connecting the different tissue units to a motherboard with integrated sensors to enable real-time measurements.
- Although promising and potentially revolutionary, multiple challenges must still be overcome to produce a reliable joint-on-chip model that could be used in arthritis research and drug development programmes.

to various external stimuli[12]. Animal testing is still an important element in OA and RA research for the elucidation of disease mechanisms and drug testing. The various animal models used in arthritis research have been reviewed elsewhere[13,14]. Undoubtedly, animal studies have considerably increased our understanding of OA and RA and provided new insights into human disease pathophysiology[15]. However, the considerable differences in joint loading, size, shape and physiology between widely used animal models (such as rodents) and humans could account for differences in their response to drugs and contribute to the limited translational power of animal models to accurately predict successful clinical introduction.

The development of drugs to treat RA and OA would benefit strongly from studies in more representative in vitro models of these human diseases, and of the human joint itself. Such models could provide novel insights into disease onset and pathophysiological mechanisms, facilitate biomarker discovery to enable distinct disease phenotypes to be distinguished, and be instrumental for drug development and testing before clinical trials in patients.

In this Review, we suggest that OoC technology, in the form of a joint-on-chip (JoC) model, has the potential to provide the next generation of in vitro models for studying RA and OA pathophysiology and might prove indispensable for drug development. We compare JoC models with 2D and 3D in vitro cell or tissue culture models and present some of the advantages and disadvantages of these platforms (TABLE 1). Although other approaches and models are being developed for the same purpose (for example, ex vivo cultures of human joint tissues), we focus solely on OoC platforms in this Review (alternative approaches have been reviewed elsewhere[16–18]). We propose a JoC model design, focusing on the minimal elements that we consider indispensable for such a model (for example, the individual tissue units that must be included to yield a representative model of the human joint). We discuss the development status of individual on-chip tissue units and the efforts to combine them in a JoC platform. We also discuss strategies for connecting individual tissue units, implementing an immune component and innervation, and integrating sensors for real-time, non-invasive longitudinal measurements. Finally, we reflect on engineering challenges that must be resolved for the technology to be broadly adopted and conclude by highlighting the potential of the proposed JoC concept for advancing research into arthritic diseases.

The joint, a multi-tissue organ

The joint is a multi-tissue system comprising articular cartilage, subchondral bone, synovial membrane, ligaments, and, in some diarthrodial joints, the meniscus. Each of these tissues performs a specific function. Together with auxiliary tissues such as Hoffa's fat pad, muscles and tendons and the patella in the knee joint, these individual tissues are responsible for joint homeostasis through intricate and still poorly understood intra- and inter-tissue communication.

The articular cartilage is an avascular multi-zonal structure comprising superficial, middle and deep zones and consisting of chondrocytes embedded in an abundant extracellular matrix. Each zone is characterized by typical differences in the distribution, shape and directionality of both the collagen fibres and cells and in the presence or absence of glycosaminoglycans (GAGs). This unique configuration allows homogeneous mechanical load distribution and provides tissue resistance against shear forces and tissue compression[19]. The articular cartilage is separated from the underlying subchondral bone plate by the calcified cartilage. This tissue has a fundamental function in the distribution of mechanical loading over the joint and the diffusion of components from the bone to the cartilage[20,21], and as such plays a prominent role in the pathogenesis of OA. The subchondral bone consists of a mineralized bone matrix deposited by bone-forming osteoblasts, the activity of which is regulated by osteocytes that act as mechanosensors[22]. Osteoclasts are responsible for bone resorption[23]. In contrast to cartilage, the subchondral bone is vascularized and innervated, providing a protective environment for haematopoietic cells in the bone marrow. Communication between the articular cartilage and subchondral bone is instrumental in disease manifestation in both RA[24] and OA[3].

The synovial membrane comprises two main strata: a multicellular synovial lining containing synoviocytes (also known as synovial fibroblasts) and macrophages that faces the synovial fluid, and an underlying lamina propria, which is a connective tissue containing synoviocytes, macrophages, microvessels and small nerves[25]. Owing to its architecture, the synovial membrane can sustain deformation while functioning as a filter and barrier that prevents leakage of synovial fluid. The synoviocytes are responsible for maintenance of synovial fluid composition by releasing the lubricants lubricin and hyaluronic acid, which allow partial dissipation of the forces generated during joint movement. Synovial macrophages have a prominent role in the regulation of joint homeostasis by clearing the synovial fluid of possible debris[26].

Ligaments are dense collagenous bands of different sizes, shapes and locations that are anchored to the bones. Fibroblasts are the main cell type responsible for maintenance of the ligaments. Ligaments passively stabilize joints by limiting the freedom of movement of bones. Ligaments are subjected to repetitive cyclic stretching, the direction of which depends on the specific movement being performed.

The meniscus is a three-layered horseshoe-shaped fibrocartilaginous structure that stabilizes and constrains movement of both bones in some diarthrodial joints. The high water (72%) and collagen (22%) levels in the meniscus dissipate mechanical load by allowing proper distribution of multi-directional forces on cartilage and bone during movement. The meniscus contains two main cell types: fibrochondrocytes, which populate the middle (lamellar) layer and inner (central main) layer, and fibroblast-like cells that occur in the outer (surface) layer. The surface layer consists of vascularized connective tissue with fibroblast-like cells; whereas the lamellar and central main layers contain fibrochondrocytes embedded in an avascular, aneural and alymphatic matrix[27].

Hoffa's fat pad (also known as the infrapatellar fat pad) is an intracapsular and extra-synovial fibrous adipose tissue comprising adipocytes with low metabolic activity. Hoffa's fat pad functions as a cushion to absorb shocks and prevent the patella from contacting the femur during movement[28,29].

Table 1 | **Comparison of in vitro 2D, 3D and organ-on-chip models**

Type of model	Advantages	Disadvantages
2D model	High throughput	Oversimplified systems
	Cheap	Static condition
	User-friendly	No control of physical parameters at the single-cell level
	Real-time observation	Insufficient maturation of the tissue constructs
	Cells can be synchronized to study discrete mechanisms	Lack of translational power
	No ethical concerns	
3D model	More complex system incorporating multi-cellular environment	No control of physical parameters at the single-cell level
	Better disease model representation compared with 2D culture	Low throughput
		Not representative of human physiology (differences in genetic and physiological responses)
		Expensive
		Requires trained personnel
		Lack of translational power
Organ-on-chip model	More reliable and better predictive models than 2D or 3D models	Technical usability (requires trained personnel)
	Cost reduction in safety testing and in the development and efficacy testing of new drugs	Complex manufacturing
		Insufficient maturation of the tissue constructs
	Reduction in animal testing	Lack of a universal system and standardization
	Medium throughput	Low throughput when systems are combined
	Improved testing of therapies and diagnostics (advanced safety testing)	
	Fully humanized model	
	Higher translational power	

Organ-on-chip

OoC platforms are powerful bioengineered in vitro models that have the potential to faithfully emulate the physiology of human organs and their pathophysiology in disease states[30]. OoC systems incorporate cell–cell and cell–matrix interactions, and can also sometimes include architectural elements of the modelled organs[31]. These platforms can be extended with dynamic components, such as continuous or timely perfusion of nutrients and/or soluble molecules (for example, biochemical stimuli or drugs)[32–34] or application of various biophysical stimuli (for example, mechanical or electrical stimulation)[35–37]. OoC devices are produced mostly from optically transparent materials (for example, polydimethylsiloxane (PDMS)) that allow longitudinal and real-time imaging, to visualize cell and tissue responses to various stimuli, in some cases even at the single-cell level. In addition, OoC devices typically use small volumes (in the microlitre range), and thus require substantially lower amounts of analytical reagents and cells than 2D and 3D cell culture models. These models have been used to recreate virtually any functional tissue or organ, including, amongst others, the lung[38,39], liver[40–42], kidney[43,44], intestine[45,46], heart[47,48], oviduct[49], endothelial barriers[50–53] and tumours[54–56]. Finally, several OoC platforms can be connected to each other to yield more complex multi-OoC models[57] or even body-on-a-chip models[58], which enable the study of multi-tissue or even systemic diseases. Consequently, owing to the multi-tissue nature of arthritic diseases, there is major interest in engineering multi-OoC models that emulate the human joint.

A modular joint-on-chip platform

In this section, we review the minimal elements (that is, tissue units) to create a JoC platform and the current status in engineering these individual tissue units, after identifying some of the essential design features that need to be considered for each tissue (TABLE 2).

Minimal components of a functional JoC model. To engineer an accurate model of the human joint to study the intricate interactions between tissues, a modular approach has been proposed, in which each tissue in the joint is first modelled as an individual OoC device that is then connected to yield the JoC model[59]. When deciding on which tissues or components to incorporate, a compromise must be found between ensuring the biological and/or physiological relevance of the entire JoC model while keeping the model as simple as possible and easy to use to encourage broad adoption. Although dependent on the main purpose of the study, the JoC should minimally include the cartilage, subchondral bone and synovial membrane, all of which are considered to be crucial in regulating joint homeostasis and in the pathophysiology of OA and RA[60]. This minimal JoC model can subsequently be extended to include other tissue units, such as ligaments, meniscus and auxiliary tissues (for example, Hoffa's fat pad), if relevant.

Table 2 | **Minimal content for engineering tissue units in a joint-on-chip model**

Tissue	Important cell types	Important extracellular matrix components	Vascularization	Biomechanical stimulation
Cartilage	Chondrocytes	Collagen fibres and proteoglycans (GAGs)	None	Compression and shear strain
Bone	Osteoblasts, osteoclasts, osteocytes, nerve cells and endothelial cells	Hydroxyapatite and collagen fibres	Extensive	Compression
Synovial membrane	Synovial fibroblasts, macrophages, lymphocytes, nerve cells and endothelial cells	Collagen fibres	Extensive	Multidirectional stretching
Ligament	Fibroblasts and endothelial cells	Collagen fibres	Partial	Unidirectional stretching
Meniscus	Fibrochondrocytes, fibroblast-like cells and endothelial cells	Collagen fibres	Partial	Compression and shear strain
Hoffa's fat pad	Adipocytes and endothelial cells	Fibrous adipose tissue and collagen fibres	Extensive	Compression and shear strain

Sources of cells. Cells from various sources can be used to engineer individual tissue units, including primary human cells or well-established, well-characterized cell lines[61]. First-generation OoC devices initially relied on these sources of cells, although they have some limitations. For example, well-established culture protocols are available for primary chondrocytes[62] and osteoblasts[63], but culturing primary adult nerve cells is still not possible. Furthermore, healthy human chondrocytes are difficult to obtain, so many experiments have used cells isolated from healthy-appearing regions of diseased joints. Given that OA is a whole-joint disease, the 'healthy' status of these cells is questionable.

Ex vivo culture models of human joint tissues have been advocated as an alternative source of cells, but these models lack reproducibility owing to the scarcity of (healthy) human joint tissue for research purposes[18]. Consequently, the development of various other stem cells for creating OoC models is an active area of research[64]. Obtaining the different cell types required for engineering the JoC model from the same patient would allow the inclusion of a functional immune system without the risk of inducing immune responses resembling graft-versus-host disease owing to immunophenotype mismatches. For cartilage and bone, the use of mesenchymal stromal cells (MSCs) that can be readily differentiated into chondrocytes and osteoblasts is a viable option[65], as multiple in vitro techniques and protocols are available for this purpose[65–69]. However, MSCs are not suitable for derivation of immune cells (such as macrophages), nerve cells or endothelial cells; thus, alternative sources are needed for these cell types.

Pluripotent stem cells, such as embryonic stem cells and, in particular, induced pluripotent cells (iPSCs), could provide a solution to the aforementioned challenges in obtaining the cell types needed to engineer a physiologically relevant JoC model[70]. iPSCs have the same differentiation potential as embryonic stem cells but without the ethical constraints, and fairly successful protocols are available for the differentiation of iPSCs into chondrocytes[71], osteoblasts[72], endothelial cells[73], nerve cells[74], tenocytes[75] and various immune cells[76]. However, improvements in efficiency, yield and cell maturation are clearly needed as, for example, most protocols result in a cell phenotype resembling fetal or postnatal tissue cells rather than the adult cell type[77]. To the best of our knowledge, no protocols are yet available for the derivation of synoviocytes. An additional advantage of iPSCs is their amenability to genetic modification using CRISPR–Cas9 technology[78], creating the possibility of engineering tissue-specific reporter cell lines[79,80], as well as studying the effect of genetic variants in an otherwise genetically identical background.

Ultimately, the use of iPSCs will enable the reconstitution of an isogenic human JoC model that could be used to investigate patient-specific treatments. However, further development is needed to ensure that iPSCs can serve as a reliable and reproducible source of all the necessary cell types and in sufficient quantities.

Cartilage-on-chip unit. A model of cartilage should contain a compartment for the 3D culture of chondrocytes in a matrix (for example, a hydrogel), which mimics the physical and chemical properties of the cartilage matrix, and a mechanical actuation module for cyclical mechanical loading of the tissue, which is essential for the maintenance of joint health[81]. Hydrogels can be prepared from natural or synthetic sources: natural polymers include alginate[82], agarose[83] or polysaccharides, such as hyaluronic acid and/or dextran[84], whereas synthetic hydrogels can be produced with polyethylene glycol (PEG), for example. However, synthetic polymers are biologically inert and thus have limited capacity to interact with cells, which is a major disadvantage for tissue formation[85]. By contrast, hydrogels based on polysaccharides closely resemble the GAG composition of the cartilage matrix and have excellent biocompatibility. Temperature-based gelling of agarose has been used for decades because it offers a reliable 3D environment for chondrocytes[86–89]. However, chondrocytes cannot degrade agarose, which hampers cell movement and would therefore prevent its use for modelling vascularization during OA[90]. Therefore, hydrogels based on hyaluronic acid have clear advantages, as chondrocytes can interact with this polymer and, because they express hyaluronidase, they can remodel their extracellular environment. Of note,

Mechanical actuation module
The part of a microfluidic device that enables repeated (cyclic) application of mechanical loading on the cell-laden 3D hydrogel.

Microfluidic device
Module or system used to precisely control and manipulate fluids in micrometre-sized structures. Microfluidics is at the crossroad of different fields such as engineering, physics, chemistry, nano- and micro-biotechnology.

Uniaxial loading
Mechanical stimulation of the joint in one direction only (for example, compression or stretching). Referred to as uniaxial mechanical actuation when a tissue (cell-laden hydrogel or 3D cell construct) is stimulated in vitro.

Microfluidic chamber
Chamber of a microfluidic device of miniaturized dimensions in the micrometre range that is typically filled with a fluid (liquid or air) or a hydrogel material supplemented with cells.

the distribution of forces generated during mechanical stimulation and perceived by chondrocytes is highly dependent on the physical and chemical properties of the polymer and how they are cross-linked to generate a stable hydrogel[91]. Injectable and in situ gelling hydrogels are of great interest for the production of OoC models. These features greatly facilitate the introduction of the tissue unit in the chip compartment by simple co-injection of the polymers, cells and cross-linker; for example, dextran-tyramine and hyaluronic acid tyramine conjugates that crosslink using an enzymatic reaction. This mixture can be co-injected with cells from various sources, settles in minutes and has a track record in supporting cartilage matrix formation[92,93].

This generic approach to generating cartilage-on-chip models by embedding chondrocytes in a 3D hydrogel (FIG. 1) has been reported in three studies, which collectively demonstrated that physiological compression[94,95] and/or shear stress[96] enhances the expression of typical cartilage markers such as type 2 collagen. A microfluidic device was developed that can generate different compression conditions using an array of balloons[95] (FIG. 1d). The system comprises two chambers, a cell–hydrogel chamber and a mechanical stimulation chamber, separated by a horizontal thin elastic PDMS membrane. Mechanical stimulation is applied by deforming the membrane using air pressure that inflates the balloons and thereby compresses the cell-laden hydrogel. A similar approach was used to create a model that mimics both physiological and hyper-physiological compression (FIG. 1b), revealing that pathophysiological mechanical stimulation enhances matrix metalloproteinase (MMP) production and cartilage breakdown[94]. The disease model in this study reflected various aspects of joint overloading as a driving cause of OA and was also successfully employed as a drug screening platform[94]. Finally, a 3D equine cartilage model based on pellets of chondrocytes embedded in a hydrogel used medium flow to apply shear stress on the surface of the cell-laden hydrogel and was notably employed to study the effect of shear stress on an inflammatory response[96] (FIG. 1c). Each of these models is capable of applying hyper-physiological mechanical stimulation (thereby introducing OA features into the cartilage system) as well as triggering inflammation (for example, by exposing the tissue to pro-inflammatory cytokines), and thus could be used to evaluate anti-inflammatory drugs. However, a disadvantage of these models is the mainly uniaxial loading that can be applied, whereas in the moving joint, chondrocytes experience a combination of compression and shear strain owing to the 'rolling' motion of the two bone surfaces in diarthrodial joints. To better mimic rolling motion, a mechanical stimulation unit was developed that is capable of exerting multi-modal, multi-axial mechanical cues on a chondrocyte-laden hydrogel in a microfluidic chamber[97] (FIG. 1a).

Subchondral bone-on-chip unit. A suitable OoC model of bone should combine osteoblasts, osteoclasts, a microvasculature and neural cells in a hydrogel that emulates the mineralized bone matrix. Of the many hydrogel systems that support bone formation, those based on collagen or the collagen derivative gelatin are an obvious choice, as they can be co-injected with cells and resemble the collagenous matrix in bone. This hydrogel matrix of collagens or gelatins may be supplemented with hydroxyapatite[98] or osteoinductive calcium phosphate crystals[99] to promote bone formation. Alternative choices of biomaterials for bone tissue formation, their hierarchical structure and their biochemical and functional properties have been extensively reviewed elsewhere[100]. These materials can be co-injected with osteoblasts, which can differentiate into osteocytes on-chip when fully embedded in a mineralized matrix. Endothelial cells and osteoclast precursors can be provided via a microfluidic channel acting as a blood vessel to support the bone compartment by supplying nutrients. Previously reported bone-on-chip platforms have focused primarily on recapitulating the haematopoietic stem cell niche or studying tumour metastasis[101] and have not yet been used to study rheumatic diseases. Nonetheless, these studies revealed that combining multiple types of cells greatly enhances bone mineralization[102,103]. In other examples, a mature immune system was successfully introduced into a bone-on-chip unit designed for toxicity testing[104], and a microvasculature was incorporated into a hydroxyapatite-supplemented model[98]. All these examples can serve as a blueprint for incorporating the bone compartment into the JoC system, particularly when the bone compartment is connected to a cartilage-on-chip or synovial-membrane-on-chip unit.

Osteochondral unit. Combining cartilage and bone into a single osteochondral-unit-on-chip rather than engineering separate units would arguably simplify the design process while recreating the interface between cartilage and bone that is crucial for osteophyte formation in OA. This single unit could be engineered as a two-compartment device (FIG. 1f): each compartment would contain a suitable hydrogel matrix supplemented with cells (chondrocytes in one chamber and osteoblasts, osteoclasts, osteocytes and endothelial cells in the other chamber), and both compartments would be exposed to mechanical actuation. In particular, the two hydrogel matrices should have distinct stiffnesses to optimally support the formation of the specific tissue while promoting vascularization of only the bone compartment. The compartments could be separated by a thin, porous membrane that facilitates communication between the cartilage and bone. In such a device, both compartments could be filled separately by simple injection of an in situgelling hydrogel supplemented with cells, with each hydrogel optimized to support the formation of the respective tissue. Alternatively, a temporary layer or element[105] could be used that enables direct cell–cell contact between cartilage and bone, which is removed after loading of the cartilage and bone compartment with their respective hydrogels and cells. It is conceivable that a mineralized cartilage matrix will form at the interface through self-organization of the tissues, or the device could be engineered with a third, intermediate compartment optimized to form the mineralized cartilage matrix. However, this extra compartment would increase the complexity in engineering the device and

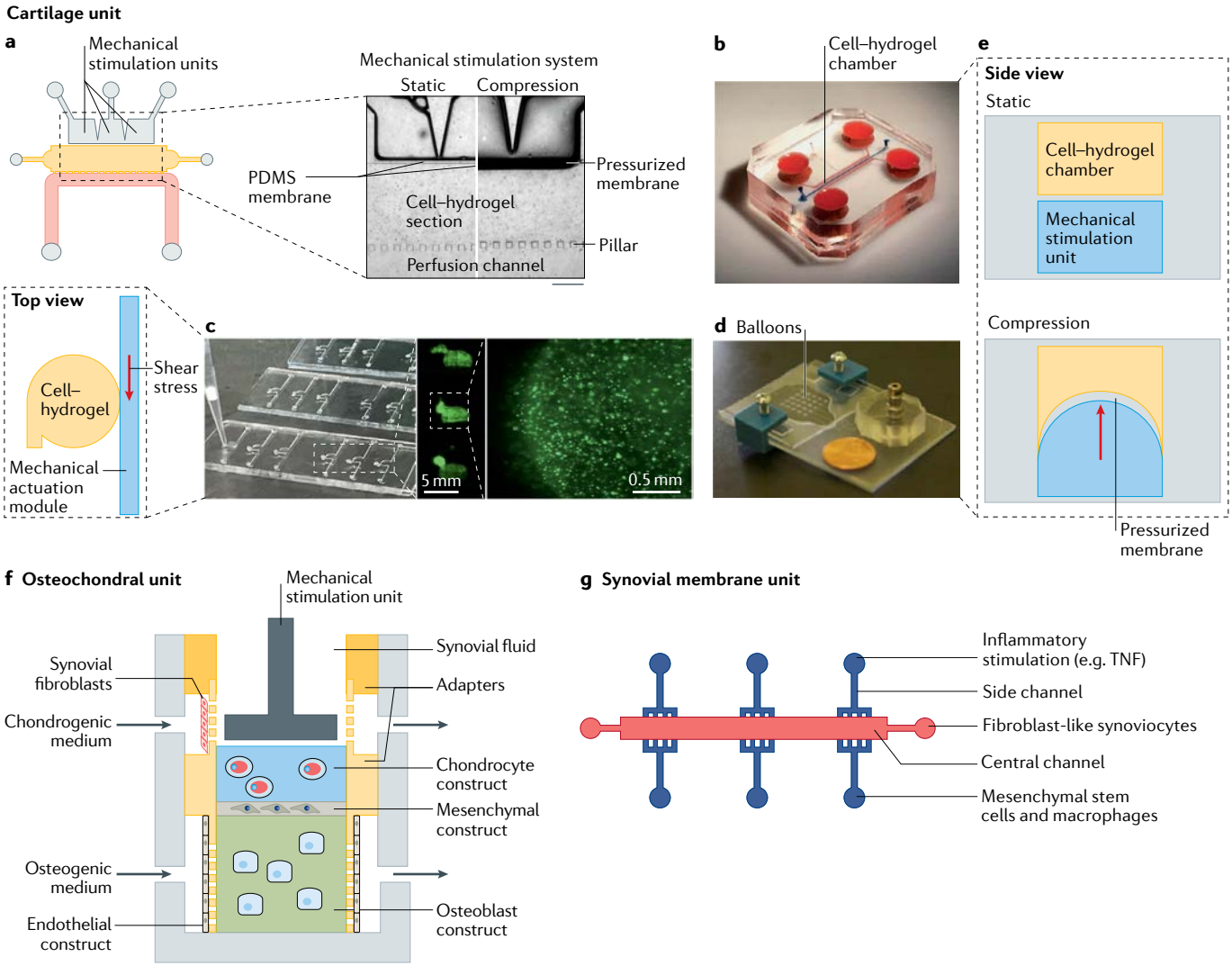

Fig. 1 | **Tissue units for establishing a joint-on-chip system. a** | Schematic (side view) of a monolithic microfluidic platform comprising a mechanical actuation unit that can generate multi-axial mechanical stimulation. A cell-laden hydrogel can be mechanically loaded by sequentially pressurizing the three independent actuation chambers with air[97]. Magnified view shows a microscopy image of the static and compressed states. Scale bar: 500 µm **b** | Microfluidic system for mechanical compression of 3D cell-laden hydrogels. The system consists of a top chamber filled with hydrogel-containing cells and a bottom chamber that acts as a mechanical actuation unit. By applying air pressure to the bottom chamber, the membrane that functions as a separator is deformed towards the top compartment, thereby applying compressive forces to the cell–hydrogel construct[94]. Magnified view shows a schematic depicting the static and compressed states. **c** | Cartilage-on-chip system comprising a circular chamber in which a chondrocyte-hydrogel construct is loaded. Cells are exposed to fluid shear stress by controlling fluid flow in the adjacent channel. Fluorescence microscopy image (right) shows the cell viability in the hydrogel construct based on calcein staining (green)[96]. Magnified view is a schematic depiction of how the model in part **c** functions. **d** | Pneumatic cell compression device comprising deformable membranes (balloons) of different diameters. Similar to the device in part **b**, this system comprises two compartments, one filled with cells and hydrogel and a second acting as a pressure chamber to apply compressive stimulation[95]. **e** | Schematic depiction of how mechanical stimulation is applied in the models in part **b** and part **d**. **f** | Osteochondral unit (not on-chip) (side view) presenting two distinct 3D hydrogels that mimic the cartilage and bone compartments[174]. **g** | Synovial membrane unit (on chip model) allowing the study of the interactions between fibroblast-like synoviocytes, osteoclasts and osteoblasts[113]. PDMS, polydimethylsiloxane; TNF, tumour necrosis factor. Part **a** is reprinted from Paggi et al.[97], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **b** is reprinted from Occhetta et al.[94], Springer Nature Limited. Part **c** is reprinted with permission from Rosser et al.[96], Elsevier. Part **d** is reprinted with permission from Lee et al.[95], Royal Society of Chemistry. Part **f** is adapted from Lozito et al.[174], Springer Nature Limited. Part **g** is reprinted from Ma et al.[113], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

in loading the microfluidic compartments with cell–hydrogel matrices. The presence of a calcified section separating the two hydrogels could be used to mimic the tidemark[106] and might be achieved by, for example, injecting a hydroxyapatite-supplemented hydrogel (used to mimic articular cartilage) between the cartilage and bone sections. Of note, the composition of the tidemark is still not fully elucidated, which arguably increases the complexity in accurately modelling this zone[107].

Few examples of an osteochondral unit-on-chip have been reported[108]. For example, a model was created that comprises chondrocytes differentiated from MSCs, which were cultured on top of a poly(ε-caprolactone) or a poly(ε-caprolactone)/hydroxyapatite scaffold

embedded in a photocrosslinkable methacrylated gelatin hydrogel that contains endothelial cells and MSCs mimicking the bone compartment[109]. In another study, a cartilage and a bone section were successfully generated with biochemically and biomechanically stimulated iPSCs, and communication was established between these two tissues; this unit was used to show a beneficial effect of the NSAID celecoxib in blocking induced inflammation[110]. Although not fully representative of OoC models and lacking a tidemark, this model illustrates the added value of mimicking the functional crosstalk between bone and cartilage in a single unit, under both healthy and OA conditions, and its potential use in drug screening (FIGS 2,3).

Synovial membrane-on-chip unit. To accurately model the in vivo structure of the synovial membrane, synovial fibroblasts (or synoviocytes) and macrophages are

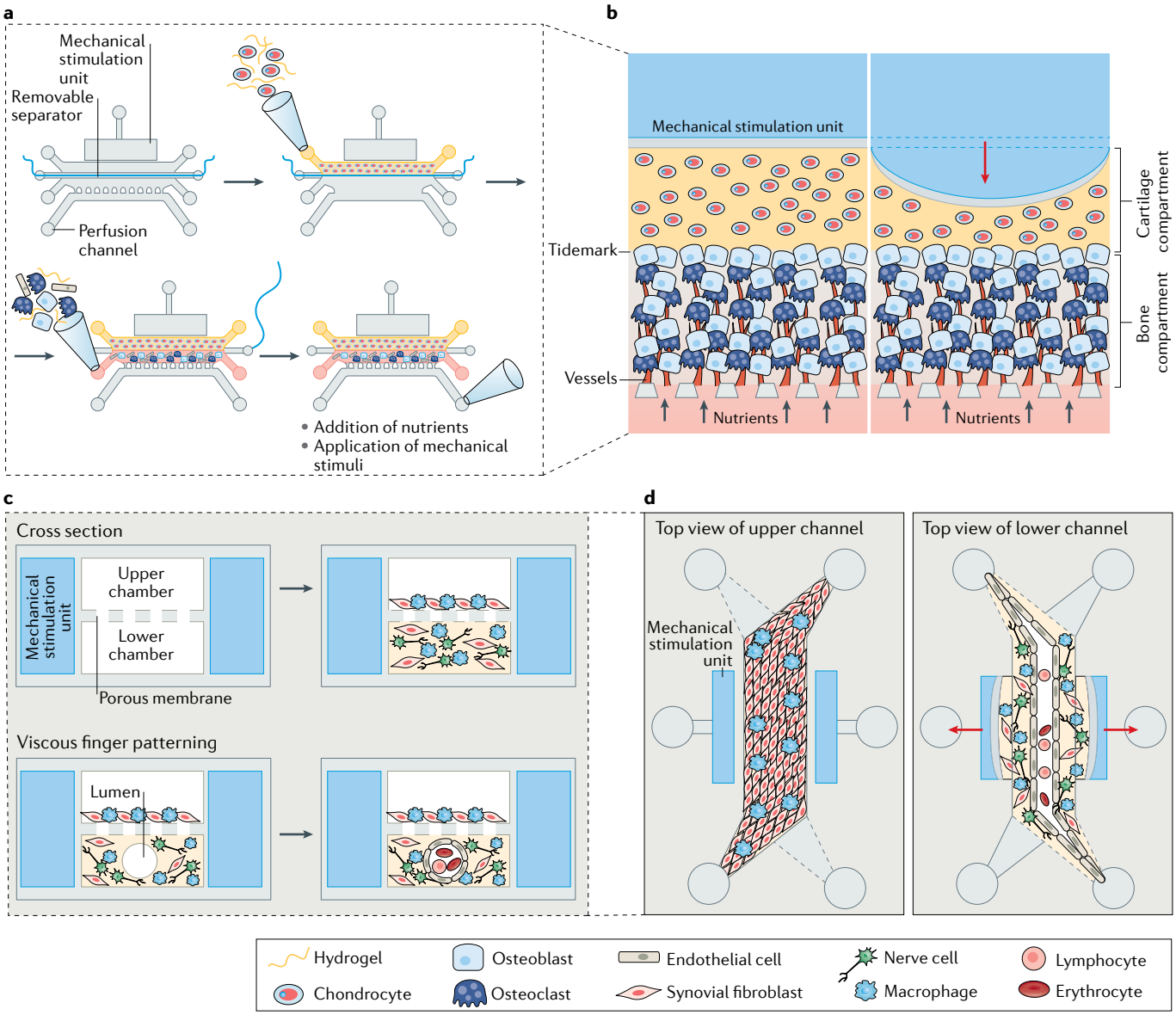

Fig. 2 | **Preparation and organization of osteochondral and synovial membrane units of the joint-on-chip platform. a** | Schematic overview of the steps for preparing the osteochondral unit (side view). **b** | Side view of the osteochondral unit in static conditions (left) and with mechanical stimulation (right). The system includes upper and lower layers of 3D hydrogels of different properties, separated by a porous membrane. The upper cartilage compartment contains chondrocytes and the lower bone compartment contains osteoblasts, osteoclasts and endothelial cells (to form a microvasculature). The hydrogel used for the bone compartment could also be supplied with neural cells to mimic innervation. Nutrients are supplied to the entire system via a perfusion channel, and the entire tissue is exposed to mechanical actuation. Alternatively, the perfusion channel can be used to introduce immune cells into the system. **c** | Schematic overview of the steps for preparing the synovial membrane-on-chip unit (cross section). **d** | Top view of the upper (left) and lower (right) chambers of the unit. The system consists of a sandwich structure with an upper chamber containing a layer of synovial fibroblasts or synovial fibroblasts and macrophages, separated by a porous membrane from a lower chamber containing a 3D hydrogel with embedded synovial fibroblasts, macrophages, nerve cells and endothelial cells. The vascular channel can be used to add lymphocytes. By applying a vacuum in the mechanical stimulation unit, the porous membrane with the attached cell layer can be stretched. Red arrows indicate the direction of deformation of the membrane.

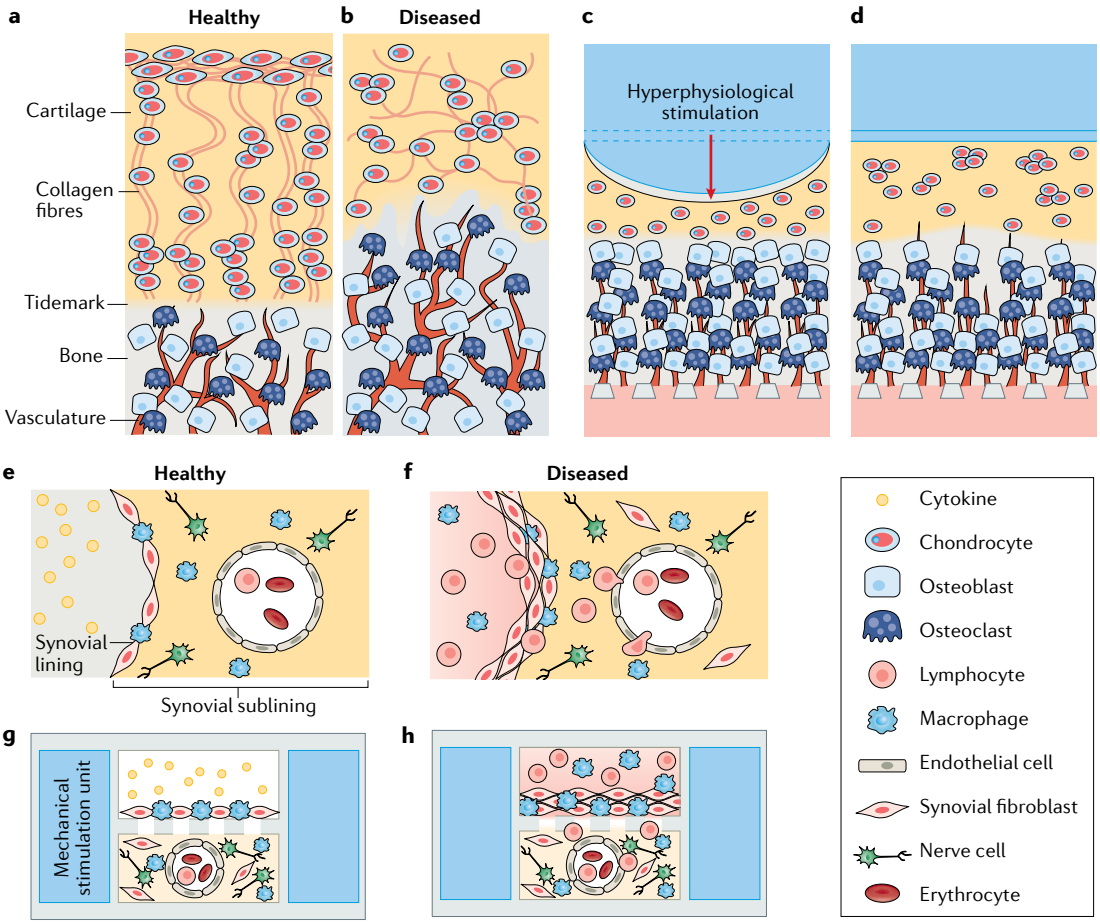

Fig. 3 | **Using organ-on-chip models to mimic pathogenetic features of rheumatoid arthritis. a–d** | Modelling hyper-physiological mechanical stimulation in the osteochondral unit. Upper schematic depicts a side view of the osteochondral unit during application of hyper-physiological mechanical stimulation (part **a**) and consequent bone outgrowth and chondrocyte death (part **b**). The schematics to the right depict a simplified view of healthy (part **c**) and diseased (part **d**) osteochondral section. **e–h** | Modelling the effects of cytokines on the synovial membrane unit. Upper schematic depicts a side view of the synovial membrane unit on addition of cytokines (part **e**), which results in proliferation of synovial fibroblasts, extravasation of lymphocytes and macrophage polarization (part **f**). Lower schematic depicts healthy (part **g**) and diseased (part **h**) synovial membrane.

co-cultured in the presence of an endothelium, which can be achieved with, for example, a compartmentalized device comprising two microfluidic chambers separated by a porous membrane that supports cell–cell communication (FIGS 2,3). The endothelial compartment can be loaded with various types of immune cells that, depending on disease-specific triggers, may extravasate to the synovial cell layer[111], a process that is characteristic of psoriatic arthritis and RA, which would be facilitated by using a membrane with an appropriate pore size compatible with cell migration. Partial or continuous perfusion in both compartments can be used to emulate shear stress created by the intra-articular fluid during joint movement and blood flow. Furthermore, mechanical stretching of the supported porous membrane could be an important feature given the profound impact that mechanical cues can have on the phenotype and behaviour of cells. Although often neglected, the synovial membrane is exposed to cyclic stretching during joint movement.

To the best of our knowledge, no OoC model that mimics all these features of the synovial membrane has been reported. However, a few OoC platforms have been developed to study the role of the synovial membrane in rheumatic diseases. For example, a synovium-on-chip model was developed using synovial fibroblast spheroids from patients with RA, which, although using only one cell type, showed clear upregulation of catabolic markers and thickening of the synovial lining layer after TNF stimulation[112]. Another study investigated the cross-communication between fibroblast-like synoviocytes and both osteoblasts and osteoclasts in bone resorption, as well as the migration of fibroblast-like synoviocytes[113] (FIG. 1g). However, although these models have elucidated some aspects of the diseases, such as catabolic activation, they still lack some fundamental features of the tissue. Addition of immune, endothelial and nerve cells would provide a better representation of the synovial membrane. Conversely, incorporating too many cell types could become cumbersome and require substantial optimization of culture conditions (for example, medium composition), shifting the balance between complexity and the accuracy of the

Microfluidic motherboard
Module for controlling nutrient supply to single-tissue units, which can include analytical modalities, to characterize tissue communication and integrated sensors for real-time monitoring. Individual tissue units could be connected to the motherboard, which thereby provides a standardized connection between units.

Pumping module
In organ-on-chip, a module for pressure or flow control that allows application of constant or cyclic pressure for regulating fluid flow in the nutrient compartment and mechanical actuation.

Plug-and-play solution
System that allows easy addition or removal of a single organ-on-chip unit from the overall joint-on-chip device.

Sensing units
Devices and/or modules for detecting events or changes in the physical environment, such as pressure, temperature, oxygen or biomolecules.

Microfluidic circuitry
Micrometre-sized tubing or channels connecting a series of microfluidic and/or organ-on-chip platforms with each other, and possibly incorporating devices for molecular analysis and biochemical sensing.

model. Furthermore, as previously mentioned, mechanical stimulation might also be an important factor to include in a synovium-on-chip unit. Stretching could be achieved with actuation chambers flanking the membrane[114,115] (FIG. 2b), which could be pressurized as described for lung-on-chip and intestine-on-chip devices[114,115], where membrane stretching is used to mimic breathing or peristalsis. In these devices, nutrients and biochemical stimuli are typically perfused into the bottom compartment, which often includes an endothelium emulating a blood vessel[116], whereas the top compartment contains the epithelial cell layer. Perfusion of lymphocytes in the bottom chamber could mimic the extravasation of immune cells observed in different arthritic diseases (FIGS 2,3). Altogether, combining a stretchable porous membrane separating endothelial cells and possibly lymphocytes in one chamber from a co-culture of macrophages and synoviocytes in an adjacent chamber could serve as a starting point for engineering a synovial-membrane-on-chip unit.

Ligament, meniscus and Hoffa's fat pad units. To date, no OoC models of the ligament have been reported. However, based on the architecture and cellular content of the ligament, this tissue could be modelled as a 3D culture of fibroblasts in a hydrogel matrix with a microvasculature[117]. To promote cellular alignment, type I collagen fibres could be pre-organized and aligned, as previously reported[118]. As for the tissues discussed earlier, mechanical actuation should be included, with a strategy similar to that for the synovial membrane, to provide cyclic and/or prolonged stretching to increase ligament matrix production and mimic joint movement.

A tissue model of the meniscus should comprise at least two sections based on two hydrogels of different stiffness and composition: one section that emulates the vascularized outer layer and another that emulates the avascular fibrocartilaginous middle and inner layers. The outer layer would be engineered from fibroblast-like cells co-cultured with endothelial cells, which eventually self-assemble into a vascular network[119,120], whereas the non-vascularized section would contain fibrochondrocyte-like cells. For mechanical stimulation, a combination of compressive and sliding forces should be applied, which could be achieved using the same rolling motion principle described for the cartilage-on-chip model in FIG. 1a.

Hoffa's fat pad can be modelled as a 3D vascularized hydrogel construct loaded with adipocytes and exposed to sliding forces. For example, an adipose tissue model successfully survived for up to 3 weeks, with interstitial shear stress modelled using controlled flow[121]. In addition, macrophages, lymphocytes and granulocytes present in Hoffa's fat pad in patients with OA contribute to disease progression[122,123]. Although developed to study type 2 diabetes mellitus, an adipose–immune cell interaction-on-chip unit has been previously reported[124,125] and results with this system were more reliable than conventional 2D culture. The design of such devices could be used as a blueprint for engineering a Hoffa's fat pad tissue unit.

Combining OoC units into a JoC model

Once these single tissue units have been fully characterized functionally (that is, they display the desired key features of the modelled tissue) (FIG. 4), they can be combined to establish the proposed JoC system. An additional consideration when combining units is the total culture medium volume in the complete JoC device, as a compromise must be found between ensuring adequate nutrient supply to all tissues and preventing dilution of secreted factors to ensure that their effects on other tissues remain intact, while being measurable to analyse inter-organ communication[57].

Connecting individual tissue units while optimizing perfusion. Three main strategies for connecting individual tissue units have been reported[57]: connecting the tissue-specific units using external capillary tubing connected to an external pump[126,127]; connecting tissue-specific units to a microfluidic motherboard that integrates all fluidic connections[128]; and including all organ models in one single plate complemented by an integrated pumping module for managing fluid flow[129–131]. Although the single-plate design might seem to be more a user-friendly approach as it offers a more plug-and-play solution with fewer engineering and connection hurdles, it lacks modularity and requires synchronized maturation of the individual tissues, a fairly difficult task. By contrast, the first two approaches provide more freedom for on-demand adjustment of the JoC model configuration, by only including specific tissue units depending on the specific purpose of the experiment. Similarly, additional units for in situ and online molecular analysis, such as sensing units for measuring environmental factors or biomarkers, can be easily inserted into the microfluidic circuitry. Furthermore, these approaches allow independent engineering and handling of tissue units, making it possible to assemble the JoC from fully mature tissue units.

An important additional feature to consider in integrating tissue units is establishing inter-tissue communication (via the synovial fluid) between the synovial membrane, cartilage, subchondral bone, meniscus, and ligaments, a process that is pivotal for healthy joint homeostasis. To mimic a fluid-filled intra-articular space, a dedicated closed recirculation loop could be included, in which, for example, an artificial synovial fluid-like liquid (that is, with a high salt concentration and elevated viscosity)[132] is perfused. Alternatively, it is conceivable that patient-derived synovial fluid could be perfused in the same fluidic circuit, to study the impact of changes in synovial fluid composition on the various joint tissues simultaneously. The ability to mechanically stimulate different tissue units, as discussed previously, is likely to facilitate the diffusion of cytokines and growth factors between tissues. Furthermore, tissue units comprising two fluidic compartments require two independent perfusion systems: one to mimic the blood supply, for tissues such as the synovial membrane, ligaments and subchondral bone, and the other to mimic the synovial fluid, which ensures recirculation flow between all tissues in the joint.

The flow rates in the combined devices should be jointly optimized to provide all tissues with the correct

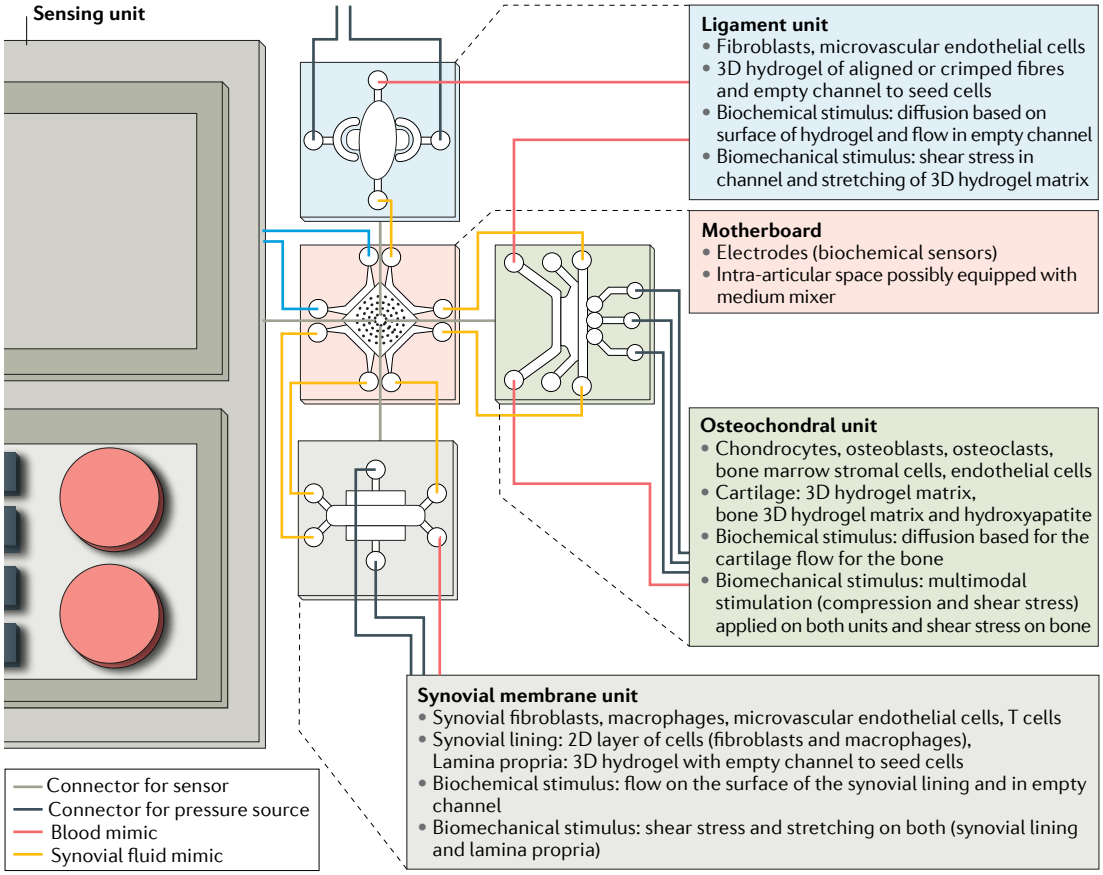

Fig. 4 | **Architecture of a proposed joint-on-chip platform.** Schematic depicting the proposed joint-on-chip device, which comprises tissue-specific units, sensing units or an analyser and a microfluidic motherboard mimicking the intra-articular space that could be complemented with sensing electrodes and/or a medium mixer. The yellow capillary tubing, when connected to the motherboard, mimics the intra-articular fluid. The black tubing is connected to pressure sources for mechanical actuation and the gold tubing connects an electrode to the analyser or sensing unit for measuring variables, such as oxygen tension or pH. The green tubing represents the medium going to an additional sensing unit that could measure changes in the concentration of biomolecules. The red tubing mimics the blood flow and is connected to an external reservoir of medium.

shear level, when applicable, while supporting (dual) inter-organ communication and keeping organ scaling (that is, the spatial relationship between organs, in terms of both fluid and metabolic demand) in mind[133,134]. The integration of mechanical actuation in the JoC system to synchronize tissue loading in the various joint tissue units will be a particular challenge.

Environmental conditions. The different tissues in the joint experience different microenvironmental cues in vivo, in terms of temperature, O_2 tension and pH, to name a few. In healthy joints, the temperature of cartilage at rest is ~33 °C, rising to 37 °C after prolonged loading and approaching 38–39 °C under disease conditions, while the ligament and synovial membrane, which are both perfused by blood, are at 37 °C. These differences in temperature could be achieved by surrounding the tissue units with individual dedicated chip-holders that include a Peltier element[135]. Similarly, as cartilage is an avascular tissue, chondrocytes reside in a hypoxic environment; thus, oxygen levels could be tuned by either having a system constructed from a transparent gas-impermeable material[136], and supplying oxygen in the perfused culture medium, or by maintaining the system at a normal oxygen level in an environmental chamber while adding oxygen-scavenging molecules to the perfused culture medium[137].

These strategies allow oxygen tension to be individually regulated in each tissue unit. However, an osteochondral unit, in which hypoxic cartilage is co-cultured with normoxic bone, presents a challenge, but might be achieved using a combination of oxygen-blocking material and perfusion of medium with distinct oxygen levels to the cartilage and bone sections.

Cartilage contains high levels of GAGs, which attract free cations (such as H^+, Na^+, K^+ and Ca^{2+}), leading to a cation accumulation and consequent decrease in pH[138] (from pH 7.1–7.3 in the superficial zone to pH ~6.9 in the deep zone)[139]. Mineralized bone contains a large reserve of alkaline minerals that is used to maintain the pH in the body[140] (at pH 7.4). In chronically inflamed joints, the pH drops to 6.5 (REF.[141]) owing to, amongst others, the high synovial metabolic rate, which increases cartilage tissue degradation by MMPs[142]. Furthermore, in bone the osteoclasts create an acidic environment to excavate resorption pits. To modulate the alkalinity or

Peltier element
A thermoelectric component capable of a temperature shift from one side of the system to the other using electrical energy.

Electrochemical microsensors
Micromachined, micrometer-sized (10^{-3}–10^{-5} m) sensing structures for detecting and quantifying specific chemical and biochemical substances in fluids, through application of a potential to induce an oxidation or reduction reaction, and recording of a current. Typically fabricated from metal materials.

neutrality of the microenvironment, the metabolic rate could be tuned (for example, an increase in metabolic rate of the synovial membrane-on-chip platform to mimic disease conditions such as in RA) or the pH of the perfused medium could be adjusted. Thus, modelling these specific environmental cues demands adequate fine-tuning of metabolic rates in each of the individual tissue units of the JoC system.

The immune system. The immune system has a crucial role in the development and/or progression of many rheumatic diseases[143] and should therefore be incorporated into the proposed JoC model. The role of the immune system is well established in RA but is less clear in OA. However, cumulative data point to a role for inflammation in disease onset and progression in OA as well[144].

Activation of the immune system in RA leads to self-sustaining inflammation that aggravates the patient's condition. In particular, $CD4^+$ T cells and macrophages release pro-inflammatory cytokines that can alter the balance between catabolism and anabolism in the joint. However, our understanding of the pathophysiological response of the immune system in rheumatic diseases is still incomplete, which further highlights the need to integrate this immune component into the JoC model to provide insight into its role. Ideally, the bone unit of the JoC should contain a fully functional haematopoietic compartment that supplies the individual tissue units with relevant immune cells. Alternatively, the model could be complemented with purified immune cell populations, derived from either differentiated iPSCs or directly isolated from whole blood, which allows the involvement of specific immune cells in the disease process to be studied.

The central role of the immune system in many disease processes has been investigated using several different OoC platforms (some of the main models that focus on the immune response are reviewed elsewhere[145]). For example, bone marrow[102,104,146,147], skin[148], gut[149], lung[114] and synovium[150] OoC units have been used to study immune responses and/or investigate immune activation during inflammation[151,152]. A JoC model that includes immune cells (for example, macrophages) could contribute to elucidating the immune response during inflammation and how this influences homeostasis of the various tissue units. For example, cytokines could be supplied to the synovial membrane model to determine the extent to which they activate macrophages and synovial fibroblasts, or to the osteochondral-on-chip unit to determine whether these cytokines lead to progressive degradation of the extracellular matrix, as is observed in vivo in RA by activating osteoclasts. An advantage of such a JoC model is its modular character, which enables dissection of interactions in specific tissue units and in the entire joint.

Innervation. Innervation is a poorly understood yet key aspect in joint homeostasis and pain sensation in all joint diseases, making inclusion of a functional nervous system in bone[153] and synovial membrane[154] compartments an important goal. At present, engineering correctly innervated OoC models of these two tissues has not been reported and remains challenging, although models investigating neural tissues and/or axonal activity have been described[155–158]. In these neural tissue models, activation of neurons could be measured in real time using a microelectrode array. Combining these models with bone and synovial membrane tissue units could better emulate the joint and further strengthen the reliability of a JoC system as a disease model.

Measuring inter-organ communication. To monitor inter-organ communication and have access to underlying molecular information, measurements must be conducted in the microfluidic circuitry that connects the individual tissue units. For example, sensing capabilities can be integrated into a JoC model for non-invasive longitudinal assessment of relevant parameters, which would substantially increase the value of a JoC model in any drug development pipeline, while being essential to acquire further insights into tissue communication and disease onset and progression. Measurement of relevant parameters in single-organ models or multi-organ platforms has been achieved using various approaches[57], relying mostly on off-line analysis (for example, comprehensive -omics analyses or immunoassays). However, real-time measurement calls for in situ or online approaches. A handful of sensors have been integrated in OoC devices[127], such as electrochemical microsensors for monitoring cell metabolism[159], oxygen concentration[160], pH[161] or glucose[162], colorimetric or fluorescent sensors for measuring oxygen concentrations[163], or plasmonic sensors for measuring cytokines[164]. Alternatively, as JoC devices are often constructed from transparent materials, it is also possible to engineer tissues using genetically modified cells that express either fluorescent or bioluminescent reporter constructs. Indeed, genetically modified iPSCs expressing green fluorescent protein (GFP) under the control of the type II collagen promoter have been used to study cartilage formation on chip by imaging of GFP[79]. Similarly, a JoC system could be complemented with reporter constructs that reveal relevant biochemical processes such as inflammation and/or MMP-mediated cartilage degradation. Eventually, this approach could allow cellular responses to be longitudinally monitored at the single-cell level.

Creating disease-specific JoC models

Arthritic diseases have disease-specific impacts on individual joint tissues. However, despite the distinct manifestations of different arthritic diseases, all joints start out as healthy. Consequently, the initial emphasis should be to accurately model the healthy joint, which is then followed by the introduction of disease type-specific triggers. For example, post-traumatic OA could be modelled by introducing a trauma into the cartilage unit followed by applying prolonged mechanical stimulation, and disease development in the chip could be followed under distinct loading regimes (FIG. 3). Hyperphysiological mechanical stimulation of the JoC system could be used to model some features of OA, as previously mentioned for the cartilage-on-chip device[94]. The JoC device provides the possibility of studying the

Non-fouling coatings
Chemical coatings that stop the interactions of molecules in solution with surfaces to notably prevent their non-desired adsorption on the surface.

impact of hyperphysiological stimulation of cartilage on other joint tissues. Alternatively, the addition of either pro-inflammatory cytokines (such as TNF) or activated immune cells to the synovial tissue unit would mimic synovial inflammation (FIG. 3), which is a feature of RA pathogenesis and could ultimately result in deterioration of the cartilage unit. These stimuli could also be injected directly into the unit to mimic intra-articular recirculation and thereby have an impact on multiple tissue units simultaneously. Furthermore, introduction of calcium phosphate crystals into the intra-articular space of the device could yield a model for gout[165].

Modelling specific features of disease might also require specific design modifications in individual tissue units. Extravasation of lymphocytes from the microfluidic channels resembling the bloodstream to the superficial layer of the synovial membrane will require the presence of a porous membrane with pores large enough to allow free movement of cells[166]. Rheumatic diseases are multi-factorial disorders, so it is important to include all of the parameters, stages, readouts and components of a disease. With the progression in engineering technologies, it is anticipated that models that can realistically emulate a diverse set of rheumatic diseases will be attainable.

Engineering challenges

Before the JoC model proposed here can be broadly adopted, there are specific engineering challenges that must be overcome[7]. If on-chip tissue models are not fully mature and thus represent fetal rather than adult tissues, they might not accurately model the in vivo tissues and as such introduce a bias into the experimental and/or assay outcome. Importantly, PDMS remains the material most commonly used to fabricate these systems but it is not suitable for mass production[167]; thus, exploration of alternative materials for fabricating OoC devices is needed. In addition, PDMS acts as a 'sponge' for small hydrophobic molecules[168], which could affect experimental outcomes in drug-dosing studies. The use of non-fouling coatings may reduce this effect[169]. Experimentation with OoC systems still requires dedicated training, owing to the technical proficiency required to work with them and, because the OoC field is still young, devices lack standardization[170,171]. Although industry is developing a range of OoC platforms, it does not always adopt the same engineering approaches as academic researchers and, most importantly, it uses different 'connection' strategies, which limits interchangeability between devices. Last, the size and costs of the auxiliary equipment required for microfluidic systems, such as pumps, tubing and controllers, might be a limiting factor for widespread implementation. Considering the rapid expansion in the OoC field, it is anticipated that many of these hurdles will be almost entirely resolved in the years to come and that easier-to-use devices and integrated solutions will become available to the wider research community.

In summary, the existence of a robust, physiologically relevant, reliable high-throughput system would radically change the way drugs are developed and tested before they are evaluated in clinical trials on patients[172]. In the following section, we highlight specific areas for which a JoC platform would have a relevant translation.

Basic research and drug development studies. As the JoC platform should mimic inter- and intra-tissue communication, it might provide new insights into how individual tissues contribute to joint homeostasis and disease onset and progression. New key molecular factors could be identified and used as targets for the development of drugs or as biomarkers for diagnosis and prognosis. Similarly, the influence of essential environmental factors such as oxygen tension, temperature and various biochemical and biomechanical stimuli could be systematically investigated in the JoC device to determine how they affect the catabolic and/or anabolic activity of cells and which parameters are optimal for recovery from a rheumatic disease. Taking advantage of the modularity of a JoC platform, tissue–tissue interactions in the joint could be investigated after triggering a disease condition in one of the tissue units, which would give new insights into the mechanisms underlying the convergence to a common OA phenotype in the joint. Similarly, the role of the immune system could be better elucidated; for example, the threshold for persistent inflammation could be established.

The JoC device could also be multiplexed and fully automated to create a fully humanized in vitro system for screening new drug candidates before they are further tested in clinical trials. Here again, the modularity of the system will allow studying the effect of the drug candidates in each tissue unit and at the level of the complete joint. Once established, the JoC device might represent an alternative to animal testing of drugs. However, realistically, a JoC device that could be used in high-throughput screening is still a long way off. Given the complexity in both the engineering and the cell biology of obtaining functional tissues, we postulate that JoC devices will find their first use as more sophisticated models for testing drug candidates that have been identified using other technologies more amenable to screening high-content drug libraries. Alternatively, drug screening and testing could first be performed with the individual tissue units before moving to the more comprehensive multi-tissue JoC device, which would significantly reduce the engineering challenge.

As patients present with different disease phenotypes and respond differently to drug treatments, patient-specific models could be used to personalize treatment. Owing to advances in iPSC culturing and differentiation, the entire JoC could be customized for individual patients, starting from a few cells collected from, for example, urine[173]. Drug retention could be analysed in individual tissue units or in the entire JoC model and it is feasible that the JoC device or the individual tissue units could be combined with other organ models, such as the liver, gut or kidney, which would allow absorption, distribution, metabolism and excretion studies and the acquisition of systemic data. Drugs currently on the market for other diseases could be scrutinized to investigate their potential for drug repurposing to treat arthritic joint diseases. Last, cell-based therapies, such as autologous chondrocyte implantation or intra-articular

injection therapies using adipose-tissue-derived mesenchymal stem cells, could be screened to determine their potential in disease modification.

Conclusions

Promoting animal-free testing using other solutions, such as the proposed JoC system, with the ability to control multiple variables, could provide deeper insights into the pathophysiology of RA and OA. The development of a JoC platform will be instrumental in understanding tissue–tissue, immune system–tissue and drug–tissue interactions, offering new opportunities to discover effective treatments and increase our fundamental understanding of the pathophysiology of joint diseases. Although substantial hurdles still remain to be overcome in the engineering of a mature, comprehensive JoC model in a standardized and user-friendly format, the rapid pace of OoC research, exemplified by the emergence of multiple start-up companies, is likely to facilitate overcoming these challenges. The implementation of a JoC system will open a new era of in vitro models for rheumatic diseases.

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Regulation of activated T cell survival in rheumatic autoimmune diseases

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Abstract | Adaptive immune responses rely on the proliferation of T lymphocytes able to recognize and eliminate pathogens. The magnitude and duration of the expansion of activated T cell clones are finely regulated to minimize immunopathology and avoid autoimmunity. In patients with rheumatic autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, activated lymphocytes survive and exert effector functions for prolonged periods, defying the mechanisms that normally curb their capacities during acute and chronic infections. Here, we review the molecular mechanisms that limit the duration of immune responses in health and discuss the factors that alter such regulation in the setting of systemic lupus erythematosus and rheumatoid arthritis. We highlight defects that could contribute to the development and progression of autoimmune disease and describe how chronic inflammation can alter the regulation of activated lymphocyte survival, promoting its perpetuation. These concepts might contribute to the understanding of the mechanisms that underlie the chronicity of inflammation in the context of autoimmunity.

The development of immune responses against self-antigens is normally avoided by a group of mechanisms, collectively referred to as immune tolerance[1]. Even during infections, when immune cells become activated and antigens derived from pathogens are presented along with antigens derived from self-molecules, immune tolerance steers the response towards components of the invading pathogen and away from molecules present in our own cells. In some instances, these mechanisms fail and a self-directed immune response is generated, for example, the development of autoantibodies after an infection. However, in most cases, the autoimmune response is transient, autoantibodies fade away and no harm is inflicted[2]. As tolerance is safeguarded by multiple mechanisms, the development of chronic autoimmunity represents a complex process.

Autoimmune diseases are usually conceptualized as the pathological result of breaches of immune tolerance. In order for a pathological condition to occur, however, the loss of tolerance must be persistent and the ensuing autoimmune response must become chronic[3,4]. In the case of systemic autoimmune diseases, which are driven by responses directed towards antigens that are not tissue restricted, an additional element is essential: that chronic inflammation develops in target organs. For example, in seropositive rheumatoid arthritis (RA), a response against citrullinated proteins drives autoimmunity, but the establishment of synovial chronic inflammation is what causes disease[5]. In systemic lupus erythematosus (SLE), the autoimmune response is directed against nuclear autoantigens, but lupus nephritis occurs only in patients who develop a renal chronic inflammatory response[4]. In this sense, the failure of immune tolerance represents a foundation that enables the development and establishment of target organ chronic inflammation. This second phase is what ultimately determines the clinical expression of disease and the extent of organ damage.

T cells define the qualitative and quantitative characteristics of the immune response and represent, along with macrophages, key drivers of chronic inflammation. Antigen-specific T cells that become activated in secondary lymphoid organs produce a progeny of cells that migrate into tissues where they exert effector functions. Research has demonstrated that complex mechanisms limit the pro-inflammatory capacity and the lifespan of effector T cells in non-lymphoid tissues, in an effort to limit the damage to bystander cells caused by infiltrating immune cells[6–9]. Whereas these mechanisms act as therapeutic barriers in contexts where stronger immune responses might be desirable (for example, in cancer or chronic infections), their breakdown might represent steps that enable the establishment of chronic inflammation in the setting of autoimmune disease. Here, we review the mechanisms that normally limit function and survival of activated T cells in peripheral tissues and

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Key points

- Chronic target-organ inflammation directly affects disease expression.
- The quantity and temporality of antigen modulates the ensuing immune response.
- The balance between stemness and exhaustion might underlie the chronicity of self-directed immune responses.
- Resistance to apoptosis and/or exhaustion might enable self-reactive T cells to persist in target organs and perpetuate local disease.

discuss how defects in these processes can facilitate the development and perpetuation of the chronic inflammatory responses that underlie organ damage in systemic rheumatic diseases.

Initiation of the immune response

Most information about the activation and regulation of the immune system has been generated using models of infection. In this setting, activation of naive T cells in secondary lymphoid organs induces antigen-dependent expansion and differentiation of effector and memory cells. Effector cells migrate to inflamed tissues and eliminate the inciting pathogen. Memory cells that establish in secondary lymphoid organs are known as central memory T (T_{CM}) cells, whereas memory cells that persist in the tissue where the pathogen was detected are known as resident memory T (T_{RM}) cells[10].

The number and lifespan of effector cells directly affects the magnitude and length of the inflammatory response and, therefore, the amount of immune-mediated damage that is exerted in the inflamed tissue[11]. Protection against future encounters with the same pathogen relies on the existence of adequate numbers of memory cells, but their presence, in particular the presence of memory cells that reside in non-lymphoid tissues (that is, T_{RM} cells), is also associated with a risk of immunopathology triggered by subsequent local activation[5].

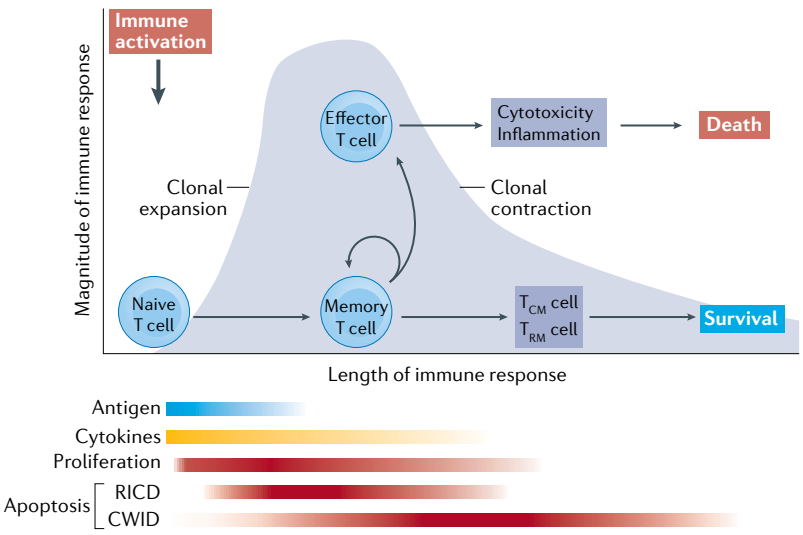

Fig. 1 | **Proliferation and apoptosis determine the magnitude and length of immune responses.** When an immune response is mounted, antigens and cytokines influence the proliferation and differentiation of T cells. Clonal expansion results from intense proliferation of T cells and is modulated by restimulation-induced cell death (RICD) that eventually dominates, limiting the magnitude of the response and generating a plateau. As the abundance of IL-2 and other cytokines declines, apoptosis of effector cells is triggered by cytokine withdrawal-induced cell death (CWID) and clonal contraction eliminates activated T cells. Memory cells home to secondary lymphoid organs, where they are known as central memory T (T_{CM}) cells, and non-lymphoid tissues, becoming resident memory T (T_{RM}) cells, where they persist for prolonged periods.

The magnitude of the immune response depends on the balance between cell proliferation and death. During the first few days, intense T cell proliferation exceeds death rates and antigen-specific cells accrue, giving rise to the process known as clonal expansion[12]. However, activation makes T cells susceptible to restimulation-induced cell death (RICD; also known as activation-induced cell death), a process whereby repeated stimulation through the T cell receptor (TCR) and/or FAS triggers apoptosis. RICD coupled with decreased T cell proliferation enables clonal expansion to reach a plateau that is maintained until cytokines, and presumably other environmental elements, decrease, triggering cytokine withdrawal-induced cell death (CWID)[12]. Activated T cells are thus removed and the remaining antigen-specific cells represent long-lived memory cells (FIG. 1).

Traditionally, clonal expansion and memory development were thought to represent consecutive linear processes where the activation of a naive cell would give rise — through cell proliferation and differentiation — to a progeny of antigen-specific effector cells that would home to the inflamed tissue and orchestrate a protective local immune response[13]. Memory cells would then arise from a few surviving high-affinity effector cells and persist in secondary lymphoid organs and non-lymphoid tissues[14–16]. This view was mostly based on findings from experiments that evaluated the behaviour and gene expression of populations of cells. For example, T cells bearing a transgenic TCR known to be specific for a viral peptide would be adoptively transferred into mice that would then be infected with the virus, so the population of virus-specific T cells could be tracked amid the diverse repertoire of native T cells of the host[14]. In humans, proliferation kinetics, turnover rate, and transcriptional profiling of virus-specific cells from vaccinated volunteers have been analysed[17]. These experimental systems have been fundamental for our understanding of how populations of specific $CD8^+$ T cells act during an infection, but assume that cells behave in a homogeneous manner during the immune response and their results are strongly biased when large populations of cells dominate.

Single-cell RNA sequencing has enabled us to analyse the transcriptome of seemingly similar cells during immune responses and has revealed a high degree of heterogeneity in T cells, even among cells that bear the same receptor and are in the process of responding to the same pathogen[18]. Importantly, these analyses indicate that early during immune responses, some responding cells activate transcriptional programs that guide their differentiation towards a memory cell phenotype[19–23]. In other words, memory cells are selected during the first few days of an immune response and, rather than being derived from effector cells that survive clonal contraction (activated cell death), represent precursor cells that give rise to effector cells during the ongoing immune response and afterwards, in recall responses[24,25] (FIG. 1).

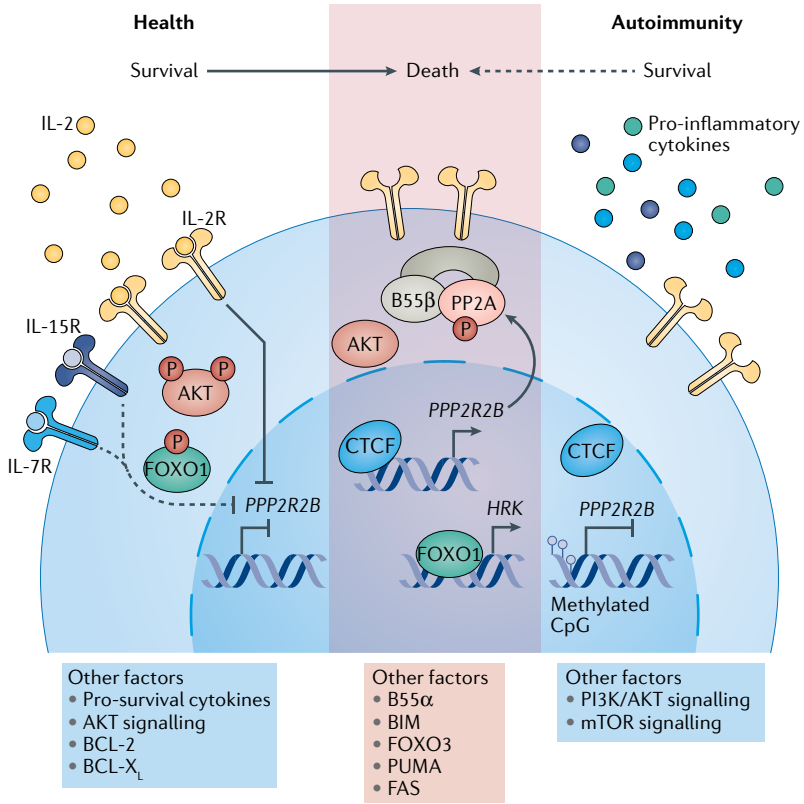

Fig. 2 | **Cytokines and other environmental stimuli regulate activated T cell survival.** IL-2, IL-7 and IL-15 promote the proliferation and survival of activated T cells through several signalling pathways, including the active suppression of *PPP2R2B* transcription. At the end of an immune response, decreased cytokine levels enable transcriptional regulator CTCF (transcription factor CCCTC-binding factor) to promote *PPP2R2B* transcription. The encoded protein, B55β, facilitates AKT inactivation by the phosphatase holoenzyme PP2A. Decreased AKT activity triggers FOXO1 nuclear shuttle and transcriptional activation of pro-apoptotic factors such as harakiri (*HRK*). In patients with SLE and RA, inflammation (partly caused by TNF) promotes inhibitory methylation of *PPP2R2B* regulatory regions, preventing the expression of B55β and impairing cytokine withdrawal-induced cell death. Additionally, activation of other pro-apoptotic mechanisms fails in autoimmunity.

Consequently, the magnitude of the immune response is not only determined by the ratio of proliferation and death of responding cells, but also by the number of cells that become memory cells and the fraction of them that generate effector cells[13]. These data indicate that factors present during T cell priming and during the first few days of an immune response determine the extent of the response and also the quality and quantity of memory developed towards the inciting pathogen.

Survival of activated T cells

Effector cells are terminally differentiated cells that have acquired functional properties that enable them to exert cytotoxic activities and promote inflammation in tissues[26]. Factors present during the early stages of the immune response — including TCR signalling strength, co-stimulation, and inflammatory cytokine abundance — determine the number of cells that become effectors[27]. However, once cells have become effector cells, their lifespan regulates the extent of their actions. The survival of activated T cells depends on the balance between pro-apoptotic and anti-apoptotic factors and is thought to be controlled by the abundance of cytokines and growth factors, in particular IL-2, IL-15, and other cytokines whose receptors include the common γ chain (γ_c (also known as CD132), encoded by *IL2RG*)[28–30]. IL-2 signalling leads to the activation of AKT, a kinase that has a central role in the regulation of cell metabolism and survival[31]. In effector T cells, AKT is phosphorylated in two residues that control its function and activity, promoting cell survival[32]. The interruption of IL-2 signalling leads to AKT dephosphorylation, nuclear translocation of FOXO1, and expression of the pro-apoptotic proteins BIM, harakiri (HRK) and PUMA, a process that depends on the serine/threonine phosphatase PP2A B55β[6,33]. Lack of B55β in murine CD8$^+$ T cells decreased apoptosis during the contraction phase of an immune response induced by *Listeria monocytogenes*, and this reduction in apoptosis greatly increased the abundance of antigen-specific activated and memory T cells after infection[6].

Expression of B55β is controlled at the transcriptional level, and its forced expression triggers apoptosis in human and murine CD4$^+$ and CD8$^+$ T cells[34]. Therefore, transcription of *PPP2R2B*, which encodes B55β, might represent a threshold after which T cells commit to apoptosis by actively maintaining AKT in an inactive (dephosphorylated) state. In this model, IL-2 concentrations would differentially regulate cell behaviour by inducing variable degrees of AKT phosphorylation, but the reduction of IL-2 under a certain level or during a certain time period would trigger B55β expression and consequently cell demise (FIG. 2). IL-2 deprivation also induces apoptosis in T cells activated in vitro[34,35]. In this system, other cytokines with a γ_c receptor (such as IL-7 or IL-15) can substitute IL-2 and prevent apoptosis[36]. Which cells are relevant sources of IL-2 and other cytokines that can inhibit apoptosis of effector cells in inflamed tissues is poorly understood. In lymphoid organs, IL-2 is mainly produced by recently activated CD4$^+$ and CD8$^+$ T cells; however, in non-lymphoid tissues, other cells, in particular innate lymphoid cells, represent an important source[37]. Regulatory T (T_{reg}) cells have been proposed to limit effector cell survival by consuming IL-2 and triggering apoptosis through CWID[38,39], but whether this mechanism occurs in tissues or is exerted during the expansion phase of the immune response is not clear. AKT functions as a node that integrates signals derived from a large number of environmental stimuli and modifies cell metabolism accordingly. Whether factors distinct from cytokines regulate the survival (and function) of effector T cells by modulating AKT activity is not well established.

Thymus-derived FOXP3$^+$ T_{reg} cells probably modulate the magnitude and duration of immune responses at different times through different mechanisms. T_{reg} cells might induce CWID in activated T cells by depleting IL-2 levels[38–40], although the relevance of this process has been challenged by demonstrating that CWID-resistant T cells (deficient in the pro-apoptotic molecules BIM and PUMA) are adequately suppressed by T_{reg} cells in in vitro and in vivo systems[41]. T_{reg} cells possess cytotoxic

capacities and have been shown to kill activated T cells (and other immune cells) through pathways that depend on FASL[42], TRAIL[43], perforin[44], or perforin and granzyme B[45]. However, the role of these mechanisms in a normal or pathological immune response is still debated[46].

T cell fate and memory development

Depending on their differentiation stage, T cells use distinct metabolic pathways and express different amounts of pro-apoptotic and anti-apoptotic proteins. Consequently, their susceptibility to programmed cell death induced by surface receptors (such as FAS) or environmental cues varies greatly. Activation of naive T cells induces the generation of memory cells that possess stem cell properties, including self-renewing capacity and prolonged survival, and terminally differentiated effector cells that will live for short periods of time[22,47,48]. Because production of effector cells might not be limited to the initial proliferative burst but might continue during the response, the duration of the immune response is not only determined by the survival of effector cells but also by the rate with which they are generated from memory precursors (FIGS 1,3). Memory cells comprise T_{CM} cells, effector memory T (T_{EM}) cells, and T_{RM} cells that exhibit differential gene expression and homing capabilities. T_{CM} cells remain in secondary lymphoid organs, where they can rapidly become a source of new effector and memory cells upon antigenic rechallenge[13]. By contrast, T_{EM} cells recirculate and T_{RM} cells migrate to epithelia or other non-lymphoid tissues where the pathogen that elicited the response was initially detected. By implementing immune surveillance at pathogen entry sites, T_{RM} cells provide local and swift immune memory[49]. In humans, T_{RM} cells represent the main T cell component of non-lymphoid tissues[50] and account for a relatively large proportion of activated T cells found in target organs in patients with autoimmune diseases[51–54].

Early work, in the 2000s, demonstrated that memory $CD8^+$ T cells express higher levels of the anti-apoptotic molecule BCL-2 and survive for longer periods of time than do naive or effector $CD8^+$ T cells with the same specificity[55,56]. However, among memory cells, survival is heterogeneous. Murine memory $CD8^+$ T cells that reside in non-lymphoid tissues were shown to live longer than those from spleen and lymph nodes[57]. When human $CD8^+$ T_{CM} and T_{EM} cells were deprived of IL-2, T_{CM} cells survived longer[28]. Interestingly, cytokine withdrawal activated autophagy, but this response was more sustained in T_{CM} cells[28]. Inhibition of autophagy annulled the differential susceptibility to CWID of T_{CM} and T_{EM} cells. Because autophagy was associated with decreased expression of BIM, the authors proposed that autophagy might regulate CWID by supplying amino acids and promoting the degradation of pro-apoptotic proteins[28].

As cell survival is required for immunological memory, memory T cells must persist for prolonged periods of time[58]. The relative resistance of memory cells to cell death is associated with their expression of anti-apoptotic molecules and differences in metabolic programming[17,59]. In contrast to effector cells, which rely on anaerobic glycolysis as their principal source of ATP and biosynthetic material, memory cells use mainly fatty acid oxidation to feed oxidative phosphorylation and, like naive cells, are relatively quiescent[60]. However, memory cells have a larger mitochondrial mass than naive cells, which provides greater spare respiratory capacity[61]. These characteristics give memory cells a survival advantage and, at the same time, enable them to rapidly produce large quantities of ATP upon TCR re-engagement[61].

These data indicate that different subsets of memory T cells are programmed to respond differently to death-inducing signals relayed by immune and non-immune cells and by the environment in which they are located. As mentioned earlier, T cell differentiation is influenced by antigen qualitative and quantitative characteristics and by signals received during antigen presentation (for example, cytokines and co-stimulation)[27]. The capacity of memory cells to divide and give rise to effector cells has been called 'stemness', because it is analogous to the capacity of stem cells to self-renew while giving rise to cells that will continue their differentiation into effector subtypes[62]. Stemness is relevant in the setting of protective immune responses, because the capacity of memory T cells to proliferate and generate effector cells depends on it[13]. However, stemness represents an unwanted function in T cells activated in response to

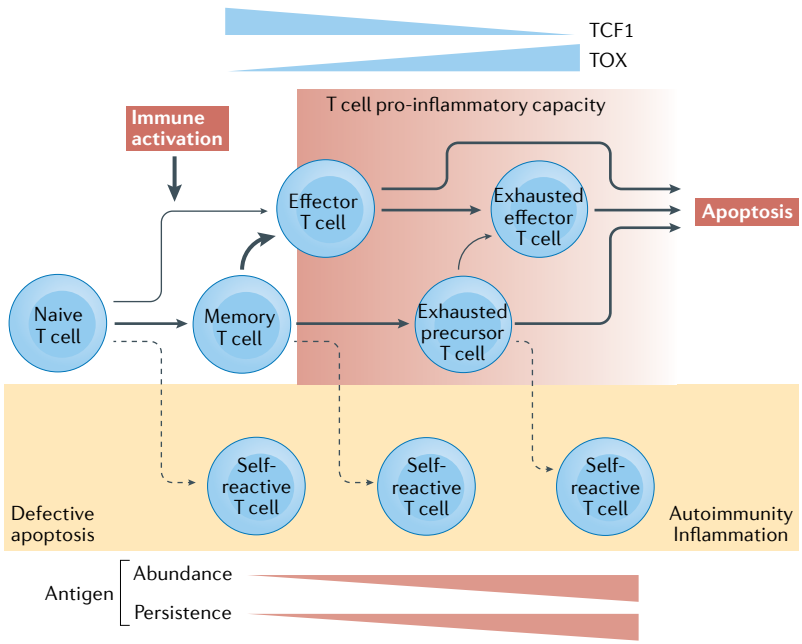

Fig. 3 | **Antigen abundance and persistence induce exhaustion and limit pro-inflammatory functions in T cells.** Persistent and abundant antigens impair sustained T cell effector function by inducing a transcriptional programme known as exhaustion. Exhausted memory T cells exhibit decreased capacity to self-renew and to produce effector T cells; exhausted effector cells express inhibitory co-receptors and have limited effector function. These features have been associated with the gradual loss of the transcription factor TCF1 and with the expression of the exhaustion-associated transcription factor TOX. When the mechanisms promoting apoptosis of naive self-reactive T cells (deletional tolerance), of activated and expanded effector/memory T cells (cytokine withdrawal-induced cell death (CWID) and restimulation-induced cell death (RICD)), and of exhausted and/or tolerized T cells are faulty, pro-inflammatory lymphocytes persist in tissues, promoting immunopathology.

Box 1 | **Mechanisms of T cell inactivation**

During T cell priming, input from the antigen (signal 1), from co-stimulatory molecules (signal 2) and from cytokines (signal 3) determine the outcome. When any of these components is absent or grossly abnormal, the T cell will acquire a dysfunctional state characterized by defective proliferation and effector function, reduced metabolic fitness and impaired survival. Other less well understood inputs to lymphocytes during activation can also affect their functional capacities. T cell competition, the presence of inhibitory co-receptors and ligands, as well as access to oxygen and nutrients from the microenvironment, can lead to different dysfunctional states known as anergy, tolerance and exhaustion[198].

Anergy
Anergy is induced by activation with an antigen in the absence of adequate co-stimulation. Anergy, which has been best defined in CD4$^+$ T cells, is programmed early during T cell priming by unbalanced NFAT signalling and is characterized by the development of a blockade in TCR signalling and specific transcriptional and epigenetic signatures[65].

Tolerance
The term tolerance is sometimes used to indicate a dysfunctional state in CD8$^+$ T cells that have become unresponsive following an encounter with self-antigen. Tolerance resembles anergy in many molecular and transcriptional aspects, including the fact that it can be induced by tolerogenic dendritic cells[199].

Exhaustion
Exhaustion is a gradual process induced by antigens that are persistently expressed. It progressively limits the function and proliferation capacity of T cells. Exhausted T cells express inhibitory co-receptors (such as PD-1, VISTA, CD244 and TIM3), which maintain their dysfunctional state[200]. The importance of exhaustion-associated inhibitory receptors as a mechanism of tolerance is evident in patients with cancer who develop autoimmune manifestations after receiving therapeutic antibodies that block PD-1–PD-L1 interactions[201].

self-antigens because, in that context, it could enable autoimmune responses to become persistent.

Responses to self

T cell activation by self-antigens. The information on long-term T cell fate and memory development described above was derived from experiments using pathogens that are cleared after a few days and no longer present by the end of the first week after infection. This temporary presence of the antigen has been shown to be a key element that shapes the development of memory and the phenotype of effector cells, because when an antigen is present for persistent periods of time, T cells lose effector capacities[62]. In other words, the paradigm where immune activation generates a large number of short-lived efficient effector cells that die after a few days only occurs when the immune response is induced by a short-lived pathogen[62]. Infection with pathogens that are able to avoid immune clearance induces T cell genetic programmes associated with limited effector function and limited proliferative capacity[7,8,63] (BOX 1). In the setting of infectious disease, such an occurrence could be considered pathological, because it contributes to failed clearance of the invading agent. However, the mechanisms that limit T cell functionality in the presence of antigen persistence might represent a failsafe system of avoiding the perpetuation of autoimmune responses.

Anergy and exhaustion (BOX 1) are two states in which T cell proliferative and effector capacity are reduced. Both anergy and exhaustion are established through discrete transcriptional programmes maintained by epigenetic modifications[7,9]. Anergy was described in CD4$^+$ T cells primed in the absence of co-stimulation[64]. However, anergy can also develop in CD4$^+$ T cells exposed to persistent antigen[7,65]. Exhaustion was described in CD8$^+$ T cells obtained from mice infected with a strain of lymphocytic choriomeningitis virus that causes chronic infection[62,66]. Repetitive stimulation caused by persistent and high levels of antigen (and perhaps other signals) triggers CD8$^+$ T cells to develop exhaustion during the first days of activation[67–70]. Interestingly, exhaustion has also been documented in T cells that infiltrate tumours[71]. In addition to these two states that impose a functional blockade in T cells through transcriptional mechanisms, CD4$^+$ T cells can undergo differentiation into IL-10-producing type 1 regulatory T (T_R1) cells[72] or FOXP3-expressing peripheral T_{reg} cells[65,73,74], and CD8$^+$ T cells can downregulate CD8$^+$ T cell expression[75]. Alternatively, both CD4$^+$ and CD8$^+$ self-reactive cells can undergo apoptosis, particularly in settings where antigen expression is high[7,76] (BOX 1). Together, these observations suggest that the activation of T cells that recognize antigens expressed persistently and at high levels induces inactivation rather than pro-inflammatory effector differentiation.

When CD4$^+$ or CD8$^+$ T cells are transferred into mice that express their cognate antigen as self, the transferred cells become activated and proliferate, but after a few days they lose pro-inflammatory properties and/or die[75]. In some systems, high antigen expression favours deletion, whereas lower levels (or interruption) of antigen expression leads to a reversible state of anergy[7]. In 2021, Wong et al. described that when CD4$^+$ T cells become activated by self-antigens, they are rapidly controlled by T_{reg} cells and, after a few rounds of proliferation, undergo apoptosis[40]. Thus, functional inactivation and apoptosis (that is, deletional tolerance) are intimately associated with each other. In fact, surface molecules linked to inactivation can promote apoptosis. For example, PD-1 has been shown to induce death in gastric adenocarcinoma-infiltrating T cells[77]; in addition, TIM3 (REFS[78,79]) and VISTA[80] can mediate apoptosis.

Because antigen concentration and persistence directly affect the balance between T cell effector differentiation and tolerance mechanisms, signalling through the TCR represents a central element in the regulation of these processes. Accordingly, the transcription factor nuclear factor of activated T cells (NFAT), activated by TCR-induced calcium influx, has been linked with transcriptional signatures of anergy[7,81] and exhaustion[82]. Intriguingly, a number of single-nucleotide polymorphisms (SNPs) linked with rheumatic autoimmune diseases map to genes whose products modulate TCR signalling (TABLE 1). For example, *RASGRP3*, associated with SLE[83], regulates the activation of the RAS signalling pathway, which is fundamental for T cell proliferation and differentiation[84]. Functional blockade of this pathway is a feature of anergic CD4$^+$ T cells[85,86]. *PTPN22* (REF.[87]) encodes a tyrosine phosphatase that regulates TCR and B cell receptor transduction and has been linked with several autoimmune diseases, including SLE and RA[88,89]. PTPN22 was shown to contribute to T cell exhaustion in the context of chronic lymphocytic

choriomeningitis virus infection[90]. This effect, however, has been proposed to depend on type I interferon production by plasmacytoid dendritic cells rather than through modulation of T cell function[90]. *CSK* (associated with SLE)[91] encodes a tyrosine kinase that regulates TCR signalling by inhibiting Lck signalling in $CD4^+$ T cells[92]. *GIMAP5* (REF.[93]) regulates MYC and NFAT transcriptional activity following T cell activation[94]. How these variants affect T cell signalling and how they interact with each other is poorly understood. Moreover, their presence might cause different effects depending on the affinity of the T cell for the antigen and the context in which presentation occurs (for example, types of cytokines and co-stimulatory signals). In SLE, T cell signalling has been extensively studied and a conspicuously disturbed TCR signalling process that results in altered behaviour upon stimulation has been well described[95] (FIG. 4).

Behaviour of self-reactive T cells in autoimmunity. As self-antigens are continuously expressed, self-reactive T cells would be expected to exhibit some degree of exhaustion in patients with systemic autoimmune diseases. In fact, the expression of exhaustion-associated gene signatures in peripheral blood $CD8^+$ lymphocytes was associated with fewer relapses in SLE and ANCA-associated vasculitides[96,97], and with slower disease progression in autoimmune diabetes[98]. Compared with healthy donors, the frequency of T cells displaying markers of exhaustion was higher in patients with systemic sclerosis[99] and SLE[100], in particular in those with prolonged clinical remission[101]. Finally, the expression of exhaustion markers in peripheral blood and synovial fluid $CD8^+$ T cells was associated with improved response to treatment in patients with RA[102].

A 2019 study that analysed gene expression at the single-cell level in kidneys affected by lupus nephritis, however, reported no expression of exhaustion-associated genes in infiltrating T cells, suggesting that exhaustion might not be induced adequately (or persistently) in T cells that infiltrate target organs of patients with SLE[100]. Importantly, peripheral blood from the same patients did display an exhaustion-associated gene signature, suggesting that even in patients where blood lymphocytes develop exhaustion, potentially pathogenic tissue-infiltrating T cells might fail to do so[100]. Accordingly, $CD4^+$ and $CD8^+$ T cells isolated from kidneys and urine of patients with lupus nephritis displayed effector memory markers, produced cytokines, and were clonally expanded[103–105]. One study that examined repeat renal biopsies from patients with lupus nephritis found persistent T cell clones, suggesting that kidney-infiltrating T cells are not only activated but represent long-lived T_{RM} cells[103]. In support of this idea, Zhou et al. reported relatively large numbers of $CD8^+$ T_{RM} cells in kidneys from MRL/lpr lupus-prone mice and showed that these cells produced IFNγ and TNF after ex vivo stimulation[51]. The abundance of $CD8^+$ T_{RM} cells in the kidney strongly correlated with proteinuria and decreased renal function[51]. These data agree with findings from Chen et al.[106], but contrast with findings from Tilstra et al.[107], who found phenotypic and metabolic features of exhaustion in kidney-infiltrating T cells from lupus-prone mice.

These conflicting results might be the result of an important aspect of exhaustion: that cells acquire the exhaustion phenotype in a gradual manner and progressively lose functionality (FIG. 3). Therefore, exhaustion features in T cells can co-exist with residual function and T cells that appear exhausted are still able to exert

Table 1 | **Genes that regulate cell survival and their association with autoimmune diseases**

Gene	Disease	Gene function	Variant effect	Refs
BCL2	SLE	Anti-apoptotic protein	Anti-DNA antibodies	147
TYK2	SLE and RA	Modulates T cell receptor signalling	Modulates signalling of IL-6, IL-23, IL-12 and interferon	148–151
PTPN22	SLE and RA	Modulates T cell and B cell receptor signalling	Affects production of pro-inflammatory cytokines	87,152–158
			Associated with lupus nephritis and anti-phospholipid syndrome	
			Associated with RF and anti-CCP antibodies	
ETS1	SLE and RA	Associated with aberrant stability and function of regulatory T cells	Associated with high levels of C-reactive protein and anti-C1q antibodies	130,159–162
			Associated with high expression of RANKL	
IKZF3	SLE and RA	Modulates T cell differentiation and function	Associated with lupus nephritis and production of anti-SSB antibodies	117,163–169
			Associated with early arthritis and absence of anti-CCP antibodies	
			Regulates differentiation and function of T_H17 cells	
TCF7	SLE and RA	Regulates T cell development and differentiation	Associated with early-age diagnosis and vasculitis	170–172
		Associated with memory T cell stemness	Associated with increased IL-17A levels in plasma	

CCP, cyclic citrullinated peptide; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

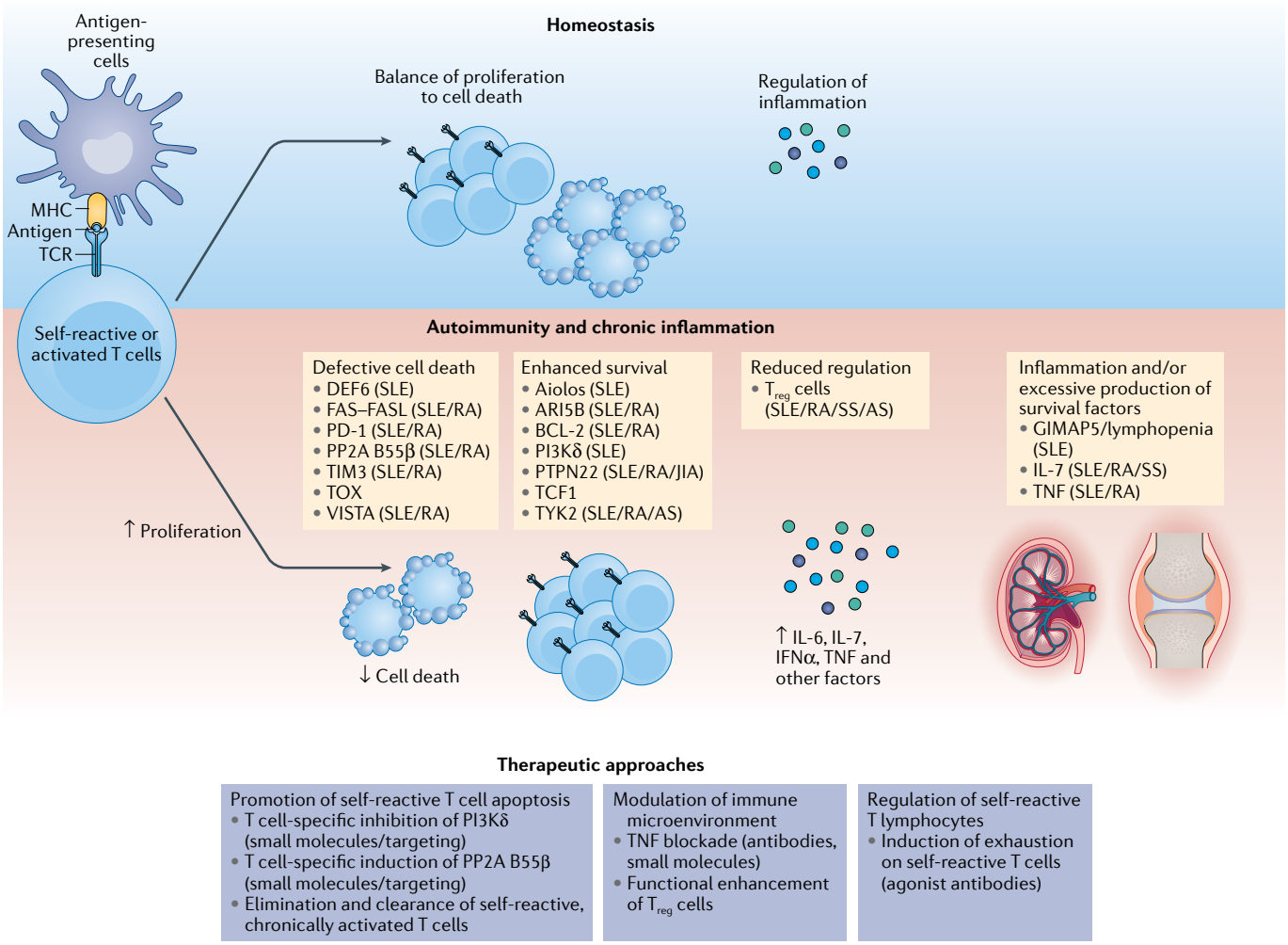

Fig. 4 | **Proposed mechanisms that promote T cell survival in the setting of autoimmune disease and possible therapeutic targets.** Under healthy physiological conditions, immune responses present a finely tuned balance between expansion (proliferation and/or survival) and contraction (cell death) of T cell clones responding to antigens. During the onset and progression of autoimmune diseases (such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)), T cells become resistant to apoptosis, promoting inflammation and immunopathology. Resistance to apoptosis is most often the result of combined genetic and environmental causes, mainly defective cell death, enhanced survival and imbalance of pro-apoptotic and anti-apoptotic external cues associated with inflammation. Therapeutic approaches that modulate the behaviour of pathogenic pro-inflammatory T lymphocytes might prove critical for the treatment of rheumatic diseases. AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis; SS, systemic sclerosis; TCR, T cell receptor; T_{reg} cell, regulatory T cell.

pathogenic effects, even if these effects are relatively limited. In support of the pathogenic capacity of exhausted T cells, a study compared the transcriptional and epigenetic landscape of CD8$^+$ T cells infiltrating the central nervous system of mice after an acute viral infection or during central nervous system autoimmunity[108]. They found that, in contrast to memory cells induced by acute viral infection, self-reactive CD8$^+$ T cells exhibited clear evidence of exhaustion[108]. Importantly, the exhaustion-associated transcription factor TOX was necessary for autoimmune disease to occur because in its absence, self-reactive CD8$^+$ T cells lived less and could not induce disease[108,109]. Analogously, a single-cell transcriptomic analysis of synovial-infiltrating T cells in patients with juvenile idiopathic arthritis revealed the presence of CD4$^+$ and CD8$^+$ T cells that co-expressed genes encoding exhaustion-associated molecules (such as PD-1 and TOX) and pro-inflammatory cytokines (such as IL-21, TNF and GM-CSF), further supporting the concept that exhausted cells are still able to drive autoimmune inflammation[110]. These data illustrate the complex relationship between self-reactive T cell activation, survival and exhaustion in the context of systemic autoimmune disease.

Persistence of self-reactive T cells. The development of a chronic autoimmune response entails the avoidance of the mechanisms that normally curb autoimmunity but also the differentiation of cells able to give rise — intermittently or continuously — to self-reactive effector cells. The capacity to persist, self-renew and produce effector cells is limited to memory cells. Therefore, patients with chronic autoimmune diseases probably have self-reactive memory T cells that perpetuate inflammation[54]. As mentioned earlier, antigen

persistence inactivates effector functions through T cell exhaustion and anergy[7]. However, experiments performed in models of chronic infection and cancer have demonstrated that, at least in $CD8^+$ T cells, exhaustion-associated transcriptomic and epigenetic signatures appear in memory cells before they proliferate and acquire effector functions[68,111].

TCF1 (encoded by *TCF7*) is a transcription factor expressed by naive T cells. It is downregulated during effector differentiation but remains expressed in memory T cells that possess stem cell-like properties[112]. TCF1 represses effector differentiation and promotes cellular survival by facilitating MYB-dependent BCL-2 expression[113] and by promoting STAT5 expression[114]. $CD8^+$ T cells exposed to antigen in a persistent manner gradually lose stemness and acquire an exhaustion phenotype[63]. In models of chronic infection and tumours, TCF1 expression is an indicator of the residual capacity of antigen-specific cells to respond to antigen, in particular following PD-1 blockade[115]. As T cell exhaustion progresses, TCF1 expression is lost and T cell effector function and stemness become severely compromised (FIG. 3). These $CD8^+$ T cells are now considered 'terminally exhausted'. Accordingly, in the context of cancer immunology, TCF1 expression is a marker of good prognosis and response to PD-1–PD-L1 blockade[116]. In the setting of autoimmunity, continued expression of TCF1 could contribute to the perpetuation of the autoimmune response. SNPs in the vicinity of *TCF7* have been associated with SLE[117] and a report has suggested that TCF1 expression might be abnormally high in T cells from patients with SLE[118], raising the possibility that increased expression of this transcription factor might have a role in the maintenance of the autoimmune response in human SLE (TABLE 1). An additional aspect to consider is that TCF1 is expressed by other cell subsets, for example, T_{reg} cells[114] and T_H17 cells[119]. Therefore, TCF1 could contribute to the pathogenesis of autoimmune diseases through different mechanisms.

Disturbances in signalling pathways have been proposed to affect the sensitivity of potentially pathogenic T cells to cell death in autoimmune diseases (TABLE 2). In some experimental settings, T cells from patients with autoimmune diseases have shown increased rates of spontaneous apoptosis[120–122], whereas in other settings they have exhibited resistance to apoptosis induced by cytokine withdrawal[34] or restimulation (RICD)[123,124]. On the other hand, increased activity of phosphoinositide 3-kinase δ (PI3Kδ)[123] or decreased function of ERK2 (REF.[124]) have been associated with resistance to RICD

Table 2 | **Defects in proteins that regulate cell survival and their association with autoimmunity**

Protein expression	Effects	Disease	Mouse phenotype	Refs
↓ YB1	↑ PUMA and caspase 3 activation ↓ BCL-2 and AKT1 pathways	SLE	KO mice exhibit embryonic or perinatal lethality; embryos have growth retardation and cells exhibit premature senescence	173,174
↓ SRSF1	↓ BCL-X_L and PTEN ↑ mTOR activity	SLE	T cell cKO mice develop spontaneous systemic autoimmunity and lupus-like nephritis. KO mice develop T cell lymphopenia with increased apoptosis and low expression of BCL-X_L	175,176
↑ Nectin 4	↑ BCL-2, BCL-X_L and caspase 6 ↓ BAX	SLE	Not described	177
↑ p110δ/PKB	↓ RICD	SLE	Mice with a mutation that inhibits the catalytic subunit (p110δ-D910A) develop autoimmunity. They have impaired clonal expansion and differentiation of T_H cells, as well as lymphopenia	178,179
↑ FAS–FASL	↑ Caspase 3 ↑ Caspase 8 ↑ BCL-2	SLE and RA	Loss-of-function mutations cause spontaneous autoimmunity (glomerulonephritis, arthritis and autoantibodies) and lymphoproliferation	180–185
↑ PD-1	↑ Proliferation and aberrant cytokine production	SLE and RA	KO mice develop late-onset lupus-like glomerulonephritis and destructive arthritis	186–190
↓ PP2A B55β	↓ CWID	SLE and RA	T cell cKO mice present progressive accumulation of central memory T cells and IFNγ-producing T cells	6,144
↑ TIM3	↑ T cell activation and cytokine production	SLE and RA	KO mice exhibit defects in antigen-specific tolerance	186,191,192
↑ VISTA	↓ TCR activation	SLE	Female KO mice spontaneously develop dermatitis, glomerulonephritis and elevated autoantibodies	193,194
↑ Aiolos	↑ BCL-2	SLE and RA	KO mice develop lupus-like disease with high autoantibody production and glomerulonephritis	195–197

cKO, conditional knockout; CWID, cytokine withdrawal-induced cell death; KO, knockout; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SRSF1, serine/arginine-rich splicing factor 1; TCR, T cell receptor; T_H cell, T helper cell; YB1, Y-box-binding protein 1.

in patients with SLE and RA, respectively. Additionally, increased mTOR activity[125] and other primary[126] or acquired[127] complex metabolic changes have been proposed to modulate survival signals and susceptibility to apoptosis that could affect activated T cell survival in inflamed tissues. In fact, metabolism — evaluated through different parameters — has been consistently found to be abnormal in T cells from patients with autoimmune diseases[128,129]. Such defects represent complex phenotypes attained by autoimmune T cells through the interplay of cell-intrinsic hereditary factors with signals derived from chronic inflammation and the specific tissue where the inflammation is taking place. Importantly, aberrant metabolism has been shown to promote pathogenic T cell capacities[126]; however, a thorough analysis of the topic is beyond the scope of this Review and has been published elsewhere[128,129].

Several genes with risk alleles linked with SLE and/or RA regulate T cell activation, differentiation, and survival (TABLE 1). One of these genes, *ETS1*, which is associated with SLE[83] and RA[130] encodes a transcription factor expressed in T cells and its absence is associated with increased apoptosis[131]. *TYK2* encodes a member of the Janus kinase (JAK) family that phosphorylates STAT1 (REF.[132]). This pathway has a large number of effects, including a BCL-2-dependent pro-survival function[133]. Deficient expression of IRF4-binding protein (encoded by *DEF6*) has been associated with autoimmunity in humans[134] and mice[135]. Interestingly, expression of IRF4-binding protein is decreased in patients with SLE that carry *DEF6* risk alleles[136]. A number of mechanisms, including abnormal susceptibility to T cell apoptosis[135,137], have been proposed to explain the link between *DEF6* and SLE. *ARID5B*[138] encodes a DNA-binding protein that interacts with histone demethylases and deacetylases. In human leukaemic T cells, it promotes leukaemogenesis by modulating MYC, a key transcription factor involved in cell cycle regulation[139]. In NK cells, ARID5B increases metabolic

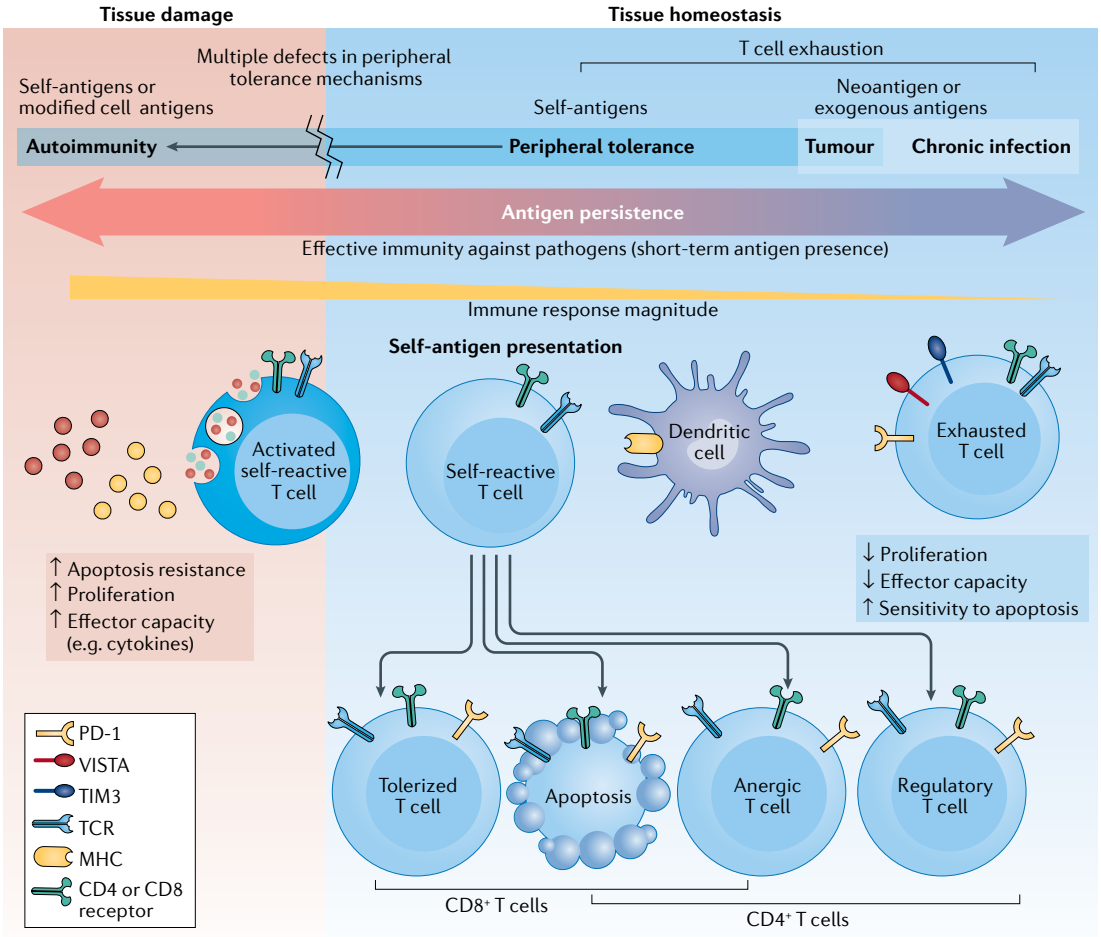

Fig. 5 | **Persistently expressed antigens induce T cell inactivation through different mechanisms.** Antigen persistence is linked to mechanisms that induce T cell inactivation in order to avoid autoimmunity and lessen immune-mediated tissue damage. These mechanisms curb the pathogenicity of self-reactive T cells through a variety of processes (apoptosis, anergy and exhaustion) that depend on the cell type and context (BOX 1). In the setting of tumours and pathogens that escape clearance, immune inactivation through persistent antigenic presence, might facilitate disease. By contrast, in patients with autoimmune diseases, inherited and acquired defects enable self-reactive T cells to avoid inactivation and instead differentiate into memory and effector subsets. These self-reactive pathogenic cells, which elude inactivation, represent the foundation of autoimmune memory that promotes chronic inflammation and perpetuates the expression of disease. TCR, T cell receptor.

Box 2 | **Unresolved questions regarding T cells in autoimmune disease**

- How do self-reactive cells evade exhaustion and apoptosis in patients with autoimmune disease?
- Is the survival of memory and effector T cells affected by genetic variants associated with autoimmune disease? Is the predisposition imposed by those variants exerted through their effect on cell survival?
- How do tissue-resident immune and non-immune cells contribute to the abnormal survival and pathogenic behaviour of self-reactive T cells in patients with autoimmune disease?
- What are the effects of chronic tissue inflammation on the survival and behaviour of infiltrating T cells? How are these effects exerted?

fitness and survival[140], which could contribute to NK cell or T cell persistence in lupus nephritis[100]. SNPs in *IKZF3*, a gene that encodes the transcription factor Aiolos, which regulates BCL-2 expression in response to IL-2 (REF.[141]), have been linked to SLE[142]. Thus, a large number of genetic variants could potentially affect the regulation of activated T cell susceptibility to apoptosis, contributing to the accumulation of long-lived self-reactive cells.

Effects of target organ inflammation

A large number of pathological conditions can lead to chronic inflammation and, disregarding its aetiology, T cells represent a key cellular component of the infiltrate that perpetuates inflammation and promotes local damage. Because the lifespan of activated T cells is modulated by environmental cues, the behaviour and survival of tissue-infiltrating lymphocytes is affected by local conditions including cytokines, oxygen and nutrient concentration, and pH. For example, tissue oxygen is lower in the kidney than in other organs[143] and capillary rarefaction induced by immune-mediated damage reduces local oxygen levels even further[4]. In order to survive in the hypoxic kidney, infiltrating T cells upregulate hypoxia-inducible factor 1 (HIF1). This transcription factor alters T cell metabolism and function, favouring the expression of pro-inflammatory T_H1 cytokines and T cell lifespan through the inhibition of apoptosis[106]. These effects were demonstrated in two mouse models of lupus nephritis (B6.*Sle1.Yaa* and MRL/*lpr*) and, importantly, transcription of *HIF1A* and HIF1-regulated genes were enriched in kidney-infiltrating T cells from patients with lupus nephritis[106]. Thus, organ inflammation and ensuing tissue damage can dramatically modify the signals received by local myeloid and lymphoid cells and, in some cases, promote pathogenic T cell function.

Transcription of *PPP2R2B*, the gene that encodes B55β, is induced in activated T cells when cytokine levels decrease[34]. B55β transcription induced by cytokine withdrawal is abated in patients with SLE and RA[144]. This defect was not linked with autoimmune-associated genetic variants but represented an acquired defect controlled by methylation of a CpG island located in the vicinity of the promoter region of the gene[144]. Importantly, resistance to apoptosis, failure to transcribe *PPP2R2B*, and CpG hypermethylation could be induced in T cells from healthy individuals by activating them in the presence of the pro-inflammatory cytokine TNF, suggesting that exposure of activated T cells to an inflammatory milieu could affect their behaviour during cytokine withdrawal, perhaps in a pathogenic manner. This notion was further supported by data that showed that the *PPP2R2B* CpG island was methylated in patients with active RA, but not in patients with low scores in the Disease Activity Score 28 (DAS28) index[144]. Interestingly, *PPP2R2B* hypermethylation and the resultant failed gene transcription has been reported in patients with colorectal cancer, a malignant disease, development of which is facilitated by chronic inflammation[145]. These data illustrate one mechanism through which chronic inflammation can affect the regulation of a gene in immune and non-immune cells. By limiting B55β expression, inflammation prolongs the functional life of activated T cells, thus promoting more inflammation and creating a vicious cycle (FIG. 2). Interestingly, other pro-inflammatory cytokines (IFNα, IL-6 and IL-21) were able to induce resistance to CWID in healthy human T cells through an independent mechanism[144] and IL-17 inhibited FAS-mediated apoptosis[146].

Conclusions

T cell activation and differentiation, as well as the establishment of central and tissue-resident T cell memory, are tightly regulated by a variety of partially redundant mechanisms. Proliferation and acquisition of effector and memory capacities are balanced by cell death induced by repetitive stimulation and growth factor withdrawal[12]. In health, these mechanisms promote the development of immunity and memory towards pathogens and curb responses aimed at self-antigens. A distinction between self-derived and pathogen-derived antigens cannot be unambiguously defined, so the immune system relies on other aspects of antigen presentation. Antigens expressed at persistent and high levels induce T cell inactivation and/or death, irrespective of the source of the antigen[7,76]. Pathogens that establish chronic infection and cancer cells silence specific T cell responses through mechanisms that probably evolved to protect the host from autoimmunity and immune-mediated tissue injury (FIG. 5). These processes involve the integration of complex signals sensed through the TCR and other receptors (such as cytokine receptors, co-stimulatory and co-inhibitory molecules); thus, it is believed that individual variation, acquired through common genetic variants and epigenetic modifications induced by the environment, affects these processes in a qualitative and quantitative manner. Therefore, the genetic predisposition to the development of autoimmune diseases might in part be caused by resistance to the mechanisms that would normally limit the activation of T cells and their differentiation into effector and memory cells. However, prolonged survival and function of immune cells in target organs is probably influenced by local environmental signals that promote pro-inflammatory T cells through modifying the T cell phenotype and facilitating T cell survival[106,144]. Thus, autoimmune disease entails not only the activation of self-reactive T cells but also the perpetuation of their inflammatory potential. Many questions, some of which are currently being addressed, remain open (BOX 2). Solving them will undoubtedly help us to better understand this complex field.

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