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Osteoporosis

Piezo1 as a therapeutic target for glucocorticoid-induced osteoporosis

Individuals undergoing treatment with glucocorticoids can develop glucocorticoid-induced osteoporosis (GIOP), which is characterized by progressive bone loss and an increased risk of fracture that correlates with low bone mineral density. Osteocytes have a crucial role in bone remodelling and reports suggest that inducing mechanical stress in osteocytes could increase bone marrow density and therefore improve GIOP. A study has now investigated the role of Piezo1, a mechanosensitive ion channel involved in osteocyte sensing of mechanical stress, in the pathogenesis of GIOP.

Both Piezo1 and the lacunocanaliculi network (LCN) are reduced in osteocytes from individuals with GIOP; similarly, in a mouse model of GIOP, expression of Piezo1 in osteocytes is reduced; however, treating these mice with the Piezo1 agonist Yoda1 prevented Piezo1 loss and detrimental alterations to bone structure and fragility. Administration of Yoda1 also reversed the loss of the mechanical stress response in osteocytes from GIOP mice.

Treating cortical bone samples obtained from hip arthroplasty procedures with glucocorticoids with or without Yoda1 showed that Yoda1 could prevent glucocorticoid-induced alterations in gene expression, osteocyte morphology and AKT phosphorylation via Ca^{2+} influx.

RNA sequencing analysis of tibial samples from mice with or without GIOP that had undergone mechanical loading demonstrated that the transcription factor Hes1 is crucial for Yoda1-mediated upregulation of Piezo1 in osteocytes; Hes1–Piezo1 signalling also resulted in an increase in calcium/calmodulin-dependent protein kinase II (CaMKII) and phosphorylation of AKT. Activation of this pathway by Yoda1 augmented the LCN and decreased factors associated with bone loss.

When comparing RNA sequencing data from samples obtained from the cortical bone of age-matched individuals with or without GIOP, *OSTN* (which encodes osteocrin, a protein that promotes the differentiation of osteoblasts during mechanical loading) was identified as the most significantly downregulated gene in those with GIOP. Integration of the mouse and human RNA sequencing datasets revealed alterations in the expression of 10 genes related to the function of osteoblasts and chondrocytes.

The authors then investigated the effects of Yoda1 on glucocorticoid-treated periosteum-derived cells as their RNA sequencing data suggested that osteoblast differentiation might be impaired in GIOP. Osteoblast differentiation was inhibited in glucocorticoid-treated periosteum-derived cells, but in cells treated with both glucocorticoids and Yoda1 osteoblast differentiation was unaffected.

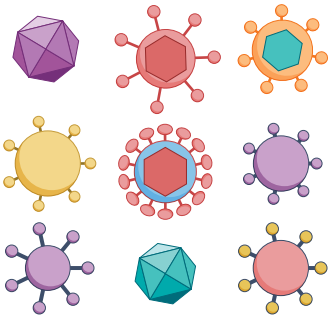
"Moving forward, we aim to develop novel, more effective and safe therapeutic agents for GIOP by specifically targeting the induction of Hes1 and Piezo1 in osteocytes", comments Kosuke Ebina, the corresponding author of this article.

Holly Webster

Original article: Ochiai, N. et al. The pivotal role of the Hes1/Piezo1 pathway in the pathophysiology of glucocorticoid-induced osteoporosis. *JCI Insight* **9**, e179963 (2024)

Genomics

Virome associations in autoimmunity and COVID-19



Viral infections have been implicated in the onset or exacerbation of autoimmunity. In a study published in *Nature Genetics*, Sasa et al. investigate associations between the human blood DNA virome and autoimmunity or COVID-19.

The authors analysed whole-genome sequencing data from the blood of more than 6,000 Japanese donors, including donors who had been diagnosed with autoimmune diseases or COVID-19. In this dataset, the genomes of anelloviruses, which are members of the healthy virome, and endogenous human herpesvirus 6B (eHHV-6B) — a heritable virus with the potential to reactivate — were significantly associated with autoimmunity or severe COVID-19.

The authors introduced a computational approach to accurately measure the total abundance of all genetically diverse anelloviruses, and found that anelloviral loads were significantly increased in individuals with systemic lupus erythematosus (SLE), rheumatoid arthritis or COVID-19 compared with healthy controls. Whereas anellovirus infection in SLE might reflect the patients' immunosuppressed state, in COVID-19, high anelloviral loads (viraemia), but not the mere presence of anelloviruses, were correlated with disease severity, indicating potential contribution to disease exacerbation.

Moreover, the full-length eHHV-6B genome emerged as a risk factor for SLE in the study cohort and in the 'All of Us' data repository, and the presence of eHHV-6B in the human genome correlated with high SLE disease activity. Whereas most individuals with SLE tested carried HHV-6B-specific antibodies, probably as a result of previous infection with exogenous HHV-6B, it was only the carriers of eHHV-6B that had antibodies against an epitope of the immediate-early A (IE-A) transactivator. In addition, monocytes and dendritic cells (DCs) from individuals with SLE that carried the eHHV-6B genome had higher expression of interferon response-related genes than monocytes and DCs from individuals with SLE that were negative for eHHV-6B. These findings indicate that a distinct immune response is established against the inherited endogenous virus, which the authors termed 'endoimmunity'.

Collectively, although the results of this study require further validation and the mechanistic links between infection with anelloviruses or eHHV-6B and autoimmunity remain to be investigated, Sasa et al. highlight the importance of including endogenous viral genomes in genetic association studies and suggest that an individual's virome might be a useful source of clinical biomarkers in rheumatology.

Maria Papatriantafyllou

Original article: Sasa, N. et al. Blood DNA virome associates with autoimmune diseases and COVID-19. *Nat. Genet.* **57**, 65–79 (2025)

Immunopathogenesis

T cells in Sjögren disease

Autoreactive T cells are associated with Sjögren disease (SjD, also known as Sjögren syndrome), but their role in disease pathogenesis is unclear. The store-operated calcium entry pathway is important for the function of T cells, and the calcium sensors STIM1 and STIM2 are involved in mediating this pathway.

Wang et al. report that mice lacking *Stim1* and *Stim2* in regulatory T cells (*Stim1/2*^{Foxp3} mice) develop SjD-like disease that accurately reflects the human disease, according to the ACR and EULAR classification criteria for SjD. The salivary and lacrimal glands of these mice had high immune cell infiltrates and gene expression signatures associated with type 1 helper T cells and IFNγ signalling. These T cell transcriptional profiles were similar to those described in SjD in humans. Adoptive transfer of CD4$^+$ T cells from *Stim1/2*^{Foxp3} mice induced SjD-like disease in recipient mice, which was IFNγ dependent; treatment with baricitinib diminished disease.

As the SjD-like phenotype of *Stim1/2*^{Foxp3} mice reflects human SjD, this model could be used to test new treatments.

Holly Webster

Original article: Wang, Y.-H. et al. IFN-γ–producing T_H1 cells and dysfunctional regulatory T cells contribute to the pathogenesis of Sjögren's disease. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.ado4856 (2024)

Bone

A direct link between SARS-CoV-2 and bone loss

Research suggests that SARS-CoV-2 infection directly contributes to bone loss in individuals with chronic immune-mediated inflammatory diseases (IMIDs). The findings demonstrate that the SARS-CoV-2 accessory protein ORF8, which mimics IL-17 signalling, intensifies inflammation and accelerates bone resorption. "These findings are significant for patients with IMIDs, as they identify a molecular mechanism through which SARS-CoV-2 exacerbates disease pathology", notes Weiqiang Chen, one of the corresponding authors of the study.

The researchers first used high-throughput multiplexed proteomics to analyse 1,500 protein biomarkers in plasma samples from four groups of individuals: those with confirmed COVID-19 and a pre-existing IMID ($n = 13$); those with an IMID but no history of COVID-19 ($n = 20$); those with confirmed COVID-19 but no history of IMID ($n = 20$); and healthy control individuals with no history of COVID-19 or IMID ($n = 20$). The analysis showed that the group with COVID-19 and an IMID had the highest number of differentially expressed proteins, with increased expression of pro-inflammatory cytokines and markers of bone resorption.

One of the twenty samples (5%) from the group with COVID-19 but no IMID tested positive for ORF8; by contrast, seven of the thirteen samples (54%) from individuals with COVID-19 and an IMID were ORF8-positive. Notably, plasma levels of ORF8 in the group with COVID-19 and an IMID correlated with expression of markers of inflammation and dysregulated bone responses.

To investigate the role of SARS-CoV-2 ORF8 in modulating inflammation and bone homeostasis in the context of IMIDs, the authors treated primary human osteoblasts from individuals with rheumatoid arthritis (RA) and from healthy individuals with ORF8. They noted that the induction of pro-inflammatory and bone remodelling-associated genes was significantly increased in ORF8-stimulated osteoblasts from individuals with RA than in those from healthy controls. Furthermore, supernatants derived from ORF8-treated RA osteoblasts had pro-osteoclastogenic effects in mouse bone marrow-derived macrophages, to a greater extent than those derived from ORF8-treated cells from healthy controls.

Together, the results shed light on how SARS-CoV-2 infection might exacerbate pre-existing IMIDs and contribute to long-term post-COVID-19 sequelae, including bone loss. "These findings not only expand our understanding of SARS-CoV-2 complications, but also highligh ORF8 as a therapeutic target for mitigating skeletal damage and inflammation" says Chen.

Sarah Onuora

Original article: Melano, I. et al. SARS-CoV-2 ORF8 drives osteoclastogenesis in preexisting immune-mediated inflammatory diseases. *JCI Insight* **9**, e178820 (2024)

Osteoarthritis

https://doi.org/10.1038/s41584-024-01209-2

Precision weight management in people with knee osteoarthritis and overweight or obesity

Shiwen Yuan & David J. Hunter

Results of the STEP 9 trial show that semaglutide leads to improvements in knee osteoarthritis-related symptoms. The findings support weight-management pharmacotherapies as a feasible option for management of knee osteoarthritis, but cost-effectiveness, risk of toxicity and likelihood of rebound must be considered.

Refers to Bliddal, H. et al. Once-weekly semaglutide in persons with obesity and knee osteoarthritis. *N. Engl. J. Med.* **391**, 1573–1583 (2024).

Knee osteoarthritis (OA) is a leading cause of disability associated with enormous socioeconomic burden. Its prevalence is increasing with increasing rates of overweight and obesity. The STEP 9 trial[1] shows that semaglutide achieves superior reductions (versus placebo) in body weight and pain related to knee OA and improvements in physical function over 68 weeks. This study is the first global, randomized controlled trial (RCT) showing rigorous evidence of improvements in knee OA-related symptoms with a weight-management medication. Limitations include a lack of information on risk of weight rebound (there were just 7 weeks of off-treatment follow-up) and the absence of a comparison of the cost-effectiveness of semaglutide versus lifestyle intervention alone. Patients in both groups received counselling on a reduced-calorie diet and physical activity, but this gentle nudge probably did not meaningfully change behaviour or lead to substantive symptom relief.

Weight loss is an effective treatment option for people with knee OA and overweight or obesity, providing pain relief, improving quality of life, reducing the need for total knee replacement and decreasing all-cause mortality[2]. A dose–response relationship is seen between weight reduction and improved pain or function in patients with knee OA[3], with amelioration of weight-related comorbidities. However, the best way to provide precision approaches to weight management is unclear.

Lifestyle interventions such as diet and exercise can achieve 5–10% weight loss and should be the first-line recommendation for initiating and sustaining weight loss[4]. Lifestyle interventions are often underutilized, with barriers at patient, clinician and facility levels. Patients with OA often lack motivation to change their lifestyle and adhere to long-term lifestyle-related treatment. Minimal effectiveness of weight counsel can reflect inadequate communication between clinicians and patients[5].

Bariatric surgery is considered the last-resort option for people with class II or class III obesity (BMI 35–39.9 kg/m^2 or ≥40 kg/m^2, respectively) and notable comorbidities, attaining 20–30% weight loss. The American Society for Metabolic and Bariatric Surgery (ASMBS) and the International Federation for Surgery for Obesity and Metabolic Diseases (IFSO) have expanded their indications for bariatric surgery to include patients with class I obesity (BMI 30–34.9 kg/m^2) who have insufficient weight loss or comorbidity improvement using nonsurgical methods[6]. Risks of bariatric surgery include early complications (anastomotic leaks, stenosis and bleeding), late complications (dumping syndrome, internal hernia and marginal ulceration), the need for reoperation (15–22% of cases) and adverse effects (gastrointestinal disorders, nutritional deficiencies and negative psychological consequences). In cost-effectiveness analyses, relative to usual care alone, bariatric surgery results in a reduction in knee OA-associated opioid use and an increase in total knee replacement among patients with knee OA with class III obesity[7]. At a willingness-to-pay threshold of US $50,000 per quality-adjusted life-year gained, Roux-en-Y gastric bypass plus usual care was cost-effective in patients with knee OA with BMI ≥40 kg/m^2, while laparoscopic sleeve gastrectomy plus usual care offered good value in those with BMI 35–39.9 kg/m^2 (ref. 7). Considering the trade-off between effectiveness, risk and cost burden is important.

The STEP 9 trial suggests the usefulness of pharmacological interventions in patients with knee OA and overweight or obesity; various antiobesity and glucose-lowering medications are emerging as potential therapies. Glucose-lowering agents such as metformin, dipeptidyl peptidase-4 (DPP-4) inhibitors and sodium–glucose cotransporter 2 (SGLT2) inhibitors can achieve modest weight loss (3–5%). Several RCTs are assessing the effects of metformin on pain, function and structural progression of knee OA in patients with overweight or obesity. Limited evidence is available on the effects of DPP-4 inhibitors and SGLT2 inhibitors on knee OA-related symptoms and these glucose-lowering agents are not approved by the US Food and Drug Administration (FDA) for those without diabetes. An observational study showed that glucagon-like peptide-1 receptor (GLP-1R) agonists decreased cartilage loss velocity of the medial femorotibial joint and incidence of knee surgery[8]. Ongoing RCTs are investigating these novel hormone agents, including tirzepatide (NCT06191848) and retatrutide (NCT05931367), in people with overweight or obesity and knee OA. The level of weight loss from these agents ranges from 10% to ≥20%, approaching the level seen with bariatric surgery, but how they will change the landscape of surgical interventions remains unknown.

Keeping off the lost weight is challenging, and weight regain is very common after intervention discontinuation. In a study investigating continued treatment with tirzepatide versus placebo, weight was substantially regained in those who terminated tirzepatide, with a mean of around 50% of prior weight loss regained within 1 year, along with the reversion of multiple cardiometabolic risk markers[9]. Much weight regain seems to be fat as opposed to lean mass, and reports exist of diminished bone mass and increased fracture risk. In a study of 10-year lifestyle interventions, 31% of participants gained ≥2% of their end-of-intervention weight 2 years after programme cessation[6]. Continued counselling for weight-loss maintenance (WLM) and self-monitoring might overcome the problem[6]. Exercise is not used as a stand-alone weight-loss treatment but is important in preventing weight regain, improving knee OA-related symptoms and reducing the need for joint

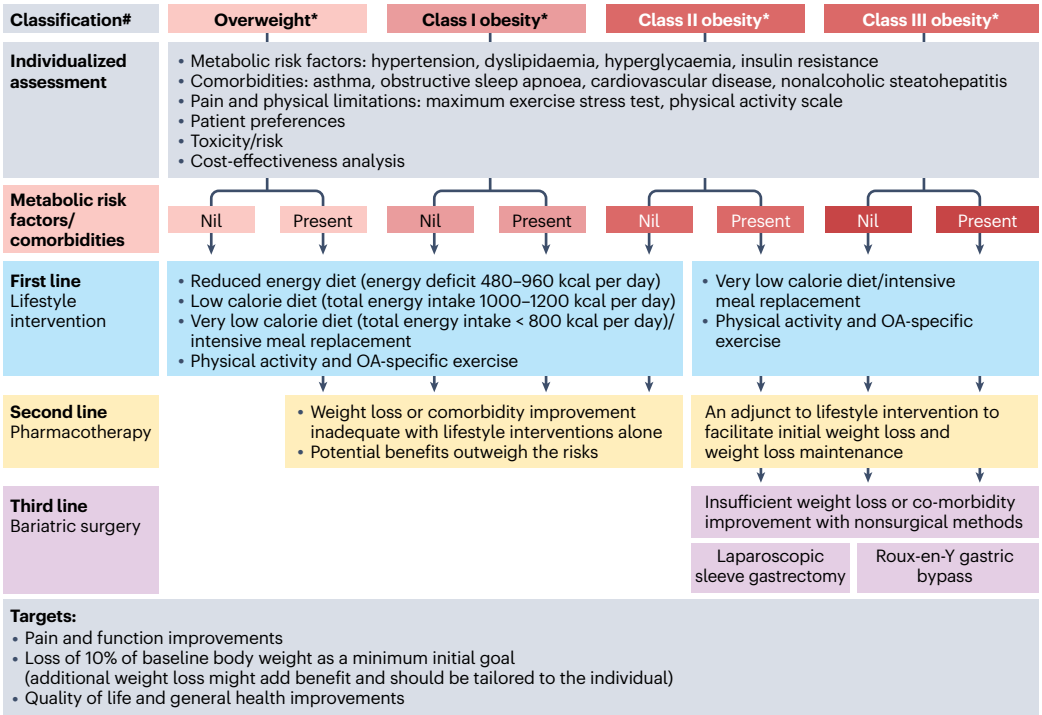


Fig. 1 | Algorithm for weight management applicable to patients with knee osteoarthritis. Flow chart showing suggested first-line, second-line and third-line treatment options for individuals with knee osteoarthritis (OA) with overweight or class I, class II or class III obesity. #World Health Organization (WHO) classification: overweight (BMI 25–29.99 kg/m^2), class I obesity (BMI 30–34.99 kg/m^2), class II obesity (BMI 35–39.99 kg/m^2) and class III obesity (BMI ≥ 40 kg/m^2). *Asian populations: overweight (BMI 23–27.49 kg/m^2), class I obesity (BMI 27.5–32.49 kg/m^2), class II obesity (BMI 32.5–37.49 kg/m^2) and class III obesity (BMI ≥ 37.5 kg/m^2). Data from https://www.diabetessociety.com.au/guideline/obesity/.

replacement. In an RCT of four WLM strategies, only the combination therapy (liraglutide and exercise) was associated with improvements in metabolic health, physical function and emotional well-being[10]. Clinical guidelines for obesity recommend the use of pharmacotherapies to facilitate initial weight loss and WLM when lifestyle interventions alone show inadequate efficacy[6], but the benefits and risks of chronic use of these agents are unclear. More studies are needed to determine whether these agents cause structural changes in knee OA if they are to be considered as disease-modifying drugs.

We believe that pharmacological weight-loss interventions should be a second-line intervention as appropriate for individual patients. A precision model considering BMI, metabolic risk factors, comorbidities, pain and physical limitations, patient preferences, toxicity and cost-effectiveness is needed to implement individualized weight-loss management. Intrinsic factors, including biological factors (leptin and ghrelin), physiological factors (obesogenic memory), epigenomic modifications, metabolomic profiles and the composition of the gut microbiome might cause the heterogeneous responses to weight-loss interventions and weight regain. Exploring these factors is the next step to implementing precision weight management in knee OA. An algorithm for weight management for patients with knee OA is suggested (Fig. 1).

Overweight and obesity and knee OA are chronic diseases requiring education, lifelong monitoring and individual weight-management goals and strategies. Lifestyle interventions are the first-line, safest and most cost-effective management approach. Judicious prescription of weight-loss medications, in conjunction with lifestyle interventions, should be a second-line approach, especially when potential benefits outweigh the risks and costs for patients with a lack of weight response from lifestyle interventions, those with comorbidities requiring additional weight loss and/or metabolic improvements, those with limited abilities to take part in physical activities to maintain weight loss, and those requiring rapid weight loss for surgery. Bariatric surgery should be a third-line approach, strictly according to eligibility criteria, based on benefit–risk, cost-effectiveness, patient preference and specialist availability. The emergence of new therapeutic options provides choices, but careful consideration of all factors is needed to facilitate precision medicine in this complex area.

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Competing interests

D.J.H. is the editor of the osteoarthritis section for UpToDate and co-editor in chief of *Osteoarthritis and Cartilage* and provides consulting advice on the scientific advisory boards for Haleon, TLCBio, Novartis, Tissuegene, Sanofi and Enlivex. S.Y. declares no competing interests.

Osteoarthritis

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Appraising the evolving landscape of protease inhibition in osteoarthritis

Muhammad Farooq Rai

The role of proteases in cartilage degradation and the development of osteoarthritis is undeniable. Despite over two decades of research on protease inhibitors, however, the transition from preclinical promise to clinical success remains elusive, underscoring the urgent need to critically appraise the challenges and limitations inherent in preclinical studies.

Refers to Latourte, A. et al. SerpinA3N limits cartilage destruction in osteoarthritis by inhibiting macrophage-derived leucocyte elastase. *Ann. Rheum. Dis.* **83**, 1781–1790 (2024)

Current treatments for osteoarthritis (OA) focus primarily on palliation, as no disease-modifying drugs are available. Pro-inflammatory mediators such as cytokines and proteases are known to drive cartilage breakdown in OA, yet efforts to target these mediators have yielded limited success. Like their predecessors, new inhibitors such as serine protease inhibitors[1] show promise in rodent models, but scepticism remains about their advancement to clinical trials. This scepticism is rooted in historical setbacks, the limitations of preclinical studies, and concern about the safety of disrupting the important pathophysiological functions of proteases.

Proteases have been linked to OA pathogenesis for decades. Seminal studies demonstrated that matrix metalloproteinase-13 (MMP13) cleaves type II collagen in cartilage and that transgenic mice expressing active human MMP13 develop lesions mirroring human OA[2]; conversely, MMP13-deficient mice are resistant to osteoarthritic cartilage erosion[3]. Early clinical trials of broad-spectrum MMP inhibitors failed owing to issues with the specificity and toxicity of these agents[4]. CYT-108, a recombinant protease inhibitor, has since been re-evaluated, but recruitment for a new phase I trial (NCT06263270) has yet to commence, dampening hopes for its near-term clinical success.

In addition to MMPs, aggrecanases — enzymes that cleave aggrecan — have been implicated in OA. Two studies published simultaneously in *Nature* demonstrated that genetic ablation of the aggrecanase ADAMTS5 prevents cartilage erosion in mouse models of injury-induced OA[5,6] and spurred efforts to develop targeted protease inhibitors. M6495, an anti-ADAMTS5 nanobody (NCT03224702), and GLPG1972, a potent, selective inhibitor of ADAMTS5 (NCT02851485, NCT03311009 and NCT03595618), have entered phase I–II clinical trials, the results of which are pending.

Many proteases are crucial for joint homeostasis and their dysregulation leads to excessive degeneration, so inhibiting them without disrupting their beneficial functions is complicated. Furthermore, systemic protease inhibition could affect other tissues in which MMPs and ADAMTSs have physiological roles, raising safety concerns. Nonetheless, several pharmaceutical companies have exhibited considerable enthusiasm for targeting proteases, MMPs and aggrecanases. However, despite numerous commitments, they have not succeeded in developing a single drug that inhibits these proteases over the past two decades. Thus, the journey to find a viable protease inhibitor is fraught with challenges, yet research efforts remain undeterred.

The serine protease neutrophil elastase, also known as leukocyte elastase, has long been implicated in inflammatory arthritic disease and its inhibitors have been well studied[7]. Latourte et al.[1] have now reported that synovial macrophages secrete neutrophil elastase upon exposure to IL-6, leading to cartilage degradation. Notably, IL-6 also induced the expression of several serine protease inhibitors, including the elastase inhibitor SERPINA3N, levels of which are elevated in OA joints; this suggests that elevated SERPINA3N levels may counteract neutrophil elastase activity, although they are insufficient to inhibit its production. The study by Latourte et al.[1] presents three key pieces of evidence for the therapeutic potential of serine protease inhibitors: first, genetic deletion of *Serpina3n* in chondrocytes renders mice susceptible to post-traumatic OA; second, intra-articular injection of recombinant SERPINA3N partially rescues cartilage loss in mice following meniscectomy; and third, sivelestat, a neutrophil elastase inhibitor, protects mice from injury-induced OA. These findings position neutrophil elastase as a promising therapeutic target and SERPINA3N as a potential treatment, although the effectiveness of the latter in OA remains uncertain.

The uncertainty stems from the limited success in bringing proteinase inhibitors to the clinic and their roles in homeostasis. MMPs have distinct, although relatively less restrictive, substrate specificities compared with other protease families. This specificity, which is partially reliant on the hemopexin domain, means that broad-spectrum inhibition could lead to off-target effects. Effective inhibition of aggrecanases requires understanding of their regulation and interaction with other proteases. Although the work by Latourte et al.[1] represents a notable advance in efforts to find new therapeutic targets in OA, critical questions about clinical translation remain. The authors noted that neutrophil elastase activates MMP3 in a paracrine manner but they did not elaborate on the underlying mechanism. They also reported that sivelestat-treated mice were protected from cartilage degradation; however, the considerable variability in cartilage degradation scores among mice in the control groups raises questions about the true disease-modifying effects of sivelestat. Additionally, whereas sivelestat subtly improved cartilage scores at a single time point, it failed to improve other critical aspects of the disease, such as synovitis, subchondral bone sclerosis and osteophyte formation. Finally, the potential of sivelestat to improve functional outcomes, such as pain and gait, was not assessed. Therefore, its therapeutic value in these important parameters remains uncertain.

Table 1 | Challenges in bringing protease inhibitors to clinical trials in osteoarthritis

Challenge	Potential solution	Effect on clinical translation
Limited understanding of candidate therapeutics	Conduct rigorous mechanistic research	Improved knowledge and identification of novel targets
Non-representativeness of existing models of OA	Adopt models of 'naturally progressing' age-related OA	Applicability of findings to the broader population of individuals with OA
Limited generalizability of existing animal models of OA	Study mouse strains with varying genetic composition	Tailored treatments that improve therapeutic efficacy
Invasiveness of existing models of OA	Prioritize and use non-invasive models of OA	Insights into long-term, disease-relevant outcomes that replicate the slow progression of OA
Neglect of functional outcomes	Integrate comprehensive evaluation of pain and overall function into studies	Improved clinical success through patient-centric therapeutic strategies
Undefined timing of interventions	Identify the optimal 'window of opportunity' to deliver treatment	Timely intervention to influence treatment efficacy
Reliance on single-treatment strategies	Explore combination therapeutic strategies	Enhanced and synergistic treatment outcomes

OA, osteoarthritis.

Several challenges hinder the successful translation of protease inhibitor studies into clinical practice. Many therapeutics are tested in models of post-traumatic OA, which represents only a small fraction of all OA. Although these models are useful for screening various therapeutics, the results of studies using them cannot be generalized. Post-traumatic OA models are often invasive and vary in terms of how well they mimic the natural progression, pathology and other features of human OA. Thus, to better assess the 'true' disease-modifying effects of protease inhibitors, a model of naturally occurring, age-related OA — which would represent the majority of OA cases — should be used.

OA is a heterogeneous disease with an important genetic component and varying manifestations. Not all individuals with OA exhibit the same features over the course of the disease, even with the exact same initiating aetiology (for example, ligament or meniscus tear), and the observation of distinct transcriptomic signatures across patients with OA indicates molecular variability. This heterogeneity has prompted efforts to classify patients with OA into clinical phenotypes and molecular endotypes, paving the way towards 'personalized' therapy[8]. Over 120 polymorphic DNA variants have been identified, accounting for 39–70% of the heritability of OA in humans[9]. The heritability of OA has also been documented in mice. In recombinant inbred lines, the characteristics of post-traumatic OA vary with genetic composition, highlighting the contribution of genetics to disease[10]. This variability underscores the need to test treatment strategies in genetically diverse mouse models to better reflect human OA. If the findings from Latourte et al.[1] and other studies in models of trauma-induced OA hold true in genetic models of primary and post-traumatic OA (or even in obesity-induced OA), the potential for clinical translation of therapies targeting neutrophil elastase remains promising. Furthermore, considering clinical phenotypes and molecular endotypes could accelerate progress towards personalized medicine targeting proteases in general and neutrophil elastase in particular.

The timing of intervention is also crucial for effective treatment of OA. Although both preventive and curative approaches have their merits, identifying a critical 'window of opportunity' in OA is desirable. Latourte et al.[1] used a curative approach after the cartilage lesions had set in and SERPINA3N levels had peaked. However, this strategy failed to alleviate key OA features (osteophytes, synovitis and subchondral bone sclerosis), emphasizing the need for timely intervention. A potentially more effective strategy could involve combination therapy that stimulates the expression of endogenous inhibitors (for example, SERPINA3N and tissue inhibitors of metalloproteinases) alongside suitable exogenous inhibitors (such as sivelestat) to address both structural and functional aspects of the disease.

Earlier studies of proteases focused primarily on understanding the molecular mechanisms of cartilage matrix breakdown by the proteases. Many rodent studies concentrate on structural changes (for example, cartilage fibrillation) without considering functional outcomes such as pain and gait. As pain is the primary reason people with OA seek medical attention, it is essential to include pain assessment when studying the therapeutic effects of protease inhibitors. As such, the focus of drug development for OA has shifted from targeting structure and extracellular matrix to targeting pain and function.

In summary, although protease inhibitors hold promise for targeting structural aspects of OA, they have not yet demonstrated an impact on pain or functional outcomes. Therefore, advancing findings from preclinical studies in small animals to human clinical trials faces substantial challenges within current research frameworks (Table 1). At the same time, it should not be inferred that all efforts to bring a protease inhibitor to the clinic are destined to fail. Strategies that address OA heterogeneity, the limitations of existing disease models, the inclusion of functional parameters, the necessity for timely intervention, and mechanistic investigations could bolster the likelihood of success. Such progress could ultimately fulfil the long-awaited promise of effective OA treatments, offering hope to the millions of people affected by this debilitating disease.

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Competing interests

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Systemic sclerosis

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European expert recommendations for managing systemic sclerosis and its complications

Robyn T. Domsic

The updated 2023 EULAR recommendations for treatment of systemic sclerosis bring notable changes to recommendations for skin, peripheral vascular disease, interstitial lung disease and pulmonary arterial hypertension therapies, based on newer evidence. These updates provide the first glimmer of personalized patient management.

REFERS TO Del Galdo F. et al. EULAR recommendations for the treatment of systemic sclerosis: 2023 update. *Ann Rheum Dis*. https://doi.org/10.1136/ard-2024-226430 (2024).

Systemic sclerosis (SSc) is a rare, complex, autoimmune and connective tissue disease that can affect multiple organs. EULAR developed recommendations for the pharmacological management of SSc in 2009 and updated these recommendations in 2017. The 2023 EULAR recommendations have now been published[1].

In terms of peripheral vascular complications, few changes have been made from the 2017 guidelines in the management of Raynaud phenomenon or digital ulcers, with phosphodiesterase 5 (PDE5) inhibitors still the recommended treatment for both conditions. Iloprost is recommended for both recalcitrant Raynaud phenomenon and digital ulcers.

The 2017 guidelines recommended fluoxetine as an alternative therapy for the treatment of Raynaud phenomenon in patients with SSc and I am disheartened that it has been removed in the new guidelines. Although the supporting literature for fluoxetine is limited to one small positive randomized trial ($n = 27$) comparing fluoxetine 20 mg daily with long-acting nifedipine 40 mg daily[2], patients with SSc often have low blood pressure and are unable to tolerate vasodilating therapies, so alternatives are needed. In addition, as SSc affects predominantly perimenopausal and postmenopausal women who can suffer from hot flashes, and is associated with concurrent depression and anxiety, fluoxetine could have dual and potentially triple benefit for some patients. By removing it from the guidelines, many practitioners might not consider it any longer as an adjunctive therapy for Raynaud phenomenon, which could be a disservice to patients.

Surprisingly absent in the new guidelines was any formal assessment of botulinum toxin injection for digital ulcers or Raynaud phenomenon, despite a growing body of supportive literature.

For pulmonary arterial hypertension (PAH), the new guidelines recommend that the combination of PDE5 and endothelin receptor antagonists should be considered as first-line treatment (level 1a evidence). Another addition is the consideration of riociguat and selexipag for PAH, with a noted lower level of evidence (level 1b). Important to mention is the new recommendation against the use of warfarin in SSc-PAH, on the basis of a meta-analysis showing increased mortality with anticoagulation (HR 1.58, 95% CI 1.08–2.31; $P = 0.02$)[3], which I am glad was added. The revised guidelines were published just prior to the approval of sotatercept for PAH treatment by the FDA, the European Medicines Agency and Japan's Pharmaceuticals and Medical Devices Agency in 2024. Although its absence is conspicuous just a year later, it was appropriate not to include at the time and is a reminder of how quickly medicine can advance.

The new therapies recommended for skin management in SSc are rituximab and tocilizumab. I feel that these therapies are probably beneficial in subsets of disease based on their molecular mechanisms. The task force felt that additional evidence of efficacy of intravenous immunoglobulin for managing skin involvement in SSc was required before being included in recommendations; trials are underway.

The recommendation for rituximab was driven primarily by the outcomes of the DESIRES trial. DESIRES enrolled 59 patients in Japan, randomized 1:1 to receive rituximab or placebo[4]. This trial did not focus on early diffuse SSc; the median disease duration was nearly 5 years (58 months) in the rituximab arm and >50% of patients were positive for anti-Scl70 antibody. The change in modified Rodnan skin score (mRSS) at 24 weeks was −6.3 in the rituximab group versus +2.1 in the placebo group (difference −8.4; 95% CI −11.0 to −5.9; $P < 0{\cdot}0001$). In the open-label extension, rituximab was associated with continual decline in mRSS irrespective of original treatment group allocation[5]. A post-hoc analysis of DESIRES published after these recommendations reported that a high $CD19^+$ cell count of $\geq 57\ \mu l^{-1}$ was associated with skin response to rituximab, more so in those with higher mRSS[6]. This finding supports biological plausibility with the drug's mechanism of action and suggests a way to potentially identify responders given the risks associated with rituximab in the post-COVID world.

In a separate open-label, randomized controlled trial of early (<3 years) diffuse SSc, patients with interstitial lung disease (ILD) and positive for Scl70 were randomized to rituximab versus monthly intravenous cyclophosphamide[7]. As a secondary endpoint, mRSS at 24 weeks declined more in the rituximab group than in the cyclophosphamide group (−9.67 versus −5.5, respectively)[7]. Although SSc-associated antibodies are not generally considered to have a role in pathogenesis, antibodies to Scl70 may well be the exception, as they have been shown to bind to the surface of fibroblasts and stimulate the activation of monocytes. Given this, it seems intuitive that a medication targeting B cells might have use in patients with positivity for anti-Scl70

antibody and might have also driven the DESIRES results given the high percentage of Scl70 antibody positivity in that study.

Tocilizumab was not supported as a first-line agent for skin involvement in SSc, but the task force suggested that it be considered for early, inflammatory skin disease. In terms of mRSS change from baseline, a phase III study showed that tocilizumab showed no significant change versus placebo, and the 2024 British Society of Rheumatology guidelines for the management of SSc do not endorse tocilizumab as an option[8]. Mycophenolate mofetil can also normalize markers of early, inflammatory skin disease clinically, making it challenging to decide if and when to use tocilizumab for skin.

For the treatment of ILD, major changes in the guidelines include the addition of four new drugs — mycophenolate mofetil, rituximab, tocilizumab and nintedanib — alongside the previously recommended cyclophosphamide.

Mycophenolate mofetil was added to ILD recommendations on the basis of the Scleroderma Lung Study II results; its use is already standard of care and is acceptable background therapy for SSc-ILD clinical trials.

Rituximab was added to ILD recommendations on the basis of the results of the RECITAL phase IIb trial (rituximab versus cyclophosphamide) and DESIRES phase II trial (rituximab versus placebo), together with several smaller, open-label trials. Rituximab is viewed as an optional first-line therapy but its combination with mycophenolate was not considered for these recommendations as no high-quality supportive evidence exists.

Tocilizumab is recommended for ILD following the results of phase II and phase III trials with multiple ILD-related secondary outcomes. In the guidelines flow chart, tocilizumab is recommended as a first-line therapy for patients in the 'early, inflammatory' subset, but the wording of the guidelines do not clearly state this. No combination with mycophenolate is considered based on lack of evidence. The flow chart for SSc-ILD is at odds with general practice in the USA, as insurance companies often demand failure of mycophenolate before authorizing tocilizumab approval, which shows the reality of how companies, not the government, decide how we treat our patients. I am personally grateful for the flow chart.

Nintedanib was added as a recommendation for ILD to be used alone or in combination with mycophenolate on the basis of results from SENSCIS and its open-label extension trial, SENSCIS-ON. The flow chart suggests consideration of nintedanib if the patient is already on mycophenolate. In SENSCIS, the relative treatment effect was similar alone or in combination with mycophenolate. However, the annual rate of forced vital capacity decline was numerically greater with nintedanib alone than in those also on mycophenolate (55.4 ml per year versus 26.3 ml per year, respectively). In practice, the high frequency of diarrhoea associated with nintedanib (75%) can lead to a dose reduction to 100 mg bid for which no supportive data exist[9].

The EULAR recommendations notably differ from the American College of Rheumatology 2023 guidelines, which strongly recommend against the use of glucocorticoids in SSc-ILD owing to risk of scleroderma renal crisis (SRC)[10]. This difference might reflect differences in the US population, where the much higher prevalence of patients with anti-RNA polymerase 3 antibodies provides an increased SRC risk.

ACE inhibitors were not recommended as a preventive option for SRC in the 2023 EULAR update — just that they be used immediately at diagnosis of SRC. No changes were made for gastrointestinal management, other than a strengthening of the level of evidence of prokinetics. Similarly, methotrexate for musculoskeletal symptom management remains unchanged in both updates. A lack of sufficient evidence existed for the task force to recommend use of tocilizumab, rituximab, abatacept, intravenous immunoglobulin, JAK inhibitors or corticosteroids. The updated recommendations are relatively comprehensive, but calcinosis management, a painful and disfiguring complication, is not addressed.

I applaud the gender balance on the task force. The methodology of these recommendations is sound and I am grateful for the time and effort of the task force. In conclusion, the updated guidelines provide a nice summary framework for applying the newer evidence for management of SSc, but I hope that in the next update, more recommendations by SSc phenotype can be included.

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Competing interests

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Systemic sclerosis

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Genome-wide mutagenesis reported in systemic sclerosis

Carol M. Artlett & John Varga

The mechanisms that drive the diverse disease manifestations and increased cancer risk associated with systemic sclerosis are unclear. Investigating the genomic alterations observed in patients with systemic sclerosis could contribute towards untangling this complex disease.

REFERS TO Vijayraghavan, S. et al. Widespread mutagenesis and chromosomal instability shape somatic genomes in systemic sclerosis. *Nat. Commun.* **15**, 8889 (2024).

Systemic sclerosis (SSc, also known as scleroderma) is an acquired chronic disease that is characterized by the presence of specific autoantibodies, vasculopathy and widespread tissue fibrosis. Currently, there is no overarching hypothesis that accounts for the diverse disease manifestations in SSc and the substantial increase in the risk of cancer that is associated with the disease[1]. Somatic chromosomal aberrations[2] and loss of telomeric DNA sequences[3] have long been recognized in patients with SSc, but the implications of these genomic changes in SSc remain unclear. Vijayraghavan et al.[4] have now uncovered widespread somatic mutagenesis and chromosomal instability in patients with SSc using whole-genome sequencing of clonal lung fibroblasts. The findings from this study highlight the linkage between lung inflammation and fibrosis in SSc and suggest a potential role of somatic mutations in the pathogenesis of SSc, including the increased risk of cancer. In addition, this study adds to the growing appreciation that somatic mutations are involved in the pathogenesis of a variety of acquired autoimmune, inflammatory and fibrotic conditions[5].

Notably, the researchers report a correlation between distinct mutational signatures in SSc lung fibroblast clones and increased activity of DNA polymerase-η (Polη, a translesion polymerase involved in DNA repair that is encoded by *POLH*) and activation-induced cytidine deaminase (AID). Cytosine deamination, an inflammation-related process driven by reactive oxygen species, causes DNA damage, which is repaired by the action of Polη; however, Polη has relatively low repair fidelity, which leads to additional genomic alterations. The study reports a strong correlation between specific *POLH*-associated trinucleotide signatures and the single base substitution pattern SBS93, a specific mutation signature identified in the Catalogue of Somatic Mutations in Cancer (COSMIC) database. This SBS93 mutation signature is caused by transcriptional strand asymmetry, and the authors postulate that SBS93 is the primary Polη mutation signature[4]. The authors found this association to be especially strong in two patients with chronic obstructive pulmonary disease.

The SBS93 mutation signature is strongly associated with oesophageal, stomach and colon cancers, which occur in patients with SSc; however, the most common cancers reported in SSc are breast and lung cancer[1].

In addition to the Polη mutation signature, the authors also describe an AID-like mutation signature in SSc lung fibroblasts. AID-associated hypermutations typically occur in germinal centres and facilitate the development of B cell diversity, which can determine the autoantibody profile of an individual; however, 'off-target' AID activity is also associated with tumorigenesis[6]. The increased AID mutation burden in SSc lung fibroblasts could also reflect a generalized defect in this DNA damage-repair mechanism; however, the limited data in this study preclude conclusions regarding the role of the AID mutation signature in SSc. Additional genomic alterations described in SSc lung fibroblasts included structural and copy number variations and loss of heterozygosity, a phenomenon that is commonly associated with tumorigenesis. Large genomic deletions in chromosomes 6, 10 and 21 were also found in a subset of SSc lung fibroblasts. The functional consequences of these genomic deletions and their role in SSc pathogenesis and disease progression are currently unknown.

Notably, although the majority of genomic changes observed in SSc lung fibroblasts were 'passenger' mutations that were spread across a variety of genes (including genes involved in cell adhesion, Wnt signalling and DNA metabolism), two oncogenic 'driver' mutations were also found, which highlights a potential mechanism that could link inflammation and cancer in SSc.

Interestingly, a study published in 2022 uncovered frequent chromosomal instability and centromeric abnormalities in SSc skin fibroblasts, further supporting the emerging theme of genomic alterations being associated with SSc. The genomic changes described in the skin fibroblasts were associated with activation of the cGAS–STING pathway[7], which might explain the augmented inflammasome activity observed in SSc[8]. Together, the observations from the present study[4] indicate a generalized and chronic inflammatory process in SSc lung fibroblasts that would promote genomic instability. Compounding this genomic instability with shortened telomere length found in patients with SSc[3] is consistent with enhanced biological ageing[9], and taken together with other predisposing factors such as environmental exposure and other intrinsic risk factors could place those with SSc at an increased risk of cancer (Fig. 1).

Limitations of the pioneering study by Vijayraghavan et al.[4] include the small cohort size (fibroblasts from six patients with SSc and five healthy individuals), the inclusion of several individuals in each group who were heavy smokers, which might have influenced the results, and the lack of other chronic lung disease controls such as idiopathic pulmonary fibrosis. Lung cells have one of the highest mutation rates in the body, presumably reflecting exposure to environmental pollutants, as indicated in a 2023 study of chronic lung diseases[10]. There was also sparse demographic information about the healthy and SSc fibroblast donors and the age of the donors was not clear[4]. Age-matching could have provided support for the enhanced genomic ageing in SSc[9].

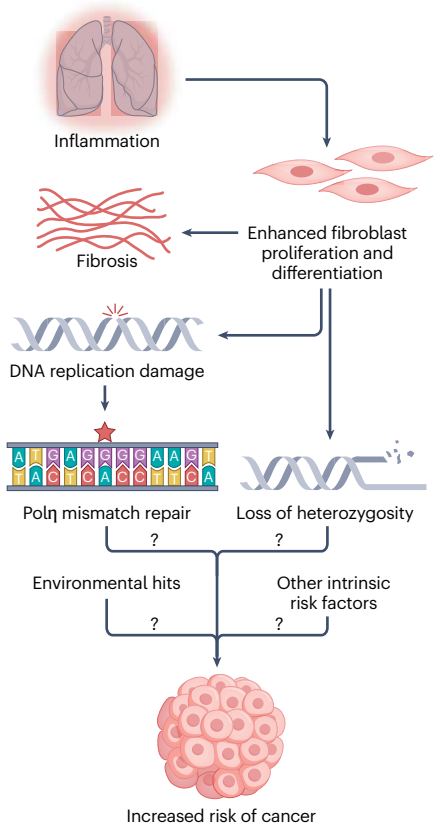

Fig. 1 | Genomic mutations in SSc fibroblasts could expose patients to an increased risk of cancer. Inflammation causes fibroblasts to proliferate and differentiate. The differentiated fibroblasts cause fibrosis. However, during fibroblast proliferation, DNA damage can occur, leading to DNA polymerase-η (Polη)-mediated mismatch repair and loss of heterozygosity. These genomic changes could lead to an increased risk of cancer in patients with systemic sclerosis (SSc). How loss of heterozygosity and mismatch repair combined with environmental hits and other intrinsic factors cause cancer in SSc remains unclear, as denoted by the question marks.

Moreover, although robust associations between certain SSc autoantibodies and cancer have been described[1], the autoantibody profiles of the fibroblast donors in this study were not reported.

The implications of this study are currently limited, and the findings might simply reflect a generalized defect in DNA repair due to inflammation[5]. Notable, but not studied, would have been the analyses of skin fibroblasts to address the question of whether their mutational burden reflects that observed in the lung fibroblasts. The patients in this cohort[4] were undergoing lung transplants and, therefore, had a lengthy disease course; the mutational burden identified in the SSc lung fibroblast samples could reflect the extensive tissue damage associated with long-term disease. Analysis of fibroblasts from patients with pre-SSc or early SSc will help to unravel the observations made in this study and address the issues raised.

Overall, Vijayraghaven et al.[4] report a general increase in the mutational burden in cloned lung fibroblasts from patients with SSc-associated lung disease. These findings might implicate somatic genomic changes in disease progression or predisposition to cancer. Understanding the full implications of these observations will require additional samples and careful selection of controls, including critical age-matching and prudent donor selection not confounded by a history of smoking or chronic obstructive pulmonary disease.

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Review article

Check for updates

Occupational dust and chemical exposures and the development of autoimmune rheumatic diseases

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Abstract

Although the association between certain occupational exposures and the development of autoimmune rheumatic disease was first described over a century ago, this association has only become more widely recognized in the past 10 years because of the use of high-silica-content engineered stone in construction and home renovation. There is now a substantial and growing body of evidence that occupational dust and chemical exposure, be it through mining, stonemasonry, building or other trades, increases the risk of various systemic autoimmune rheumatic diseases (SARDs) including rheumatoid arthritis and systemic sclerosis. Although the pathogenic mechanisms of silica-induced autoimmunity are not fully elucidated, it is thought that alveolar macrophage ingestion of silica and the ensuing phagosomal damage is an initiating event that ultimately leads to production of autoantibodies and immune-mediated tissue injury. The purportedly causal association between occupational exposure to chemicals, such as organic solvents, and an increased risk of SARDs is less frequently recognized compared with silica dust, and its immunopathogenesis is less well understood. An appreciation of the importance of occupational dust and chemical exposures in the development of SARDs has implications for workplace health and safety regulations and offers a unique opportunity to better understand autoimmune disease pathogenesis and implement preventative strategies.

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Sections

Key points

- Occupational exposure to silica dust is associated with several-fold increased risk of systemic sclerosis and also of developing rheumatoid arthritis, systemic lupus erythematosus, small-vessel vasculitis and sarcoidosis.
- Occupational exposure to solvents is associated with an increased risk of systemic sclerosis and rheumatoid arthritis, and possibly other autoimmune diseases.
- There are a large number of occupations ranging from construction and mining to petrochemical, plastics and rubber industries wherein potentially harmful exposures to silica and solvents can occur.
- The pathogenesis of autoimmune disease related to occupational exposures is not fully elucidated but is likely to involve a key role for innate immune responses.
- Occupational systemic autoimmune rheumatic diseases present a unique opportunity for disease prevention through the generation of new scientific knowledge, advocacy, workplace health and safety policy, and legislation.

Introduction

The association between occupational exposures and the development of systemic autoimmune rheumatic diseases (SARDs), though long recognized, is often neglected for this group of conditions, which are typically described as idiopathic[1]. Over the past decade, both the general public and medical professionals have become aware of this association in the context of a dramatic rise in silica-related diseases in engineered stone workers[2,3]; however, the full gamut of occupational exposures associated with the development of autoimmune rheumatic disease, and the pathogenic processes involved, are yet to be fully elucidated.

In this Review we focus on occupational exposure to dusts, in particular silica (silicon dioxide), and chemicals, in particular solvents, for which the epidemiological evidence of a link to SARDs is most compelling. We also touch on some other dust and chemical exposures for which evidence of an association with SARDs is less compelling. We list some of the many occupations in which these exposures can occur, including farming, mining and construction. We summarize and appraise the evidence for the most common SARDs encountered in this context, including rheumatoid arthritis (RA), systemic sclerosis (SSc, also known as scleroderma), systemic lupus erythematosus (SLE) and small-vessel vasculitis (SVV). We discuss specific clinical observations regarding the phenotype of SARDs encountered in this context, including serological markers. We discuss postulated pathogenic mechanisms such as dust-exposure-induced phagosomal injury, chemical-exposure-induced epigenetic modification and effects on T cell regulation and activation, depletion of antioxidant activity and formation of neoantigens. In addition to reviewing the literature, we discuss knowledge gaps that give rise to opportunities for scientific discovery. We discuss occupational health and safety implications including reducing and monitoring exposure in the workplace, health surveillance for high-risk workers and workplace policy and legislation to protect workers.

A historical perspective of occupational exposures

In a seminal paper presented to the Edinburgh Medico-Chirurgical Society in 1914, the Scottish physician Byrom Bramwell described nine individuals with 'sclerodermia', of whom five were stonemasons and one a coppersmith[4]. Bramwell incorrectly speculated that the common aetiological factor in these cases was 'the holding of a chisel in the hand in cold weather' resulting in constriction of peripheral blood vessels, later termed Raynaud phenomenon, a pathognomonic hallmark of SSc. Notably, one of the individuals described in this report was a miner. Caplan syndrome was first described in 1953 as radiological evidence of intrapulmonary nodules indicative of pneumoconiosis in coal miners with a diagnosis of RA[5]. In 1957, the description of 17 cases of 'scleroderma' in gold miners in South Africa by Erasmus led to the coining of the term Erasmus syndrome[6]; in this report, Erasmus correctly hypothesized that the aetiological factor in these cases was exposure to silica dust.

During the 1990s and 2000s, spatiotemporal clusters of cases of SSc were reported around airports in London, UK, in a village in Italy, in southwestern Ontario, Canada, and in the Wimmera region of Victoria, Australia, in genetically unrelated individuals, implicating a common environmental exposure for each cluster[7–10]. Notably, the affected people reported in Victoria were mostly farmers or in farming-related occupations that are known to be associated with exposure to inorganic dusts containing silica, as well as pesticides and fertilizers[10].

Years after the 2001 collapse of the World Trade Centre, which left large volumes of rubble and dust containing harmful substances including silica in the surrounding area, there were case reports of SSc in 'ground zero' rescue and recovery workers[11]. In a nested case–control study of World Trade Centre rescue and recovery workers, the conditional odds ratio of developing autoimmune disease was increased by 13% (conditional odds ratio 1.13, 95% CI 1.02–1.26) for each additional month worked at the site[12]. Among 59 affected workers, RA was the most commonly reported autoimmune disease (37%), with cases of spondyloarthritis, myositis, SLE, SSc, Sjögren syndrome (also known as Sjögren disease), antiphospholipid syndrome and granulomatosis with polyangiitis (GPA) also observed. In another World Trade Centre study, those with 'intense dust cloud exposure' had almost twice the risk of SARDs compared with those with less intense exposure (adjusted risk ratio 1.86, 95% CI 1.02–3.40), suggesting a dose–response causal association[13].

A resurgence of silicosis, a silica-induced form of lung disease, among stonemasons working with high-silica-content artificial stone was reported in 2012, in a study of cases referred to the National Lung Transplantation Program in Israel from 1997 to 2010 (ref. 14). This same group of researchers went on to report an 'outbreak' of autoimmune disease in artificial stone workers, with an estimated sevenfold excess risk of autoimmune disease on the basis of the expected disease prevalence[15].

The association between organic solvents and the development of SARDs was first reported in the 1950s with the publication of case reports of individuals who developed an SSc-like syndrome after exposure to vinyl chloride, epoxy resins, trichloroethylene, perchloroethylene or mixed solvents[16]. Although the global expansion in building and construction and the production of engineered stone has seen a rise in occupational exposure to silica over the past two decades, changes in the composition of products used in the cleaning, painting and plastics industries have led to an overall decrease in occupational exposure to organic solvents since the 1990s[17].

Global burden of disease studies report over 20,000 new cases of silicosis per year worldwide, indicating that occupational exposure

to silica remains prevalent and that there are likely to be many cases of SARDs related to silica exposure for which this association has not been made or documented[18,19]. Although there are no published studies specifically evaluating the global burden of SARDs resulting from occupational exposure to silica, it is likely that silicosis and silica-related SARD frequencies track in parallel, albeit with different magnitudes of risk associated with exposure. Also, the proportion of cases of SARDs associated with occupational exposure to chemicals, including organic solvents, remains unquantified despite many occupations posing a risk of exposure. Accordingly, it is possible that a much higher number of cases of SARDs result from occupational exposure than is appreciated by patients and treating physicians alike.

We acknowledge that a multitude of other exposures can occur in various occupations, and these include exposure to epoxy resins, heavy metals, pesticides, metallic welding fumes and ultraviolet radiation. Sleep deprivation associated with shiftwork and psychological stress at work might also have harmful effects on health. However, this article focuses on occupational exposures to dusts and chemicals for which epidemiological evidence of association with the development of SARDs is most compelling. We briefly describe some of these other exposures in order to highlight the broad scope of the field.

Occupations associated with exposure to silica dust and chemical solvents

Box 1 presents a list of some occupations wherein there is a risk of exposure to silica dust and organic solvents. However, it is important to note that exposure levels have changed over time in many professions; for example, among painters, exposure to organic solvents was high up until the 1980s, when paints containing organic solvents were gradually replaced by water-based paints[17]. A European study of plastics industries showed that styrene exposure was around 850 mg/m^3 in the 1950s and 85 mg/m^3 in the 1980s, representing a tenfold reduction over three decades[17]. As alluded to earlier, the converse trend is noted for silica content in stones used for construction, whereby natural stones generally have silica content that is <50% by weight, whereas manufactured stone, which has become increasingly popular in the past two decades, has >90% silica content by weight[20]. We postulate that, globally, there are many millions of people working in professions that entail exposure to silica, and also potentially solvents (Box 2), although there are currently no formal estimates of numbers.

Silica exposure-associated systemic autoimmune rheumatic diseases

Among all occupational exposures, the association between silica dust exposure and SARDs has the strongest and most robust evidence. Exposure to silica dust has been linked to a variety of SARDs, with the main risks being for SSc, RA, SLE, SVV and sarcoidosis (Table 1 and Supplementary Table 1). Although lung silicosis and SARDs can coexist in an individual exposed to silica dust, rheumatic disease related to occupational silica dust exposure can occur even without evidence of silicosis[21]. Supplementary Table 2 provides a summary of study design and quality of articles quantifying risk of SARDs in association with occupational dust exposures[22].

Systemic sclerosis

SSc is characterized by vasculopathy and fibrosis of the skin and internal organs, has a 5-to-1 preponderance in women, a global prevalence ranging from 0.02% to 0.05% and a median age of disease onset of around 50 years[23–25].

Box 1 | Occupations that can be associated with exposure to silica or solvents

A broad range of professions and industries are associated with exposure to silica or solvents. This list of occupations provides numerous examples but is not exhaustive.

Professions and industries involving exposure to silica dust

- Stone and brick masonry
- Engineered stone cutting and installation
- Mining
- Quarrying, excavation and earth moving
- Agriculture and farming
- Tunnelling
- Road construction and maintenance
- Demolition and construction
- Concreting
- Sandblasting
- Hydraulic fracking
- Foundry work
- Pottery and ceramics
- Jewellery production
- Glass manufacture and recycling
- Dental technicians

Professions and industries involving exposure to chemical solvents

- Painting, staining and lacquering
- Furniture and cabinet making
- Floor laying
- Dry cleaning
- Petrochemical, oil and gas industries
- Printing
- Plastics and rubber industries
- Chemical refining
- Pharmaceuticals
- Adhesives industry
- Mechanics
- Chemists

The prevalence of silica exposure in SSc cohorts (Supplementary Table 1) is striking, ranging from 7.5% in an Australian SSc cohort[26], on the basis of self-recalled exposure, to 54% when exposure was evaluated on the basis of serum markers[27]. Despite SSc predominantly affecting women, most workers in occupations associated with a risk of silica dust exposure are men[26,28–30], with a substantially higher frequency of silica dust exposure in men than in women (58% versus 1%) in a French SSc cohort[31].

Two meta-analyses have evaluated the association between occupational silica dust exposure and the development of SSc[32,33] (Table 1). The first meta-analysis was published in 2010 and included 16 studies from the USA, Europe and Australia[32]: 9 case–control studies, 3 cohort studies, 3 mortality studies and 1 case series spanning the period between 1967 and 2007. When all 16 studies were pooled, the combined estimator of relative risk (CERR) was 3.20 (95% CI 1.89–5.43), indicating that occupational silica dust exposure was associated with a greater than threefold risk of the development of SSc. There was substantial heterogeneity between studies (I^2 97.2%) owing to different study designs and different methods of ascertaining silica exposure, including the use of a self-reported occupational history, job title or using the presence of silicosis as evidence of silica exposure. To reduce this heterogeneity, the authors further assessed the association by study design and gender. Regardless of study design, the overall CERR indicated an increased relative risk of the development of SSc: case–control studies CERR 2.24 (95% CI 1.65–3.31), mortality studies CERR 1.01 (95% CI 0.9–41.08) and cohort studies CERR 15.49 (95% CI 4.54–52.87). When assessing this risk by sex, the CERR for silica dust exposure in

Box 2 | Occupational dusts and chemicals associated with systemic autoimmune rheumatic diseases

Among occupational dusts, silica has the largest body of evidence in support of an association with systemic autoimmune rheumatic diseases. Among occupational chemicals, solvents have the largest body of evidence in support of an association with systemic autoimmune rheumatic diseases.

Silica

Silica (silicon dioxide) exists in both non-crystalline and crystalline forms[20]. Silica dust particles that are less than 10 µm in diameter are respirable, which means that these particles are small enough to penetrate deep into the lungs and drive an inflammatory response[172]. Silica is found in soil, sand, concrete, asphalt, bricks, terracotta tiles and pavers, sandstone and granite, with small amounts present in cement[20,173]. The typical concentrations of crystalline silica vary from up to 95% in sand and sandstone to 20–45% in granite and 5–15% in bricks[20].

Engineered or artificial stone is a composite material made of crushed stone bound together by either polymer resin or cement[174]. This material was developed as a more affordable, durable and versatile alternative to natural stones. Unlike natural stone, engineered stone has a very high concentration of silica[174]. Moreover, analysis of the constituents of engineered stone has revealed that it also contains polycyclic aromatic hydrocarbons and metals such as aluminium that could also contribute to its toxicity[175]. Italy, Spain and Israel were among the largest producers of engineered stone products from the 1980s to the early 2000s; however, China is now the source of the largest overall quantity of engineered stone produced and the largest market for this product[176]. Engineered stone has greater than 90% respirable crystalline silica (RCS) content by weight[20].

Solvents

Solvents are usually liquids that can dissolve other substances, creating a solution. Organic solvents can be divided into aliphatic-chain compounds (n-hexane), aromatic compounds (benzene, xylene) and chlorinated compounds that are commonly used in dry cleaning and degreasing (tetrachloroethylene), paint thinners (toluene), nail-polish removers, glue (acetone, methyl acetate, ethyl acetate) and spot removers (hexane)[36,177].

men was higher than that in women, indicating that silica dust might be a stronger risk factor for SSc disease in men (CERR 3.02, 95% CI 1.24–7.35) than in women (CERR 1.03, 95% CI 0.74–1.44). Overall, the substantial difference in the CERR among case–control and cohort studies is likely to point to differences in ascertaining exposure retrospectively compared with prospectively, with retrospective assessment of exposure being subject to recall bias and prospective studies being susceptible to overdiagnosis in milder, subclinical or incipient cases of SARDs.

The second meta-analysis, published in 2017, included 19 studies (15 case–control studies and 4 cohort studies) spanning the period from 1960 to 2014 (ref. 33) (Table 1). This study also showed a significant association between silica dust exposure and the development of SSc (odds ratio (OR) 2.81, 95% CI 1.86–4.23); and also that silica dust exposure increases the risk of SSc more in men (OR 3.06, 95% CI 1.90–4.91) than in women (OR 2.10, 95% CI 1.24–3.55).

In 2021, a Danish nationwide cohort study of over 3 million workers linked the Danish Occupational Cohort database to the National Patient Registry and once again showed an association between silica dust exposure and the development of SSc. In addition to showing an increased risk with increasing cumulative exposure since entering the workforce, an incidence rate ratio (IRR) for SSc of 2.62 (95% CI 0.87–7.90) per 50 µg/m^3-years (ref. 34) (Table 1) was reported.

The phenotype of SSc in those exposed to silica dust compared with those without exposure has been evaluated in French, Australian and Canadian cohorts. Those with silica dust exposure are more likely to be men with diffuse cutaneous SSc, that also have anti-Scl-70 antibodies, digital ulceration, tendon friction rubs, myocardial involvement and interstitial lung disease[35]. In multivariable logistic regression analysis, silica dust exposure was associated with being male, being younger at the onset of SSc skin involvement[26,35–37] and having digital ulceration, joint contractures and greater physical disability, as measured by the scleroderma health assessment questionnaire[26,36], but not with mortality[37]. Similarly, a Chinese cohort study that compared the clinical characteristics of patients with SSc with silicosis with those without silicosis found that those with silicosis were more likely to be men, who were a younger age at disease onset and were more likely to have interstitial lung disease, cardiac involvement and pulmonary hypertension[38]. Likewise, data from an Italian study demonstrated that higher levels of serum silica corresponded to a higher prevalence of diffuse cutaneous SSc, myositis, anti-Scl-70 antibody positivity and a greater extent of lung fibrosis than those with lower levels of serum silica[27]. Additionally, a French study of people with SSc also showed an association between greater silica exposure and the progression of pulmonary involvement[31].

Rheumatoid arthritis

RA, an autoimmune disease characterized by synovial inflammation in small and large joints, has an estimated worldwide prevalence of around 0.24–1.0% in adults, with women affected two to three times more often than men[39]. Similar to SSc, RA is recognized as an occupational disease in the USA, but not in other countries such as Australia. There is a similarly high prevalence of silica exposure reported in RA to that in SSc cohorts (Supplementary Table 1), with ranges between 4.6% and 21.6% in case–control studies[40,41], and a higher prevalence for those with seropositive RA than for those with seronegative RA (4.7% versus 4.1), particularly in males (seropositive RA 13.5% versus seronegative RA 10.6%)[40].

Three meta-analyses have evaluated the association between occupational silica dust exposure and the development of RA (Table 1). The first meta-analysis, which was published in 2002 and included 10 studies, demonstrated an increased risk of developing RA with exposure to silica dust (CERR 3.43, 95% CI 2.25–5.22), particularly for men (CERR 4.45, 95% CI 2.24–8.86)[42]. In 2020, a meta-analysis that included 15 studies confirmed an increased risk of RA with silica dust exposure (OR 2.59, 95% CI 1.78–3.82)[43]; however, owing to substantial heterogeneity (I^2 52.8%),

Table 1 | Studies of occupational exposure to silica dust and risk of rheumatic disease

Study design	Study population	Occupational history and exposure ascertainement	Prevalence and risk of disease exposure	Ref.
SSc				
Meta-analysis of 16 studies	Participants in nine case–control studies (1,101 patients with SSc and 2,900 healthy individuals), three cohort studies (*n*=1,681), three mortality studies (*n*=39,080) and one case series (*n*=54)	Information obtained via in-person interviews, telephone interviews and/or postal questionnaires Exposure assessment was based on a combination of job title, occupational history and diagnosis of silicosis as a proxy for silica exposure	Overall increase in risk of SSc development with exposure: CERR 3.20 (95% CI 1.89–5.43; I^2 97.2%) By study design: case–control studies CERR 2.24 (95% CI 1.65–3.31; I^2 26%); mortality studies CERR 1.01 (95% CI 0.94–1.08; I^2 0%); cohort studies CERR 15.49 (95% CI 4.54–52.87; I^2 85.5%) By sex: women CERR 1.03 (95% CI 4.54–52.87; I^2 26%); men CERR 3.02 (95% CI 1.24–7.35; I^2 55.3%) Prevalence of SSc secondary to RCS not reported	32
Meta-analysis of 19 studies	Participants in 15 case–control studies (*n*=1,336) and 4 cohort studies (*n*=247,563)	Not mentioned	Overall increase in risk of SSc development with exposure by study design: case–control studies OR 2.81 (95% CI 1.86–4.23); cohort studies (RR 17.52; 95% CI 5.98–51.37) By sex: women (CERR 1.03; 95% CI 4.54–52.87; I^2 26%); men (CERR 3.02; 95% CI 1.24–7.35; I^2 55.3%) Prevalence of SSc secondary to RCS not reported	33
Cohort study of the Danish Occupational Cohort linked to the National Patient Registry	Overall cohort 3,012,274 Danish workers (1,541,505 men, 1,470,769 women) 252 of whom had SSc	SYN-JEM	Overall increase in risk of SSc development with exposure: women IRR 1.46 (95% CI 0.65–3.27); men IRR 1.62 (95% CI 1.08–2.44) By increasing cumulative exposure (IRR per 50 μg/m^3-years): women IRR 1.14 (95% CI 0.95–1.36); men IRR 1.10 (95% CI 1.03–1.18) Prevalence of SSc in male workers ~0.02% Prevalence of SSc in female workers ~0.05% Prevalence in those with highest cumulative exposure ~0.10%	34
Prospective cohort study that included a description of disease characteristics in those with and those without RCS exposure	142 patients with SSc	Self-reported RCS exposure on standardized occupational history questionnaire Blind evaluation of occupational exposure Dichotomized by smoking	Individuals with SSc and RCS exposure (compared with those without RCS exposure) were more likely to have dcSSc, digital ulceration, ILD, myocardial dysfunction and cancer In multivariate logistic regression analysis, RCS was associated with male sex (OR 19.31; 95% CI 15.34–69.86), cancer (OR 5.97; 95% CI 1.55–23.01), digital ulceration (OR 2.42; 95% CI 1.05–5.56) Prevalence of occupational exposure in individuals with SSc was ~42.2%, 12.6% of whom had RCS exposure	35
Cohort study, which included a description of disease characteristics in those with and those without RCS exposure	1,670 patients with SSc	Self-reported RCS exposure on occupational history	Individuals with SSc and RCS exposure were more likely to be male (31.6% vs 3.7%), have dcSSc (40.5% vs 24.7%), digital ulceration (58.4% vs 44.3%), tendon friction rubs (14.6% vs 8.1%), cardiac involvement (3.2% vs 0.3%), anti-Scl-70 antibodies (27.6% vs 14.0%) In multivariate logistic regression analysis, RCS exposure was associated with younger age at the onset of SSc skin involvement (OR 0.98; 95% CI 0.96–1.0), male sex (OR 14.9; 95% CI 14.9–25.7), joint contractures (OR 1.8; 95% CI 1.0–3.3) and more physical disability on scleroderma HAQ (OR 1.4; 95% CI 1.1–1.7) Prevalence of RCS occupational exposure in SSc was 7.5%	26
Case–control study	80 patients with SSc (10 men, 70 women) and 50 age- and sex-matched healthy individuals	In-person structured questionnaire to ascertain exposure to silica micro- and nanoparticles through occupational, environmental exposure, smoking or prosthesis implants	Individuals with SSc (compared with healthy individuals) had higher occupational silica exposure (OR 8.52; 95% CI 3.26–22.25) High silica exposure in individuals with SSc (compared with healthy individuals) was associated with dcSSc, myositis, lung fibrosis, ground-glass opacification, honeycombing, higher ESR, CRP, anti-Scl-70 antibodies, and was negatively associated with ACA Prevalence of RCS exposure in SSc was ~54%	27
Belgian cohort study	91 patients with SSc (20 men, 71 women)	Categorized into construction (including electricians, masons, tilers, plumbers and pipefitters versus other industry job) Limited list for construction was based on the Belgian National Institute of Statistics occupational list The broader list of construction-related occupations was determined by an independent occupational expert	The prevalence of SSc was 10-fold higher in the construction industry than in the general male work force (50% vs 5%) No women had a construction-related occupation if the broader definition of construction was used 75% of men with SSc had a job in construction and ~53% were electricians Individuals with a job in construction (compared with those who did not have a job in construction) had a higher mRSS and disease severity scale	28

Table 1 (continued) | Studies of occupational exposure to silica dust and risk of rheumatic disease

Study design	Study population	Occupational history and exposure ascertainement	Prevalence and risk of disease exposure	Ref.
SSc (continued)				
Belgian cohort study	103 men with SSc	Baseline occupational history categorized into four exposure groups	The prevalence of occupational exposure in men with SSc was ~72.9% By exposure type: 57.3% had probable silica exposure, 11.5% had probable solvent exposure, 2.1% had combined silica and solvent exposure, 2.1% had probable asbestos exposure and 27.1% had no occupational exposure There was a higher prevalence of past or current smokers in individuals exposed to solvents (90.9%) vs the silica-exposed (81.8%) and no-exposure groups (53.8%)	29
French multicentre retrospective cohort study	210 patients with SSc (55 men, 155 women)	Standardized quantitative assessment of occupational exposure through a CES using the JEM	The occupational exposures included in this study were: chlorinated solvents (25.2% of the cohort), crystalline silica (14.3% of the cohort) and epoxy resins (11.0% of the cohort) There was a higher frequency of exposure in men than in women (OR 10.3; 95% CI 5.1–21.9) Men were exposed to a higher number of toxins than women (ratio of 3:1) CES was higher in those with dcSSc, correlated with mRSS and correlated independently with FVC decline of >10% from baseline. CES was not associated with DLCO decline Prevalence of occupational exposure in SSc was ~37.6% (solvents 25.2%, RCS 14.3% and epoxy resins 11%)	30
French prospective cohort study	100 patients with SSc	Exposure score was calculated based on information from self-reported occupational and non-occupational questionnaires followed by a phone interview or an in-person interview	16% of individuals with SSc had an occupation associated with high silica exposure, 58% of whom were men Occupations with high silica exposure were associated with mediastinal and hilar LAD (OR 8.09; 95% CI 2.01–32.52) and worsening pulmonary involvement (OR 4.57; 95% CI 1.12–18.60) The prevalence of RCS in individuals with SSc was 58% in men and 1% in women	31
Canadian retrospective cohort study	1,439 patients with SSc	Baseline occupational questionnaire at enrolment into the database	Prevalence of RCS in individuals with SSc was ~7%, of whom 20% were men RCS exposure was associated with being under 50 years of age at diagnosis (OR 0.47; 95% CI 0.29–0.77), Raynaud phenomenon (OR 0.48; 95% CI 0.29–0.78) and a trend for mortality (HR 1.45; 95% CI 0.96–2.19)	37
Cohort study of patients with SSc, with and without silicosis	310 patients with SSc (72 with silicosis, 238 without silicosis)	Clinical diagnosis of silicosis	Individuals with SSc and silicosis (compared with those without silicosis) were more likely to be male, be of a younger age at SSc onset, have more weight loss, a history of smoking, cardiac involvement, ILD, pulmonary hypertension, and elevated BNP, CRP and ESR Prevalence of RCS exposure in individuals with SSc and silicosis was 23.2%. 76.8% of individuals without silicosis had no RCS exposure	38
RA				
Meta-analysis (10 studies)	Participants in five cohort studies (n=110), two case–control studies (n=87) and two proportionate mortality studies (n=22)	Self-reported job title and occupational history	Overall risk of RA development with silica exposure: CERR 3.43 (95% CI 2.25–5.22) Silica exposure in men CERR 4.45 (95% CI 2.24–8.86)	42
Meta-analysis of 15 studies	Participants in eight case–control studies (3,777 individuals with RA and 5,508 healthy individuals), five cohort studies (n=6,316) and two cross-sectional studies (n=1,688)	Job title, self-reported occupational history and diagnoses of silicosis as a proxy for silica exposure	Overall risk of developing RA after exposure to RCS: OR 2.59 (95% CI 1.73–3.45; I^2 52.8%) By study design: case–control (OR 2.80; 95% CI 1.78–3.82; I^2 0.0%); cross-sectional (OR 3.07; 95% CI 0.96–5.18; I^2 66.3%); cohort studies (OR 1.98; 95% CI −0.57 to 4.53; I^2 25.6%) By smoking status: smokers (OR 2.49; 95% CI 1.13–3.86; I^2 74.1%)	43
Cohort study of the Danish Occupational Cohort linked to the National Patient Registry	Overall cohort 3,012,274 Danish workers (1,541,505 men, 1,470,769 women) 3,490 of whom had RA	SYN-JEM	Overall IRR: men IRR 1.57 (95% CI 1.41–1.75); women IRR 1.10 (95% CI 0.85–1.42) By increasing cumulative exposure (IRR per 50 μg/m^3-years): men IRR 1.07 (95% CI 1.05–1.10); women IRR 1.05 (95% CI 0.98–1.11) Prevalence of RA in male workers was ~0.23% Prevalence of RA in female workers was ~0.63% Prevalence of RA in those with highest cumulative exposure was ~0.20%	34

Table 1 (continued) | Studies of occupational exposure to silica dust and risk of rheumatic disease

Study design	Study population	Occupational history and exposure ascertainement	Prevalence and risk of disease exposure	Ref.
RA (continued)				
Swedish National Registry case–control study	Overall cohort 93,417 (31,139 patients with RA, 62,278 healthy individuals)	Health-insurance and labour-market studies used to determine occupation and duration Finnish Information System on Occupational Exposure job-exposure matrix to determine occupational exposure	Prevalence of occupational exposure in individuals with RA was 13% in men and 2% in women By RCS exposure: Overall exposures 803/17,353 (4.6%) Men exposed (67.2%), seropositive RA (4.7%), seropositive RA men (13.5%), seronegative RA (4.13%), seronegative RA men (10.6%)	40
Meta-analysis of 12 studies	Participants in seven case–control studies (36,698 patients with RA and 266,136 healthy individuals), and five cohort studies (n=4,017)	Job title, self-reported occupational history and diagnoses of silicosis as a proxy for silica exposure	Overall odds of developing RA after exposure to RCS: OR 1.94; 95% CI 1.46–2.58; I^2 95% Risk according to autoantibody positivity, that is, anti-RF antibodies or anti-ACPA (data from seven studies): seropositive individuals (OR 1.74; 95% CI 1.35–2.25; I^2 59%); seronegative individuals (OR 1.23; 95% CI 1.06–1.40; I^2 0%) Risk according to smoking habits (data from five studies): seropositive smokers (OR 3.30; 95% CI 2.40–4.54; I^2 49%)	44
SLE				
Cohort study of the Danish Occupational Cohort linked to the National Patient Registry	Overall cohort 3,012,274 Danish workers (1,541,505 men, 1,470,769 women) 255 of whom had SLE	SYN-JEM	Overall IRR with increasing cumulative exposure (IRR per 50 μg/m^3-years): IRR 1.09 (95% CI 1.01–1.17) Prevalence of SLE in male workers ~0.02% Prevalence of SLE in female workers ~0.12% Prevalence in those with highest cumulative exposure 0.001%	34
Case–control study	265 patients with SLE and 355 healthy individuals	Occupational history by a trained interviewer and exposure assessed by investigators blinded to case–control status	The prevalence of RCS in the SLE cohort was 13.9%, (n=37), ~6% of whom had medium- to high-level exposure and exposure was higher in men (29%) than in women (4%) Compared with low RCS exposure, risk of developing SLE with medium RCS exposure (OR 2.10; 95% CI 1.1–4.0) and high RCS exposure (OR 4.60; 95% CI 1.4–15.4) was higher Monotonic increase in risk across exposure groups: difference in SLE clinical manifestations (RCS exposed compared with unexposed): haemolytic anaemia (OR 0.1; 95% CI 0.00–0.3), leukopenia (OR 0.3; 95% CI 0.1–1.0)	47
Meta-analysis of six studies (four case–control studies and two cohort studies)	Participants in four case–control studies (7,771 patients with SLE and 261,441 healthy individuals) and two cohort studies (n=1,662)	Job title, self-reported occupational history and diagnoses of silicosis as a proxy for silica exposure	Overall risk of SLE with silica exposure: OR 3.49; 95% CI 1.24–9.83; I^2 92.36% By study design: case–control studies (OR 1.85; 95% CI 0.96–3.59; I^2 75.92%); cohort studies (individuals with silicosis) (OR 9.71; 95% CI 1.13–83.58; I^2 72.65%)	48
Vasculitis				
Cohort study of the Danish Occupational Cohort linked to the National Patient Registry	Overall cohort 3,012,274 Danish workers (1,541,505 men, 1,470,769 women) 749 of whom had SVV	SYN-JEM	Overall risk of SVV after exposure to RCS: IRR 1.34 (95% CI 1.02–1.76) Overall IRR with increasing cumulative exposure (IRR per 50 μg/m^3-years): IRR 1.06 (95% CI 1.01–1.11) Prevalence of SVV in male workers ~0.05% Prevalence of SVV in female workers ~0.06% Prevalence in those with highest cumulative exposure ~0.003%	34
US case–control study	Overall cohort 328 (219 patients with SVV and 109 healthy individuals)	Occupational history including estimates of exposure (conducted via phone interview)	Prevalence of RCS exposure was 60.5% in those with SVV and 45% in healthy individuals (no differences were observed with sex or ethnicity) Odds of SVV, compared with no exposure, in those with low or medium lifetime exposure OR 1.0 (95% CI 0.4–2.2) and high lifetime exposure OR 1.9 (95% CI 1.0–3.5)	51
Case–control study	75 patients with PSV, 273 control individuals (220 non-vasculitis, 19 secondary vasculitis and 34 asthma controls)	Structured occupational questionnaire to measure occupational exposures and confounders, exposure risk estimated by JEM	The prevalence of PSV in individuals exposed to RCS was 24% (compared with 19.1% in healthy individuals) Risk of PSV with exposure to silica (OR 3.0; 95% CI 1.0–8.4); EGPA (OR 5.6; 95% CI 1.3–23.5); GPA (OR 2.5; 95% CI 0.8–8.5); MPA (OR 3.2; 95% CI 0.8–8.5)	52

Table 1 (continued) | Studies of occupational exposure to silica dust and risk of rheumatic disease

Study design	Study population	Occupational history and exposure ascertainement	Prevalence and risk of disease exposure	Ref.
Vasculitis (continued)				
French cross-sectional cohort study	Overall cohort 185 patients with vasculitis (120 with GPA, 35 with MPA, 30 with renal-limited vasculitis)	Spatial association between individuals with AAV and quarries was assessed electronically	Increased AAV risk in communities with quarries (OR 2.51; 95% CI 1.66–3.80); specifically for GPA (OR 3.21; 95% CI 1.96–5.25); and renal-limited vasculitis (OR 3.1; 95% CI 1.12–8.51), but not for MPA (OR 1.10; 95% CI 0.50–2.41) There was a spatial association between the number of individuals with AAV and proximity to quarries (including GPA, MPA and renal-limited vasculitis) Geospatial analysis: PR3-AAV (OR 2.95; 95% CI 1.76–4.94); MPO-AAV (OR 2.32; 95% CI 1.22–4.39) Geographic-weighted regression models analysis showed that quarries were associated with the development of PR3-AAV and MPO-AAV	53
Sarcoidosis				
Meta-analysis of 12 studies	Participants in 11 case–control studies (7,678 individuals with sarcoidosis and 311,788 healthy individuals) and 1 cohort study (n=371)	Job titles and associated exposures	Risk of pulmonary sarcoidosis in those exposed to RCS: OR 1.26; 95% CI 1.02–1.56; I^2 33.7%	56

AAV, anti-neutrophil cytoplasmic antibody-associated vasculitis; ACA, anti-centromere antibody; BNP, B-type natriuretic peptide; CERR, combined estimator of relative risk; CES, cumulative exposure score; CRP, C reactive protein; dcSSc, diffuse cutaneous SSc; DLCO, diffusing capacity of the lungs for carbon monoxide; EGPA, eosinophilic granulomatosis with polyangiitis; ESR, erythrocyte sedimentation rate; FVC, forced vital capacity; GPA, granulomatosis with polyangiitis; HAQ, health assessment questionnaire; HR, hazard ratio; I^2, heterogeneity of studies; ILD, interstitial lung disease; IRR, increased incidence rate ratio; LAD, lymphadenopathy; MPA, microscopic polyangiitis; mRSS, modified Rodnan skin score; OR, odds ratio; PSV, pressure support ventilation; RA, rheumatoid arthritis; RCS, respirable crystalline silica; RR, relative risk; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SVV, small-vessel vasculitis; (SYN-)JEM, (quantitative) job exposure matrix.

this risk was confirmed by evaluating risk according to study design. The authors combined the results from the eight case–control studies and confirmed the finding of an increased risk of RA (OR 2.80, 95% CI 1.78–3.82). An analysis of smokers exposed to silica dust also showed a higher overall risk of developing RA than non-exposed smokers (OR 2.49, 95% CI 1.13–3.86). In 2021, a third meta-analysis that combined 12 studies demonstrated risk of RA with silica dust exposure (OR 1.94, 95% CI 1.46–2.58) and calculated the risk according to autoantibody status. This study showed a higher risk of developing seropositive RA than seronegative RA in those exposed to silica dust (OR 1.74, 95% CI 1.35–2.25 and OR 1.23, 95% CI 1.06–1.40, respectively)[44]. Understanding why those exposed to silica dust are at an increased risk of seropositive RA requires further research; however, it is thought that RA-specific autoantibodies originate in the lungs in response to silica-induced citrullination of peptides[44]. A dose–response relationship between silica dust exposure and the development of RA was also shown in the Danish Nationwide Cohort study (IRR 1.20, 95% CI 0.87–1.65 per 50 μg/m^3-years)[34].

Systemic lupus erythematosus

SLE is a multisystem autoimmune disease that affects the skin, joints, kidneys and nervous system and is characterized by the presence of autoantibodies, reduced levels of complement, and in some cases, cytopenias[45]. SLE occurs with a prevalence of 0.02–0.07% and predominantly affects women of childbearing years (ratio of 9 men:1 woman)[46]. Compared with SSc and RA, there is much less literature assessing silica dust exposure in SLE cohorts; in one study the prevalence of silica dust exposure in patients with SLE was 6% (Supplementary Table 1) and this prevalence was higher for men than for women (29% versus 4%)[47].

A systematic review and meta-analysis published in 2021 assessed the relationship between silica dust exposure and the development of SLE[48] (Table 1). The combined results of the six studies that were included (four case-control studies and two cohort studies) from the USA, Canada and Europe demonstrated an increased risk of SLE (OR 3.49, 95% CI 1.24–9.83); owing to substantial heterogeneity among studies (I^2 92.36%), the risk of SLE was stratified by study design and regardless of study design, there was a positive association between SLE and silica dust exposure (case–control studies OR 1.85, 95% CI 0.96-3.59, and cohort studies OR 9.71, 95% CI 1.13–83.58).

The increased risk of SLE with increasing duration and quantity of exposure to silica dust was shown in a US-based case–control study. Compared with the group with the lowest exposure, those with medium or high exposure were at a higher risk of developing RA (OR 1.7 and 3.8, respectively)[47]. A Danish cohort study also showed that increasing exposure to silica dust is associated with an increase in the risk of developing SLE (IRR 1.09, 95% CI 1.01–1.17)[34] (Table 1).

The SLE disease phenotype in those exposed to silica dust compared with those who have not been exposed seems similar, the only notable differences being in the prevalence of cytopenias and a lower frequency of haemolytic anaemia and leukopenia in those exposed to silica[47].

Small-vessel vasculitis

SVV, specifically anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), occurs with a prevalence of 0.02–0.04%, with equal sex distribution[49]. AAV can be classified into three categories that are based on the patterns of clinical involvement. GPA is characterized by cytoplasmic ANCA, often with specificity for proteinase-3, and granulomatous inflammation in blood vessels of the nose, sinuses, throat, lungs and kidneys. Microscopic polyangiitis (MPA) is characterized by perinuclear ANCA with specificity for myeloperoxidase, and a

pauci-immune, necrotizing SVV affecting organs throughout the body. Eosinophilic GPA (EGPA) is an extremely rare autoimmune condition that causes inflammation of small and medium-sized blood vessels in persons with a history of airway allergic hypersensitivity[49,50].

Similar to SLE, the literature on the prevalence of silica dust exposure in SVV is limited but reports a frequency of 0.05% for men and 0.06% for women[34] (Supplementary Table 1). Within a US case–control study, 60.5% of individuals with SVV had been exposed to silica dust compared with 45% of healthy individuals, with no difference in prevalence noted by gender or ethnicity[51].

The association between silica dust exposure and the development of SVV, specifically AAV and biopsy-proven glomerulonephritis, has been reported in several case–control studies since 1993, which showed an increased risk of SVV with silica dust exposure[51,52] (Table 1). A large case–control study published in 2007 confirmed these findings and provided evidence of a dose–response relationship with an increased risk of SVV, specifically biopsy-proven glomerulonephritis, with cumulative dose and duration of silica dust exposure (OR 1.9, 95% CI 1.0–3.5), in those with 'high' exposure compared with 'low' or no exposure[51]. These findings were confirmed in the Danish Nationwide Cohort study, which found a significant association between silica dust exposure and SVV (IRR 1.34, 95% CI 1.02–1.76), with increasing exposure to silica being associated with an increasing risk of SVV (IRR 1.42, 95% CI 0.57–2.54 per 50 μg/m^3-years)[34]. A French study also highlighted this increased risk of SVV with silica exposure and quantified the geospatial risk for both proteinase-3 and myeloperoxidase AAV (OR 2.95, 95% CI 1.76–4.94, and OR 2.32, 95% CI 1.22–4.39, respectively)[53].

Sarcoidosis

Sarcoidosis is a multisystem disease characterized by granulomatous inflammation in lymph nodes and the lungs, with potential for heart, skin, bone and nervous system involvement[54]. Sarcoidosis occurs with a prevalence of 0.001–0.07%[55], with approximately 90% of cases involving the lungs[56]. There is emerging evidence of an association between sarcoidosis and occupational exposures.

The observation of histological and radiological similarities between sarcoidosis and silicosis has led to increased interest in examining the association between silica dust exposure and development of sarcoidosis, with numerous case–control and cohort studies published on this topic in the past decade. A systematic review and meta-analysis published in 2023 included 12 studies (11 case–control studies and 1 cohort study) and demonstrated an overall increased risk of pulmonary sarcoidosis in those exposed to silica dust compared with unexposed individuals (OR 1.26, 95% CI 1.02–1.56), with low heterogeneity reported among these studies (I^2 33.7%)[56] (Table 1).

Solvent exposure-associated systemic autoimmune rheumatic diseases

The most convincing evidence for an association between solvent exposure and autoimmune disease is for the development of SSc, although there are also reports of associations with RA, SLE and SVV[57]. Our search revealed no studies specifically assessing risk of sarcoidosis with solvent exposure.

Systemic sclerosis

The association between SSc and organic solvent exposure was first described in 1957 (ref. 58). Since then, many epidemiological studies have investigated the association between solvent exposure and risk of SSc, the majority of which report positive correlations. These studies have highlighted the substantial prevalence (ranging from 22.1% to 33%) of solvent exposure in those with SSc; this prevalence is higher in men than in women (45% versus 16.7%)[35,59,60] (Table 2). Four meta-analyses have reported the combined results of these studies, and each concluded that the risk of SSc increases with solvent exposure (Table 2).

The first of these meta-analyses was published in 2001 and included eight studies (seven case–control studies and one cohort study), showing a combined relative risk of SSc of 2.9 among people exposed to solvents (95% CI 1.6–5.3), albeit with notable heterogeneity among the studies[61]. A second meta-analysis of 11 case–control studies published in 2007 confirmed an increased risk of developing SSc among people exposed to solvents (OR 1.8, 95% CI 1.2–2.5, after adjusting for publication bias), which was higher for men than for women (OR 3.0, 95% CI 1.9–4.6, and OR 1.8, 95% CI 1.5–2.1, respectively)[60]. Neither of these meta-analyses was able to identify the risk associated with specific solvents because of the small number of studies for each solvent type. A third meta-analysis, published in 2016, specifically addressed this issue[59]. Combining the results of 14 case–control studies, the investigators found an increased odds of SSc among those exposed to any type of solvent (OR 2.07, 95% CI 1.55–2.78), which was higher for men than for women (OR 5.28, 95% CI 3.46–8.05, and OR 1.62, 95% CI 1.34–1.96, respectively), and identified an increased odds of developing SSc after exposure to aromatic solvents (OR 2.72, 95% CI 1.21–6.09), trichloroethylene (OR 2.07, 95% CI 1.34–3.17), halogenated solvents (OR 1.49, 95% CI 1.12–1.99) and ketones (OR 4.20, 95% CI 2.19–8.06), but no statistically significant association with benzene, toluene, xylene, perchloroethylene, trichloroethane and white spirit[59]. A 2017 meta-analysis that included 17 studies (13 case–control studies and 4 cohort studies) supported these findings[33].

The difference in SSc disease phenotype in those exposed and those not exposed to solvents was reported in a French cohort study showing that those with solvent exposure were more likely to have diffuse cutaneous SSc disease, anti-Scl-70 antibody positivity, digital ulceration, and pulmonary and myocardial involvement[35].

Rheumatoid arthritis

Although no systematic reviews or meta-analyses have been published investigating the association between RA and organic solvent exposure, numerous case–control and cohort studies published in the past 2 decades show mostly positive associations (Table 2). Furthermore, the prevalence of solvent exposure in RA, although lower than in SSc, is still notable at 14.8%[62] (Supplementary Table 1). To date, the largest prospective study to investigate the association between farming and RA was published in 2019 and included over 50,000 participants (male farmers (n = 27,175) and their spouses (n = 22,231, all of whom were women))[63]. The authors of this study found an increased risk of RA with exposure to chemical fertilizers (OR 1.50, 95% CI 1.11–2.02), non-gasoline solvents (OR 1.40, 95% CI 1.09–1.80) and paint (OR 1.26, 95% CI 1.00–1.59). Given the nature of the occupations wherein solvent exposure can occur, isolating the risk of RA related to exposure to one particular solvent has not been possible.

Systemic lupus erythematosus

Similarly, there have been no systematic reviews or meta-analyses published on the association between occupational exposure to organic solvents and the development of SLE. Although in case–control and cohort studies an increased risk of SLE has been found with duties involving solvent exposure, when evaluated through formal job histories and

Table 2 | Studies of occupational exposure to solvents and risk of systemic autoimmune rheumatic diseases

Study design	Study population	Occupational history and exposure	Prevalence and exposure risk of disease	Ref.
SSc				
Meta-analysis of eight studies	Participants in seven case–control studies (651 individuals with SSc) and one cohort study (n=71)	Self-reported exposure or ascertained from occupational history	Overall risk of SSc after solvent exposure: CERR 2.9 (95% CI 1.6–5.3) There was significant heterogeneity between studies, I^2 not provided By study type: case–control studies CERR 3.14 (95% CI 1.56–6.33); cohort study RR 2.1 (95% CI 0.8–5.5)	61
Meta-analysis of 11 studies	Participants in 11 case–control studies (n=4,726; 1,291 individuals with SSc and 3,435 healthy individuals)	Self-reported exposure or ascertained from occupational history	Risk of SSc with silica exposure: OR 2.4 (95% CI 1.7–3.4), after adjusting for publication bias (OR 1.8; 95% CI 1.2–2.5). By sex: men (OR 3.0; 95% CI 1.9–4.6); women (OR 1.8; 95% CI 1.5–2.1) The prevalence of solvent exposure in individuals with SSc was 24.6% and 15.5% in healthy individuals The prevalence was higher for men than for women (42.3% versus 21.7%)	60
Meta-analysis of 14 studies	Participants in 14 case–control studies (n=5,513; 1,675 individuals with SSc and 3,838 healthy individuals) Studies were from Europe (n=9), North America (n=4) and Japan (n=1)	Self-reported exposure or ascertained from occupational history Some of the studies estimated exposure duration and quantification	Overall risk of SSc after RCS exposure: OR 2.07; 95% CI 1.55–2.78; I^2 51.7% By sex: men (OR 5.28; 95% CI 3.46–8.05); women (OR 1.62; 95% CI 1.34–1.96) Solvent exposure associated with SSc: aromatic solvents (OR 2.72; 95% CI 1.21–6.09); trichloroethylene (OR 2.07; 95% CI 1.34–3.17); halogenated solvents (OR 1.49; 95% CI 1.12–1.99); ketones (OR 4.20; 95% CI 2.19–8.06) Solvent exposure with a non-significant association with SSc: benzene OR 1.02 (95% CI 0.59–1.75, P=0.951), toluene OR 1.41 (95% CI 0.78–2.54), xylene OR 1.35 (95% CI 0.72–2.52), perchloroethylene OR 2.03 (95% CI 0.44–9.27), trichloroethane OR 1.37 (95% CI 0.76–2.48), white spirit OR 2.22 (95% CI 0.65–7.63) Prevalence of solvent exposure in SSc was 22.1% and was higher for men than for women (45% versus 16.7%)	59
Systematic review and meta-analysis of 13 case–control studies	Participants in 13 case–control studies (789 individuals with SSc and 1,318 healthy individuals)	Self-reported exposure or ascertained from occupational history using JEM to ascertain exposure risk Some of the studies estimated exposure duration and quantification	Overall risk of SSc after RCS exposure by random effects model): OR 2.00 (95% CI 1.32–3.02) By sex (by fixed effects model): men (OR 2.40; 95% CI 1.44–4.01); women (OR 2.01; 95% CI 1.66–2.44)	33
Prospective cohort study	100 patients with SSc	A committee of experts blindly evaluated exposure from self-reported exposure forms and occupational history	SSc and solvent exposure were associated with dcSSc, digital ulcers, ILD, myocardial dysfunction, cancer and anti-Scl-70 antibody positivity On multivariate analysis, factors associated with SSc and exposure were male sex (OR 19.31; 95% CI 15.34–69.86), cancer (OR 5.97; 95% CI 1.55–23.01) and digital ulcers (OR 2.42; 95% CI 1.05–5.56) The prevalence of solvent exposure was 35.2% in individuals with SSc and 24% in healthy individuals	36
RA				
Meta-analysis of 10 studies	Participants in three retrospective studies (n=766), five case–control studies (1,325 individuals with RA and 128,974 healthy individuals) and two prospective studies (n=3,000)	Occupation selection and self-report	Risk of RA by occupation: farmers (RR 1.3–1.8; 95% CI 1.0–5.0); spray painters or lacquer workers (RR 2.4; 95% CI 1.1–5.4); construction workers (RR 1.4–2.9; 95% CI 1.1–5.7) and miners (RR 1.4–3.6; 95% CI 1.0–6.2)	68
Prospective cohort study	52,394 private pesticide applicators	Questionnaire given to participants renewing their licence for pesticides	By solvent type: chemical fertilizers (OR 1.50; 95% CI 1.11–2.02); non-gasoline solvents (OR 1.40; 95% CI 1.09–1.80) and paint (OR 1.26; 95% CI 1.00–1.59) The prevalence of solvent use in individuals with RA was 14.8%	63

Table 2 (continued) | Studies of occupational exposure to solvents and risk of systemic autoimmune rheumatic diseases

Study design	Study population	Occupational history and exposure	Prevalence and exposure risk of disease	Ref.
SLE				
Case–control study (identified via the Carolina Lupus Cohort study)	Overall cohort 620 (265 patients with SLE and 355 healthy individuals)	In-person interviews to assess occupational history; expert committee evaluated the exposure and job history to determine exposure	Exposure dose–response compared with those with no exposure: low exposure (OR 0.94; 95% CI 0.50–2.3); moderate exposure (OR 1.0; 95% CI 0.57–1.9) and high exposure (OR 1.0; 95% CI 0.60–1.6) Prevalence of SLE associated with exposure to solvents: moderate exposure (10.6%) and moderate–high exposure (6.1%)	64
Case–control study	Overall cohort 286 (95 patients with SLE and 191 healthy individuals)	In-person interviews to assess occupational history; an expert committee whose members were blinded to the participants' disease evaluated exposure	Prevalence of >1 year of solvent exposure was 16% for those with SLE and 13% for healthy individuals Exposure to organic solvents for more than 1 year was not associated with SLE (OR 1.04; 95% CI 0.34–3.2), and there was no exposure-response effect with longer durations of exposure to solvents	66
SVV				
UK case–control study	Overall cohort 348 (75 patients with SVV and 273 healthy individuals)	SVV was determined from prospective vasculitis registry (histologically confirmed) A structured questionnaire was used to obtain occupational exposures and JEM was used to determine exposure score	Overall risk of SVV from solvent exposure: OR 3.4 (95% CI 0.9–12.5) Prevalence of solvent exposure in those with SVV was 14.7%	52

CERR, combined estimator of relative risk; dcSSc, diffuse cutaneous systemic sclerosis; I^2, heterogeneity of studies; ILD, interstitial lung disease; JEM, job exposure matrix; OR, odds ratio; RA, rheumatoid arthritis; RCS, respirable crystalline silica; RR, relative risk; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SVV, small-vessel vasculitis.

exposure dose–response questionnaires, these associations have not been confirmed[64–67]. In these case–control studies, the prevalence of SLE associated with solvent exposure is 14.8–16% (Table 2). To date, published data provide insufficient evidence that exposure to organic solvents has a causative role in the development of SLE.

Small vessel vasculitis

Few studies have evaluated the association between solvent exposure and the development of SVV. In one UK case–control study that estimated solvent exposure among participants in a vasculitis registry (prevalence 14.7%), those with a history of high solvent exposure at any time had a higher likelihood of primary systemic vasculitis (OR 2.7, 95% CI 1.1–6.6), specifically GPA (OR 3.4, 95% CI 1.3–8.9), with no association found with EGPA or MPA[52] (Supplementary Table 1). Given the paucity of literature in this area, there is currently insufficient evidence that exposure to organic solvents has a causative role in the development of SVV, with further studies needed.

Other occupational exposures and systemic autoimmune rheumatic diseases

Owing to the paucity of studies evaluating associations between other occupational exposures and SARDs other than SSc, this section mainly focuses on SSc, with reference to associations with RA and other SARDs only where applicable (Table 3).

It must be noted that many occupations involve concurrent and successive exposure to multiple agents such as dust, paint, glues, resins and metals, thereby hampering the ability to determine the risk associated with a specific exposure in isolation. Additionally, in some occupations, such as construction, smoking is more common, adding further complexity to determining each contributing risk. Some studies have reported a cumulative exposure risk or score as a way of highlighting this multifaceted risk[30].

In 2020, a scoping literature review showed an increased risk of RA among certain occupations, including farming (RR 1.3–1.8), construction work (RR 1.4–2.9) and mining (OR 2.1–6.2), which was thought to be associated with occupational exposures[68]. This risk was highlighted in a large prospective study[63] that showed an increased risk of RA with exposure to chemical fertilizers (OR 1.50, 95% CI 1.11–2.02), non-gasoline solvents (OR1.40, 95% CI 1.09–1.80) and paint (OR 1.26, 95% CI 1.00–1.59). As the nature of these occupations involves concurrent and successive exposure to multiple agents such as dust, paint, glues, resins and metals, isolating the risk of any rheumatic disease including RA as a consequence of one particular exposure is difficult.

Epoxy resins

Epoxy resins are compounds commonly used in construction and their association with the development of SSc was first described in 1980. Thus far, there has been 1 meta-analysis of exposure to epoxy resins that was published in 2017 and included 4 case–control studies with a total of 264 patients with SSc[33]. This analysis showed an overall odds ratio of 2.97 (95% CI 2.31–3.83) of developing SSc after exposure to epoxy resins, which was higher for men than for women (OR 2.92, 95% CI 2.26–3.78, and OR 1.00, 95% CI 0.02–12.72, respectively)[33]. To our knowledge, no studies to date report an association between epoxy resins and other rheumatic diseases.

Welding or metallic fumes

To date, there has been 1 meta-analysis of 4 case–control studies on exposure to welding or metallic fumes in 2017, which included 448 patients with SSc. This analysis showed an overall OR of 1.29 (95% CI 0.44–3.74) of developing SSc after exposure to welding or metallic fumes, which was higher for men than for women (OR 5.87, 95% CI 2.49–13.86, and OR 1.52, 95% CI 0.36–6.49, respectively)[33].

Table 3 | Studies of other occupational exposure and risk of rheumatic disease

Study design	Rheumatic disease	Study population	Occupational history and exposure ascertainment	Prevalence and exposure-associated risk of disease	Ref.
Epoxy resins					
Meta-analysis of four case–control studies	SSc	Participants in four case–control studies (264 patients with SSc, only two of the studies included women (*n*=147))	Self-reported exposure or ascertained from occupational history utilizing JEM to ascertain exposure to resin Some of the studies estimated exposure duration and quantification	Overall risk of SSc with exposure to epoxy resins (by fixed-effects models): OR 2.97 (95% CI 2.31–3.83) Stratified by sex (by fixed-effect models): men (OR 2.92; 95% CI 2.26–3.78); women (OR 1; 95% CI 0.02–12.72) No prevalence data reported	33
Pesticides					
Meta-analysis of four studies	SSc	Participants in three case–control studies (264 patients with SSc, 55.7% of whom were women) and one mortality study (*n*=5,642)	Self-reported exposure or obtained from occupational history utilizing JEM to ascertain exposure to pesticide Some of the studies estimated exposure duration and quantification	Overall risk of SSc with exposure to pesticides (by fixed-effects models): OR 1.02; 95% CI 0.78–1.32 Stratified by sex (by fixed-effects models): men (OR 1.02; 95% CI 0.79–1.33); women (OR 3.06; 95% CI 0.22–43.34) No prevalence data reported	33
Meta-analysis of 12 studies	Pulmonary sarcoidosis	Participants in 11 case–control studies (7,678 individuals with pulmonary sarcoidosis and 311,788 healthy individuals) and 1 cohort study (*n*=371)	Job titles and associated exposures	Overall risk of pulmonary sarcoidosis with exposure to pesticides (OR 1.42; 95% CI 1.09–1.85; I^2 14.3%) No prevalence data reported	56
Welding fumes					
Meta-analysis of four studies	SSc	Participants in four case–control studies (448 patients with SSc, 82.8% of whom were women)	Self-reported exposure or obtained from occupational history utilizing JEM to ascertain exposure to pesticides Some of the studies estimated exposure duration and quantification	Overall risk of SSc with exposure to welding fumes (by fixed-effects models): OR 1.29 (95% CI 0.44–3.74) Stratified by sex (by fixed-effects models): men (OR 5.87; 95% CI 2.49–13.86); women (OR 1.52; 95% CI 0.36–6.49) No prevalence data reported	33
Meta-analysis of 12 studies	Pulmonary sarcoidosis	Participants in 11 case–control studies (7,678 individuals with pulmonary sarcoidosis and 311,788 healthy individuals) and 1 cohort study (*n*=371)	Job titles and associated exposures	Overall risk of pulmonary sarcoidosis with exposure to welding fumes OR 0.40 (95% CI 0.16–0.96) No prevalence data reported	56
Heavy metals					
Case–control study	SSc	Overall cohort 400 (100 patients with SSc and 300 healthy individuals)	Trained investigator interviewed all individuals Standardized questionnaire on exposure and confounders (socio-economic factors, including smoking)	Individuals with SSc compared with age- and sex-matched healthy individuals had higher median levels of antimony, cadmium, lead, mercury, molybdenum, palladium and zinc SSc in men was associated with higher levels of antimony and platinum SSc in women was associated with higher levels of antimony, cadmium, lead, mercury, molybdenum, palladium and zinc No prevalence data reported	70
Case–control study	RA	Overall cohort 156 (78 patients with RA and 78 healthy individuals)	No occupational assessment made Levels of metals in hair samples quantified	The mean values of cadmium and lead were higher in samples of scalp hair from patients with RA (both smokers and non-smokers) than in samples from healthy individuals The concentration of zinc was lower in the samples of scalp hair from patients with RA No prevalence data reported	71
Asbestos					
Cross-sectional study of individuals enrolled in the Center for Asbestos Related Disease in Montana (LAA cohort) and the steamfitters cohort	Rheumatic autoantibody positivity	Participants in the LAA (*n*=397) and the steamfitters cohort (*n*=87)	Self-reported occupational and residential exposures using a questionnaire generated by the authors	Factors that distinguish the two cohorts: ENA antibody specificity; higher levels of anti-histone antibodies were associated with a higher probability of being from the LAA cohort than from the steamfitters cohort, whereas high values for anti-Sm antibodies were associated with a higher probability of coming from the steamfitters cohort. The presence of antibodies did not predict the development of clinical disease The occupational exposure prevalence was 53.3% in the LAA cohort and 100% in the steamfitters cohort	74

Table 3 (continued) | Studies of other occupational exposure and risk of rheumatic disease

Study design	Rheumatic disease	Study population	Occupational history and exposure ascertainment	Prevalence and exposure-associated risk of disease	Ref.
Asbestos (continued)					
Montana nested case–control study	RA	Participants included those with SAIDs including SLE (*n*=30), RA (*n*=129) and SSc (*n*=4) Healthy individuals were randomly selected at a 3:1 ratio from the remaining 6,813 screening participants using frequency-matched age and sex groupings	Self-reported questionnaire answers on exposure and diagnosis of rheumatic condition	Exposure to vermiculite in mining had no increased risk of SAIDs (OR 1.03; 95% CI 0.69–1.58) In those 65 years of age or older, there was an increased risk of RA (OR 3.23; 95% CI 1.31–7.96) No prevalence data reported	75
Swedish register-based cohort study	SSc and RA	Overall cohort 515,174 (375,035 men and 140,139 women) of whom 9 individuals had SSc and 104 individuals had RA	JEM was used to calculate exposure	Risk of SSc with asbestos exposure RR 1.8 (95% CI 0.8–4.3) Risk of RA with asbestos exposure RR 1.1 (95% CI 0.9–1.3) No prevalence data reported	76

ENA, extractable nuclear antigen; I^2, heterogeneity of studies; JEM, job exposure matrix; LAA, libby asbestiform amphiboles; OR, odds ratio; RA, rheumatoid arthritis; RR, relative risk; SAIDs, systemic autoimmune diseases; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Pesticides

The association between pesticide exposure and the development of SSc was first described in 1996 in 26 individuals with occupational SSc[62]. In 2017, a meta-analysis was published that summarized 3 studies with 264 patients with SSc, and showed an overall OR of 1.02 (95% CI 0.78–1.32) that remained non-significant when stratified by gender (men OR 1.02, 95% CI 0.79–1.33, and women OR 3.06, 95% CI 0.22–43.34)[33].

A systematic review and meta-analysis published in 2023 evaluated the association between occupational chemical exposure and the development of pulmonary sarcoidosis. Three of the case–control studies that were included reported an increase in the likelihood of developing pulmonary sarcoidosis with pesticide exposure (OR 1.42, 95% CI 1.09–1.85), whereas welding, which was only assessed in one case–control study (n = 1,412) was associated with a decreased likelihood of developing sarcoidosis (OR 0.40, 95% CI 0.16–0.96)[56].

Metals

In 1988, an association was first postulated between rheumatic conditions, including RA and SSc, and heavy metals that are found in paint (such as antimony, arsenic, cadmium, chromium, cobalt, lead, manganese, mercury and tin)[69]. A 2017 case–control study compared 100 patients with SSc with 300 healthy individuals; the levels of several heavy metals were higher in the serum of patients with SSc than in healthy individuals. Additionally, this study reported an association between SSc and occupational exposure to antimony and platinum in men with SSc and an association between SSc and exposure to antimony, cadmium, lead, mercury, palladium and zinc in women with SSc[70] (Table 3).

An association between cadmium and lead exposure and RA was shown in a case–control study that evaluated the levels of these metals in hair samples collected from patients with RA from Ireland and Pakistan who were stratified by smoking status[71]. The authors found higher mean values of cadmium and lead in scalp hair samples of both smokers and non-smokers with RA compared with healthy smokers and non-smokers of a similar age (Table 3).

Asbestos

Although evidence is still under investigation, there is no clear link between asbestos exposure and SARDs[72,73]. Certain types of asbestos fibres (such as libby asbestiform amphiboles (LAA) as opposed to chrysotile asbestos) are associated with the increased risk of having certain rheumatic autoantibodies, but not necessarily clinical disease[74]. The presence of antinuclear antibodies (ANAs), including anti-histone, anti-ribosomal P protein, anti-Sm, anti-ribonucleoproteins and anti-Jo-1 (histidyl tRNA synthetase) antibodies was more frequent in a cohort that was exposed to LAA. Notably, predictive modelling demonstrated that anti-histone antibodies were the strongest predictors of LAA exposure[74]; however, the presence of these autoantibodies was not consistently predictive of the development of clinical rheumatic disease[74]. This lack of association between exposure and overall risk of rheumatic disease was also shown in a case–control study of LAA exposure[75]; however, when those older than 65 years of age were analysed separately, there was an increased risk of RA in those exposed to vermiculite mining (OR 3.23, 95% CI 1.31–7.96), but other rheumatic diseases did not carry this increased risk[75]. This increased risk of RA in those exposed to vermiculite mining was also shown in a Swedish register-based cohort study (RR 1.8, 95% CI 0.9–1.3)[76].

Postulated immunopathogenesis of silica- and chemical-induced autoimmunity

Unlike silicosis, a fibrotic lung disease in which high exposure to respirable crystalline silica (RCS) is associated with a high risk of developing disease, there is a less clear dose–response relationship between exposure and development or severity of SARDs[77]. As discussed previously, some studies report a higher risk of developing autoimmune disease with greater magnitude exposure to RCS[34,77]; however, not all workers exposed develop disease, which implicates genetic susceptibility and epigenetic modification as important, as yet unquantified, contributors to risk[78–80]. Genome-wide association studies have identified polymorphisms that are associated with autoimmune disease development. The strongest associations found with autoimmune susceptibility to environmental chemicals involve the HLA alleles and polymorphisms in the gene that encodes the IL-23 receptor[81,82]. However, the contribution of a

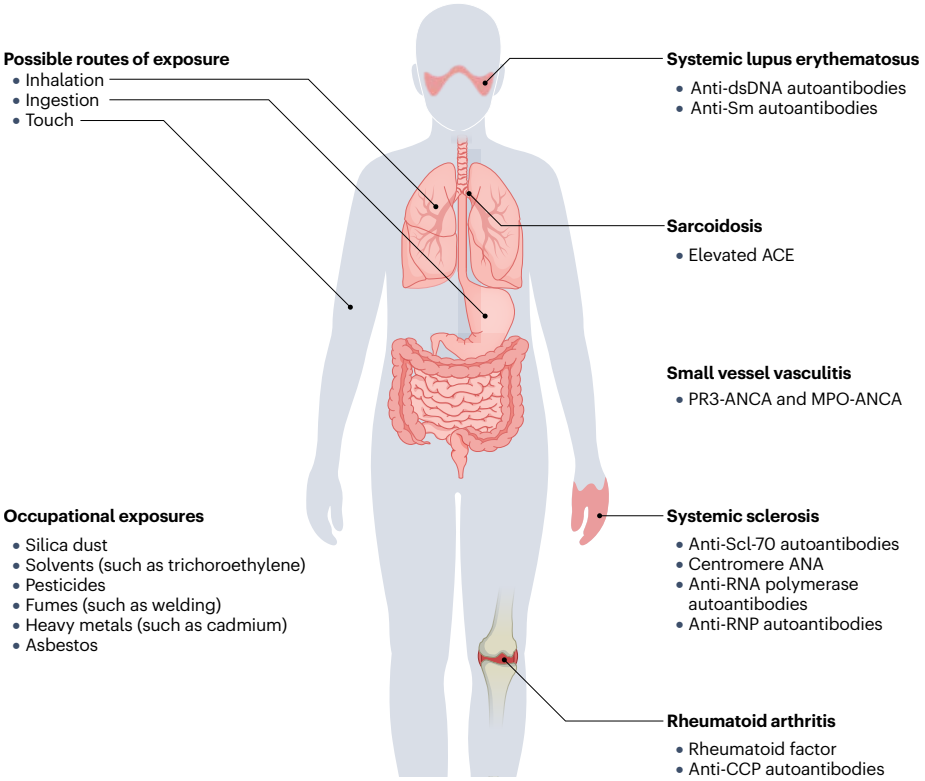

Fig. 1 | Systemic autoimmune rheumatic diseases associated with occupational exposures. There are numerous types of occupational exposure and also multiple possible routes of exposure. Occupational exposure can lead to the development of systemic autoimmune rheumatic diseases (SARDs) and the autoantibodies that are characteristic of these diseases can be identified in individuals with SARDs. ACE, angiotensin-converting enzyme; ANA, anti-nuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; CCP, cyclic citrullinated peptides; dsDNA, double-stranded DNA; MPO, myeloperoxidase, PR3, proteinase 3; RNP, ribonuclear protein.

single gene polymorphism is very small and a combination of polymorphisms and environmental triggers, such as a toxic chemical, is required to initiate or propagate inflammatory autoimmune responses[83]. This concept is further supported by twin studies that show that genetics can only account for increased susceptibility to autoimmune diseases and that an environmental trigger is necessary to turn on the genetic expression associated with disease[84]. A study of genetically heterogeneous mice showed individual variations in response to transoral doses of crystalline silica, with some mice developing silicosis and others developing autoantibodies with specificity for extractable nuclear antigens, with or without clinical autoimmune features, such as glomerulonephritis[85]. Silica-exposed male mice had more lung inflammation, inflammatory cells in the bronchoalveolar lavage fluid and higher levels of both IL-6 and autoantibodies than silica-exposed female mice. Overall, this study highlights the importance of genetic predisposition in the development of silica exposure-induced autoimmunity.

As alluded to earlier, other potentiators of risk include environmental exposures such as smoking; augmented risk of developing RA with smoking in workers exposed to silica was reported in a Swedish study[43,86]. The latency between exposure and development of clinical features of SARDs can be variable, ranging from a few months to two decades[87,88]. Although inhalation is the assumed predominant mode of exposure for silica dust, the role of skin contact and ingestion cannot be dismissed, particularly for exposure to organic solvents. The potential role of 'first responder' resident immune cells in the skin is being increasingly recognized in the development of various diseases including SARDs[89,90]. Moreover, the clinical observation that skin changes in SSc invariably begin in 'exposed' areas of the body such as the hands and face raises the hypothesis that the exposure route might not be solely respiratory[91]. Outbreaks of SSc-like conditions, such as eosinophilia-myalgia syndrome caused by L-tryptophan consumption and toxic oil syndrome caused by intake of denatured rape-seed oil, point to ingestion as a potential route of entry for toxins that can trigger autoimmunity[92,93] (Fig. 1).

Studies of silica-induced autoimmunity in animal models, including lupus-prone NZM2410 mice that are exposed to crystalline silica, have shed some light on the potential mechanisms of autoimmunity following exposure to RCS, including a key role for impaired clearance of apoptotic cells by phagocytes, a process known as efferocytosis[27,73,85,94–104].

Currently, it is hypothesized that the role of crystalline silica in the induction of autoimmunity (Fig. 2) involves the death (apoptosis and pyroptosis) of lung epithelial cells and macrophages with subsequent expression of nuclear proteins at the cell surface and the release of nuclear contents (including free DNA and DNA-associated proteins such as topoisomerase). The peptides derived from intranuclear proteins are presented to autoreactive $CD4^+$ T helper 1 cells (T_H1 cells), which in turn become activated and drive disease. Silica also induces the release of IL-1β in an NLRP3-dependent manner in macrophages, which promotes co-stimulation of autoreactive T cells and B cells in mediastinal lymph nodes with subsequent production of autoantibodies that target nuclear proteins[105].

Large-scale screening programmes of at-risk workers who have been exposed to RCS have revealed that a proportion of individuals develop autoantibodies, including those that are disease specific such as anti-Scl-70 and anti-centromere antibodies, without having clinical features of systemic autoimmune disease, indicating that full-blown disease requires a breakdown of self-tolerance at multiple levels[106–108].

The pathophysiology of immune dysregulation and autoreactivity with exposure to toxic chemicals (Fig. 2), in the context of genetic susceptibility, is thought to involve epigenetic mechanisms such as histone modifications, microRNA gene expression, and DNA methylation[109–111]. Chemicals can function as direct ligands for aryl hydrocarbon receptors, which modulate T cell differentiation, the regulatory function of T cells and interactions with antigen-presenting cells, including activation of T_H17 cell responses[112,113]. Other mechanisms involved in chemical-induced autoimmunity include depletion of antioxidant activity, particularly of glutathione, which is an important immune modulator[114–119], and loss of tolerance owing to disruption in the expression of the autoimmune regulator protein, a key transcriptional regulator of thymocytes[120–123]. Chemicals can also change the allosteric structure of self-proteins such as albumin, resulting in the formation of 'neoantigens', with binding of chemicals to these and other self-proteins including DNA, small nuclear ribonucleoproteins and chromatin resulting in a humeral response that generates autoantibodies[124–130]. Chemicals can alter post-translational modifications and promote immunogenicity of self-proteins[131]. Regulatory T cells (T_{reg} cells) are essential for the maintenance of immunological tolerance[132]; many chemicals have been shown to activate PPARγ (peroxisome proliferator-activated receptor-γ) and promote T_{reg} cell dysregulation[133,134]. Loss of tolerance in dendritic cells through chemical-induced alterations to the hydrocarbon receptors on these cells[134–139], as well as chemical-induced permeability of immune barriers in the lungs and gastrointestinal tract, are also key processes involved in chemical-induced autoimmunity[140–146].

Implications for clinical practice

Recognition of the role of occupational exposures in the development of SARDs has implications for the clinical care of affected individuals who could be eligible for compensation and for whom there could be a need to minimize or eliminate ongoing exposure to harmful dusts and chemicals.

Occupational exposure history and methods of exposure assessment

In rheumatology, the occupational history is often focused on the physicality of work and whether those affected by SARDs are still able to function in their role or need modifications to accommodate any physical impairment. We urge physicians managing patients with SARDs to take an occupational history that is also aimed at eliciting occupational exposures that could have had an aetiological role in the development of disease[147]. More broadly, including in a research setting, methods of exposure assessment include self-reporting through interviews or questionnaires, workplace health and safety records, and measuring serum concentrations of relevant substances.

Work in professions that can be associated with exposure to silica or solvents (Box 1), especially among individuals whose demographic profile is atypical for a particular condition, should raise suspicion of occupational aetiology[23,26]; for example, a young man presenting with SSc, which typically affects older women. It must be kept in mind that substantial exposure could have occurred in a previous occupation, which the individual could have held many years ago, and that within each occupation, the level of exposure varies according to the type of work performed. For example, work as a supervisor or bookkeeper in an engineered stone business is probably associated with less exposure to silica dust than work directly involving cutting and installing stone benchtops. Details of ventilation, use of personal protective equipment (PPE), and dry cutting of stone without water suppression (whereby a spray of water suppresses the dust generated as stone is cut) are important to record, as is information regarding compliance with regulatory dust control standards. For example, a malfunctioning ventilatory system will inadequately control dust, and use of PPE is often neglected.

Employers are required to provide employees with documentation from industrial partners and manufacturers regarding potentially hazardous substances in a material safety data sheet, and this data

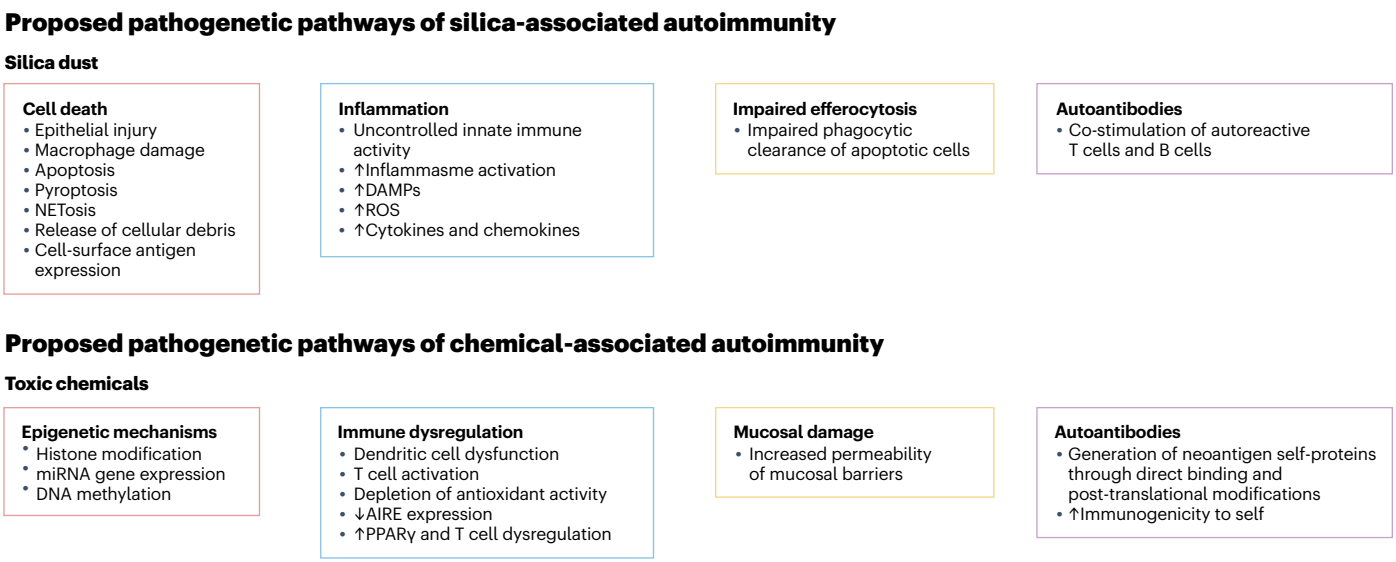

Fig. 2 | Proposed pathogenic pathways involved in silica and chemical exposure-associated autoimmune diseases. Proposed pathogenic mechanisms involved in silica-associated autoimmunity include cell death, inflammation, impaired efferocytosis and autoantibody formation. Proposed pathogenic pathways involved in chemical-associated autoimmunity include epigenetic mechanisms, immune dysregulation, mucosal damage and autoantibody formation. AIRE, autoimmune regulator; DAMPs, damage-associated molecular patterns; miRNA, microRNA; NET, neutrophil extracellular trap; PPARγ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species.

sheet can sometimes be obtained by those taking an occupational history, and yield valuable information. Eliciting a history of occupational exposures has implications for compensation as well as for the health and safety of all those who are working in that environment. We acknowledge the challenge posed by obtaining an occupational exposure history in the confines of a consultation wherein a number of other pressing medical issues need to be addressed. However, a repeat consultation could reveal valuable information, and suspicion regarding an occupational exposure aetiology could prompt referral to an occupational physician, respiratory physician and/or occupational hygienist to elicit a more detailed and nuanced occupational history[147].

Implications for occupational health and safety

The risk of SARDs posed by exposures occurring in the workplace has implications for occupational health and safety monitoring of workers. An understanding of potentially harmful exposures also has implications for workplace policy and legislation.

Reducing and monitoring exposures in the workplace

There is no clearly defined no observed adverse effect concentration (which refers to a concentration below which no harmful effects have been observed) of RCS in humans[148,149]. According to information from Safe Work Australia, chronic exposure to crystalline silica above 0.02 mg/m^3 is associated with radiographic changes in the lungs, although it is not known whether the threshold for autoimmunity in susceptible individuals is less or more than this amount[149,150].

In Australia, for example, a national agreement was reached to reduce the workplace exposure limit of RCS to 0.05 mg/m^3 with pressure to reduce this number even further, whereas in the UK and European Union the workplace exposure limit is 0.1 mg/m^3 and in the USA it is 0.025 mg/m^3 (refs. 150–153). It is therefore recommended by the Australian Institute of Occupational Hygienists that if there is a chance that the exposure level could exceed 0.025 mg/m^3 (50% of the exposure standard), there should be an investigation into the sources of exposure and implementation of suitable control strategies together with health surveillance[154].

Box 3 | Research opportunities in occupational autoimmune disease

Although knowledge regarding the association of occupational exposures and SARDs is expanding through epidemiological and population-based studies, many knowledge gaps remain, presenting opportunities for further research.

- Monitoring the global disease burden to determine the effect of health and safety measures and public policy
- Cohort studies of occupational autoimmune diseases to determine disease phenotypes and outcomes
- Cohort studies of exposed workers to quantify risk, determine dose–response relationships, likelihood and lead time for the development of various autoimmune diseases
- Studies of immunopathogenesis in animal models
- Studies of human samples to gain insights into the pathogenesis of, and genetic susceptibility to, occupational autoimmune diseases

Local exhaust ventilation and water suppression have been shown to reduce the ambient concentration of RCS[155]. Use of PPE such as fit-tested N95 masks, eye protection, face visors and clothing covering exposed areas can also reduce exposure, although handling of contaminated PPE is also an important consideration[156,157].

Health surveillance for high-risk workers exposed to silica dust

Health-surveillance programmes are predominantly focused on screening workers exposed to silica dust for the development of silicosis using chest imaging[107,158]. With the rise in prevalence of SARDs in silica-exposed workers, some of these programmes are incorporating a blood test panel for autoantibodies including rheumatoid factor, anti-cyclic citrullinated peptide antibodies, ANAs and anti-extractable nuclear antigen antibodies along with anti-double-stranded DNA antibodies and ANCAs[107,159]. However, this screening for autoantibodies is generally at the discretion of the physician and the predictive value of autoantibody screening in this context has not been established. Arguably, the detection of some autoantibodies that are more disease-specific, such as anti-Scl-70 antibodies, which have a high specificity for SSc, could be more predictive of future development of clinical disease, making expanded health surveillance for SARDs potentially advantageous for workers within high-risk occupations. In relation to the detection of SSc-specific antibodies in the absence of clinical features of SSc, nail-fold capillaroscopy can have a role in risk stratification in that those who have abnormal nail fold capillaries might have very early stages of SSc and could therefore be at a higher risk of future development of clinical disease features, warranting monitoring and follow-up[160]. However, the optimal management of workers who are exposed to silica dust and are autoantibody positive but are yet to display any clinical features of the disease is not known and the natural history of such cases is a subject for future research.

Policy and legislation

In a landmark decision, Australia became the first country in the world to ban the manufacturing, supply, processing and installation of engineered stone benchtops, panels or slabs, which became effective on 1 July 2024 (ref. 161). This decision was based on pressure brought by activists and unions who had highlighted practical difficulties in enforcing regulations such as the banning of ‘dry cutting’, which had been brought into effect some years earlier, and the rising numbers of workers with silica-associated diseases[19,161]. Worldwide, industries that involve exposure to solvents remain poorly regulated, with no industry standards, monitoring of exposure or health surveillance programmes for workers.

Challenges in quantifying the extent of the problem

In this Review we summarize the literature for SARDs that are commonly encountered in those exposed to silica and solvents, two occupational exposures for which the evidence is most robust. We also touch on several other occupational exposures, including epoxy resins, welding fumes, pesticides, metals and asbestos, for which there is some, albeit inconclusive, evidence that suggests a relationship with SARDs[16,33,36,63,68–70,72,76,147,162–164]. Case reports of other autoimmune diseases such as Sjögren syndrome, myositis, psoriatic arthritis and IgG4-related disease in exposed workers suggest that the spectrum of autoimmune rheumatic diseases associated with occupational exposures could be broader than presently appreciated[165–169]. Overall, the

global burden of SARDs related to occupational exposures remains largely uncharacterized and unquantified.

Unlike silicosis, a lung disease that has characteristic features, SARDs can also occur in the absence of occupational exposures, are generally rare in frequency, are very heterogeneous and are often complex diseases with multi-organ involvement. Moreover, although many of these conditions are associated with the presence of disease-specific antibodies, the diagnosis of SARDs rests on the presence of a constellation of clinical features that can evolve over an extended period of follow-up, with no single diagnostic test at the outset. Therefore, the justification for screening for autoimmune diseases in an occupational setting needs to be carefully considered, especially as it might be more resource-intensive than screening for silicosis.

Although beyond the scope of the present Review, we acknowledge that exposures associated with autoimmune disease might also occur in a household or recreational setting. Identifying and quantifying these exposures is very challenging as minor exposures of this kind are very common and what constitutes a 'significant' exposure amount is unclear. Moreover, recall bias often limits the reliability of self-reported exposures. Using the Dust Exposure Life-Course Questionnaire, crystalline silica dust exposure in 97 women with RA was compared with that in matched healthy individuals[170]. The investigators found that the main sources of silica dust exposure were cleaning activities and laundering of dusty clothes, with higher exposure levels from these sources in women with RA versus the general population[170]. In another study, global, occupational and non-occupational exposure to silica dust was evaluated in a cross-sectional study of French patients with SSc or RA and healthy individuals[171]. The investigators found higher lifetime general and occupational silica dust exposure, but not higher non-occupational silica dust exposure, in both men and women with SSc and RA, compared with the general population[171]. Although the questionnaire used in this study is well validated, the unreliability of self-reported exposures is a key limitation.

Future directions

The identification of occupational exposures that are implicated in the development of SARDs presents unique opportunities to better understand disease pathogenesis and develop and implement strategies to prevent disease. Research opportunities include prospective studies to characterize and quantify disease burden in exposed workers, and research that utilizes animal models and samples obtained from patients (such as skin biopsies and peripheral blood mononuclear cells) to understand pathogenic mechanisms of disease (Box 3). The field of occupational autoimmune rheumatic diseases also offers an opportunity for interdisciplinary collaboration in the delivery of care as well as research, among occupational physicians, hygienists, immunologists, lung specialists, rheumatologists and social scientists. Rheumatologists could have an important role in advocating for workplace health and safety measures to limit harmful exposures. Quantifying the effect of these measures is a key component of their successful implementation.

Conclusions

In this Review we summarize the literature supporting an epidemiological link between occupational exposure to silica dust, solvents and some other dusts and chemicals, and the development of SSc, RA, SLE, SVV and sarcoidosis. We acknowledge that there are likely to be other SARDs that might occur with these exposures, depending on genetic susceptibility and other environmental factors. The early steps in the proposed pathogenesis of silica-associated autoimmune disease are thought to mirror those of silicosis and involve alveolar macrophage phagosomal injury, leading to a cascade of events that result in the formation of autoantibodies and tissue injury. This topic highlights the importance of taking an occupational exposure history in the clinic and presents a unique opportunity for preventative medicine. Legislation is only effective through proper implementation and enforcement of workplace health and safety regulations. Through awareness and advocacy, rheumatologists can have a key role in this process, which entails working with physicians from other specialties such as respiratory and occupational medicine and beyond, such as occupational hygienists, policy makers and legislators, and, importantly, listening to the patient; collectively, these efforts could enable disease detection and prevention.

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Review article

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Antigen-driven T cell responses in rheumatic diseases: insights from T cell receptor repertoire studies

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Abstract

Advances in T cell receptor (TCR) profiling techniques have substantially improved our ability to investigate T cell responses to antigens that are presented on HLA class I and class II molecules and associations between autoimmune T cells and rheumatic diseases. Early-stage studies in axial spondyloarthritis (axSpA) identified disease-associated T cell clonotypes, benefiting from the relative genetic homogeneity of the disease. However, both the genetic and the T cell immunological landscape are more complex in other rheumatic diseases. The diversity or redundancy in the TCR repertoire, epitope spreading over disease duration, genetic heterogeneity of HLA genes or other loci, and the diversity of epitopes contributing to disease pathogenesis and persistent inflammation are all likely to contribute to this complexity. TCR profiling holds promise for identifying key antigenic drivers and phenotypic T cell states that sustain autoimmunity in rheumatic diseases. Here, we review key findings from TCR repertoire studies in axSpA and other chronic inflammatory rheumatic diseases including psoriatic arthritis, rheumatoid arthritis, systemic lupus erythematosus and Sjögren syndrome. We explore how TCR profiling technologies, if applied to better controlled studies focused on early disease stages and genetically homogeneous subsets, can facilitate disease monitoring and the development of therapeutics targeting autoimmune T cells, their cognate antigens, or their underlying biology.

Sections

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Key points

- Next-generation sequencing has enabled development of comprehensive and accurate T cell receptor profiling, allowing studies to search for expanded T cell clonotypes suggestive of shared antigenic drivers.
- Using these approaches, expanded CD8 T cell clonotypes bearing highly similar T cell receptor (TCR) β-chains and α-chains have been identified in a high proportion of patients with ankylosing spondylitis, and this information has been used to identify expanded T cell clonotypes potentially involved in the pathogenesis of the disease.
- TCR repertoire studies in other rheumatic diseases, including psoriatic arthritis, rheumatoid arthritis, systemic lupus erythematosus and Sjögren syndrome, have not found consistently expanded T cell clonotypes, although evidence of T cell expansions including at sites of inflammation have been reported.
- Further studies focusing on early disease and more clinically, genetically and immunologically homogeneous cohorts might provide more informative to identify expanded aetiopathogenic T cell clonotypes.

Introduction

HLAs are encoded by *HLA* genes, which are located within the MHC found on chromosome 6p21.3. Their canonical function is to present antigenic peptides to cells of the adaptive immune system. Conventional T cells recognize HLA-presented antigenic peptides (peptide–HLA complexes) through their T cell receptors (TCRs). The strong genetic association of HLA class I and class II antigens with immune-mediated inflammatory diseases (IMIDs), both rheumatic and non-rheumatic, and the canonical role of these proteins in antigen presentation, provides strong support for a central role of T cells in the pathogenesis of several of these diseases. This central role of T cells in IMID pathogenesis is further supported by multiple genetic associations between the various IMIDs and aspects of T cell activation and function.

Analyses of the TCR structure, and methods that accurately quantify differences in T cell repertoire between individuals, have provided useful insights into T cell dynamics in disease[1]. TCRs are highly diverse heterodimeric proteins of either α-chain and β-chain (in the case of αβ T cells, which are the majority of T cells) or γ-chain and δ-chain (in the case of γδ T cells, which are mainly found at mucosal sites). Each TCR chain consists of a constant domain and a variable domain that contain three hypervariable complementarity-determining regions (CDRs) that determine peptide–HLA specificity. The variable β-chain and γ-chain regions are encoded by variable (V), diversity (D) and joining (J) genes, whereas α-chain and δ-chain are encoded by V and J genes (Fig. 1a). TCR diversity of conventional T cells is by necessity massive in order to cover the huge variety of peptide antigens the immune system could potentially be exposed to. In comparison, other T cell populations present a reduced TCR diversity consistent with their response to antigens of constrained diversity (Box 1).

TCR diversity is generated through three mechanisms: somatic recombination of VDJ gene fragments[2]; nucleotide insertions or deletions at the junction sites between VDJ fragments (creating 'junctional diversity')[3]; and pairing of different α-chains with β-chains, or γ-chains with δ-chains[4]. CDR1 and CDR2 are entirely encoded in germline DNA, determined by V gene usage. CDR3α and CDR3β loops are also affected by junctional diversity and are therefore the most variable regions of the TCRs. Consequently, each T cell and its descendants (all constituting a clonotype) express a unique receptor with a unique spectrum of peptide–HLA affinities[5].

An initial TCR diversity of approximately 10^{11} different clonotypes is subjected to positive or negative selection at the thymus and through peripheral antigen encounter of the naive repertoire. Positive selection for affinity to peptide–HLA molecules and commitment to $CD4^+$ or $CD8^+$ T cell lineage ensures recognition of self-HLA repertoire. Negative selection for self-peptide recognition ('central tolerance') arises where a T cell reacts strongly to an individual presented peptide (reflecting 'affinity')[6] (Fig. 1b). This ensures both reactivity and self-tolerance[7]. Tolerance might also occur in the periphery through processes involving regulatory T cells, anergy and apoptotic deletion of self-reactive T cells, and through antigen-presenting cell (APC) modulation of responses towards tolerance.

When a TCR engages a novel peptide–HLA complex on the surface of APCs, TCR activation may occur (signal 1). A T cell may also be exposed to a range of peptides presented by APCs, integrating the strength of the cumulative interaction ('avidity'). TCR activation, together with engagement of co-stimulatory and co-inhibitory receptors and exposure to cytokines (signals 2 and 3, respectively), determines the result of this process (Fig. 1c). The proliferation of a particular T cell is termed clonal expansion, and the resulting abundance of such 'clonotypes', can be measured in repertoire studies through characterization of its TCR DNA rearrangement, a unique molecular signature. In instances of strong selective pressure to common antigens, such as in response to infection or disease, similar clonotypes arise among different individuals, termed public clonotypes[8]. Because the majority of TCRs are rare[7], sharing of expanded clonotypes between multiple individuals with a disease strongly supports a common antigenic drive[4,9]. Thus, resolving these interactions is essential to understanding the autoimmune contribution to rheumatic diseases.

Recent advances in bulk and single-cell TCR profiling have enabled the identification of clonal T cell expansions and have provided further support for the involvement of T cells in IMIDs. This opens opportunities to determine how HLA antigens are involved in IMID pathogenesis, identify self-antigens and environmental triggers, and potentially develop new biomarkers and therapeutic options.

In this Review, we describe TCR profiling methods, and their findings in various rheumatic IMIDs including axial spondyloarthritis (axSpA), psoriatic arthritis (PsA), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and Sjögren syndrome. Moreover, we examine how these approaches have in some cases enabled identification of TCR–peptide–HLA associations that are involved in the mechanism by which HLA drives disease pathogenesis, and discuss learnings from these studies to guide future studies and the potential use of their findings to underpin future research into disease pathogenesis and therapy.

TCR repertoire profiling

Numerous methods have been developed over the past decades[10] to evaluate repertoire diversity and biased VDJ usage, identify public clones, and track and characterize T cell clonotypes (Fig. 2). Initial low-resolution approaches evaluated TCR diversity using V-specific detection and were thus limited in their definition of the hypervariable

CDR3 sequences (Fig. 2a). An indirect approach to the analysis of TCR specificities was based on the detection of lymphocytes responding to antigen-specific stimulation, starting from peripheral blood mononuclear cells (PBMCs) or expanded T cell lines. Neither of these approaches is comprehensive in coverage, nor is clonotype quantification very precise. The development of next-generation sequencing (NGS) methods has enabled comprehensive repertoire profiling over the last decade, either by bulk sequencing of pooled immune cell populations or by single-cell approaches (Fig. 2b,c). The resolution of disease-associated TCR–peptide–HLA interactions requires further validation by antigen or TCR screening studies, as also described below.

Population-based bulk NGS

Bulk methods are used for investigation of repertoire diversity in large cohorts. These methods mostly focus on the TCRβ CDR3, which has a higher combinatorial potential than TCRα, and a key role in determining antigen specificity[11]. Multiplex PCR, target enrichment, and 5′-RACE (rapid amplification of cDNA ends) cDNA synthesis and nested PCR are the three predominant methods for TCR sequence library construction[1]. Primary analysis involves clustering of identical TCR sequences, VDJ annotation and quantification of clonotype abundance[11]. TCR sequences can also be extrapolated from untargeted transcriptome sequencing data[12], but such approaches can only detect highly prevalent clonotypes owing to transcriptome sequencing depth constraints.

Secondary TCR repertoire analysis calculates descriptive statistical indices of diversity and homology based on clonotype abundance[13–15]. Diversity depends on the number of unique TCR sequences and their relative abundance[16]. Homology among TCR repertoires of individuals with a disease reflects shared fingerprints of adaptive immune responses[17], suggesting exposure to specific disease-associated antigens. Computational approaches to resolve antigen specificity are in development, evaluating patterns in TCR sequence[18], structure[19] and physicochemical properties[20]. However, predicting the TCR epitome (the range of epitopes that a TCR might be able to recognize) remains a major computational challenge and requires additional experimental input[21].

Single-cell transcriptomics

Single-cell RNA sequencing (scRNA-seq) is used to both obtain paired TCRαβ sequences[22] and assess biological heterogeneity through transcriptional phenotyping. Further developments now allow epigenetic profiling and surface marker phenotyping[23]. Among the various methodologies[24], water-in-oil emulsion-based approaches stand out for their high throughputs (Fig. 2c). The biggest limitations of these approaches are the high cost, the limited numbers of cells that can be analysed, which makes studies of rare cell types challenging, and the requirement for fresh material for the isolation of live cells, with the subsequent loss of tissue context.

In rheumatic diseases, the results of TCR repertoire studies have so far been mostly negative or inconclusive, with the exception of axSpA. To capture autoimmune clonotypes, future studies should strongly consider the advantages and limitations of all TCR profiling techniques, as detailed in Fig. 2. These considerations are required to improve success of current methodologies in the dissection of the pathogenesis of IMIDs, as discussed below.

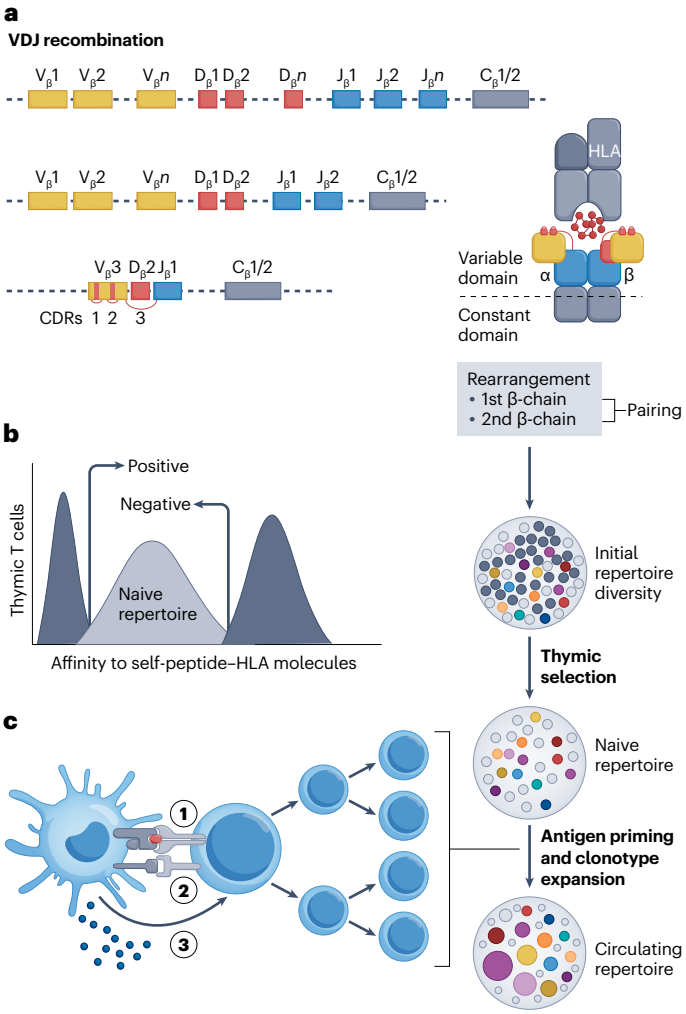


Fig. 1 | Generation of a diverse T cell receptor repertoire. a, Generation of initial T cell receptor (TCR) diversity by recombination rearrangement of genomic segments. TCR β-chains are formed by the bringing together or 'recombination' of individual V, D, J and C segments, and TCR α-chains from V, J and C segments. The two chains are paired to create a TCRαβ, which recognizes peptides presented by HLA molecules (peptide–HLA complexes) on the surface of antigen-presenting cells (APCs). **b**, In the thymus, T cells are exposed to self-antigen-derived peptide–HLA complexes. T cells bearing strongly reactive TCRs are deleted via the mechanisms of negative selection, to reduce the chance of autoimmune disease. T cells bearing moderately reactive TCRs receive survival signals and are positively selected to generate the naive repertoire. **c**, Naive T cells can mount adaptive immune responses upon priming by APCs, which involves antigen presentation and TCR activation (first signal), co-stimulation/co-inhibitory signal balance (second signal) and cytokine stimulation (third signal). T cell activation via these three signals results in the expansion of T cells that carry the same TCR DNA rearrangement (clonotypes) and reduces diversity of the circulating repertoire. CDRs, complementarity-determining regions.

TCR repertoire findings in axSpA

The axial spondyloarthropathies are a diverse group of immune-mediated arthropathies including reactive arthritis (ReA), non-radiographic axSpA and ankylosing spondylitis (AS; sometimes referred to as radiographic axSpA). These conditions share clinical features such as involvement of the spine and sacroiliac joints, pathological characteristics such as prominent enthesitis, and genetic associations particularly with HLA-B27. The collective term spondyloarthritis (SpA)

Box 1 | Diversity, reactivity and role of T cell populations in rheumatic conditions

Conventional αβ T cell receptors (TCRs) show the highest TCR diversity, matching a wider range of peptide antigens presented on MHC class I ($CD8^+$ T cells) and class II molecules ($CD4^+$ T cells). Various subtypes have been defined according to location and functional differentiation. Response to secondary infections is provided by proliferation and effector functions, which is the focus of central memory T (T_{CM}) and effector memory T (T_{EM}) cells, respectively. Both subtypes are found in lymphoid organs (T_{CM} cells) or tissues (T_{EM} cells) in addition to the blood. By contrast, tissue-resident memory cells remain in tissues for adaptive memory protection upon initial response. T cells can be further divided by function, according to involvement in coordination of type 1, 2 or 3 responses (such as the T helper 1 (T_H1), T_H2 or T_H17 subsets of $CD4^+$ T cells). $CD4^+$ T cells also support B cell maturation: T follicular helper cells are present in lymphoid follicles of spleen and tonsils, whereas T peripheral helper cells remain in circulation and tissue, mediating ectopic lymphoid formation under inflammatory conditions[223].

γδ T cells present an intermediate TCR diversity and are able to recognize a variety of antigens on butyrophilins or antigens that are not restricted to MHC presentation, including surface and stress-related proteins and phosphoantigens[224].

Mucosal-associated invariant T (MAIT) cells are abundant at mucosal surfaces. Comparatively, their TCR diversity is small, as is the range of antigenic molecules known to activate them: vitamin B_2 (riboflavin) and derivatives presented by MR1 molecules. These cells are found particularly in the gut and react to precursors of vitamin B_2 presented by the MR1 molecule rather than by HLA alleles[225]. MAIT cells produce ankylosing spondylitis (AS)-associated cytokines including tumour necrosis factor and IL-17 and have been implicated in AS[226].

Invariant natural killer T (iNKT) cells express a semi-invariant TCR with constrained TCR diversity. They respond to specific glycolipid antigens presented by CD1 molecules via the rapid production of interferon-γ and IL-4 (ref. 107).

is used to also include patients for whom the clinical features are primarily peripheral joint involvement. Over 116 non-MHC genetic loci have been robustly associated with AS, and the HLA associations of the disease are also complex, including not only HLA-B27 but multiple other alleles, the most reproduced of which is HLA-B40 (refs. 25,26). In addition to specific HLA haplotypes, other genes that are associated with antigenic peptide processing, such as the gene encoding endoplasmic reticulum aminopeptidase 1 (*ERAP1*) have been associated with a genetic risk of AS. ERAP1 is expressed in the endoplasmic reticulum and functions as a 'molecular ruler', trimming peptides down to nine amino acids in length, the optimal length for HLA class I presentation[27]. These observations support the 'arthritogenic peptide hypothesis', which proposes that HLA-B27 induces AS by binding and presenting bacterial and cross-reactive self-peptides to $CD8^+$ T cells[25], possibly via a mechanism that involves peptide processing and handling[28,29]. Alternative mechanisms such as those involving unfolded protein responses and consequent endoplasmic reticulum stress have been proposed to explain the mechanism underpinning HLA-B27-associated disease[30], though as yet these have not extended to AS associated with HLA-B40 or other HLA variants[29].

Cytotoxic T lymphocyte (CTL) reactivity to self-antigens and bacterial antigens was initially observed in patients with ReA[31,32] (Supplementary Table 1). Expanded numbers of $V_\beta1$ T cells were demonstrated in synovial fluid of patients with ReA, but not in healthy individuals[33]. $V_\beta1$ is encoded by *TRBV9*. Expanded clonotypes shared homologous CDR3 sequences (CASSV/PGLYSTDTQ) across three patients with ReA[33,32]. Subsequently, analysis of the size diversity of the CDR3 region (spectratyping) and of the *TRBV* repertoire of HLA-B27$^+$ carriers who were healthy, or had ReA, AS or RA, identified the canonical CDR3 motif TRBV9-CASSVG(V/I/L)(Y/F)STDTQYF-J2S3 among HLA-B27$^+$ carriers with SpA, but not among healthy HLA-B27$^+$ individuals[34]. This is suggestive that the HLA-B27$^+$ patients with SpA had been exposed to a shared disease-associated antigen.

The analysis of circulating T cells in 234 patients with AS (of whom 192 carried the HLA-B27 variant) and 227 healthy individuals (including 10 HLA-B27$^+$ individuals) by NGS confirmed the prevalence of 6 previously identified TCR clonotypes as well as of additional new TCR motifs[35] among $CD8^+$ T cells from patients with AS. 5′-RACE libraries from the blood and synovial fluid of an additional 24 HLA-B27$^+$ patients with AS and the blood of 107 healthy individuals (including 7 HLA-B27$^+$ individuals)[36] further confirmed the expansion of TRBV9-J2S3 clones, with a total of 8 CDR3 motifs, 7 of which belonged to $CD8^+$ T cells, having expanded in HLA-B27$^+$ patients with AS. These clones were enriched in the inflamed synovial fluid compared with paired peripheral blood samples in five patients with AS. Enrichment of specific TCR motifs was also observed by multiplex PCR and RNA-seq of $CD4^+$, $CD8^+$ and double-positive $CD4^+CD8^+$ T cells in the synovial fluid of patients with axSpA of east Asian ancestry[37], who probably carried different HLA-B27 subtypes from those of patients of European ancestry included in previous studies. Whether the same clonal expansions are also present in individuals with HLA-B27 subtypes that have not yet been associated with axSpA, such as *HLA-B*2706* and *HLA-B*2709*, is unclear but the low frequency of these clonotypes in healthy *HLA-B*27* carriers of European ancestry makes this rather unlikely. More studies looking expressly at the presence of AS-associated clonotypes in relation to different *HLA-B*27* subtypes would be of value to help further establish the potential central role of these clonotypes in AS pathogenesis.

Bulk TCR repertoire profiling of sorted $CD8^+$ and $CD4^+$ PBMCs from 47 patients with AS (including 37 HLA-B27$^+$ individuals) and 38 healthy individuals (including 20 HLA-B27$^+$ individuals) further confirmed that TCR clonotypes are specific to AS rather than to the HLA-B27 haplotype[38]. Among $CD8^+$ T cells, 10 CDR3 sequences were shown to be strongly associated with AS and were found in all 37 HLA-B27$^+$ patients with AS, but in only 4 of 19 HLA-B27$^+$ healthy individuals ($P = 2.6 \times 10^{-6}$). In some patients different *TRBV-J* gene pairings generated the same CDR3 sequences, pointing to common antigenic recognition. *TRBV9* usage was far from universal amongst AS-associated $CD8^+$ T cell clonotypes, and some clonal expansions were completely restricted to AS. For example, the CASSVGLFSTDTQYF motif was found in $CD8^+$ T cells from 12 of the 37 patients with AS but in none of the 38 healthy controls ($P = 1.6 \times 10^{-5}$).

TRA and *TRB* sequencing in matched blood and synovial fluid samples from patients with AS and PsA[39] associated HLA risk alleles with nine clonotype clusters. These clusters included the TRBV9-J2S3

motif, as well as three new TCRβ motifs that were found to be enriched in the synovial fluid of HLA-B27$^+$ patients and five TCRβ motifs that were found to be enriched in the synovial fluid from HLA-B38$^+$ patients. The axSpA-associated TRBV9-J2S3 motif was enriched in inflamed joints and present among activated and tissue resident memory T (T_{RM}) cells of the synovial fluid, further supporting the involvement of these clonotypes in local inflammation. Analysis of TRA chains identified only one cluster shared among the patients with AS and patients with PsA, which featured the invariant TRAV1-2/TRAJ33 rearrangement. This rearrangement is indicative of the presence of mucosal-associated invariant T (MAIT) cells (Box 1), and these findings potentially provide a link between gut and systemic inflammation in AS[40].

The search for autoimmune TRBV9 clonotypes has further progressed through the use of scRNA-seq for the paired analysis of TCR α-chains and TCR β-chains in sorted TRBV9$^+$ T cells isolated from peripheral blood of HLA-B27$^+$ patients with AS, and in some cases from ocular fluid of patients with AS complicated by acute anterior uveitis[41]. The TRBV9-J2S3 chain paired exclusively with TRAV21, confirming the findings of a previous study in SpA[42]. The full-length characterization of TRBV9–TRAV21 clonotypes allowed subsequent elucidation of endogenous and bacterial cross-reactive epitopes potentially involved in HLA-B27$^+$ SpA[41]. A follow-up study leveraged reactivity against one such bacterial peptide (YeiH) to identify further YeiH-reactive TCR sequences. These T cell clones were not restricted to TRBV9 usage and carried phenotypic markers that were suggestive of a mucosal origin[43].

Overall, these findings provide strong evidence of clonotype expansion amongst CD8$^+$ T cells in axSpA in line with the arthritogenic peptide model. However, they also indicate that repertoire alterations are broader than a single TRBV9 motif on CD8$^+$ T cells[38,39]. Further studies are required, particularly focusing on HLA-B27$^-$ individuals, who make up 15–20% of all patients with AS, to investigate the evolution of clonotypic expansions as disease develops including in the preclinical phase, and in relation to disease activity and treatment. A key clinical challenge is to distinguish patients with non-radiographic axSpA who have a true AS-related immune-mediated pathology from those who have non-inflammatory chronic pain-associated conditions[44,45]. While AS is preceded by a non-radiographic phase, only a minority of patients with non-radiographic axSpA go on to develop AS, either because they do not have an immune-mediated axSpA or because their disease goes into remission[46]. The presence of specific clonotypes in a high proportion of patients with a HLA-B27 genotype suggest that some TCR motifs might be useful biomarkers to distinguish between patients with non-radiographic axSpA who have a true immune-mediated pathology and are at risk of going on to develop AS, and patients with non-inflammatory conditions. Enrichment of the clonotypic expansions at the site of inflammation is consistent with their reacting to local antigens or recruitment molecules[43]. Further definition of the peptides that drive clonal T cell expansions and identification of the origins of these peptides, as well as characterization of the function and impact of antigenic peptides in local inflammation, will be of great value in resolving aetiological questions and to design novel targeted therapeutics.

TCR profiles in psoriatic arthritis

Antigen-driven disease

Genetic predisposition to psoriasis is primarily linked with HLA class I genes[47,48], which also influence clinical presentation of entheseal disease (*HLA-B*27:05:02*), PsA (*HLA-B*08:01:01*, *HLA-C*07:01:01*) or skin phenotype (*HLA-B*57:01* and *HLA-C*06:02*). Although the mechanism by which these genes influence the risk of PsA is not yet established, the restriction of the association with the antigen processing gene *ERAP1* to *HLA-C*06:02*-positive psoriasis strongly suggests that they operate to cause disease by a mechanism involving peptide processing and presentation[49], most likely to CD8$^+$ T cells.

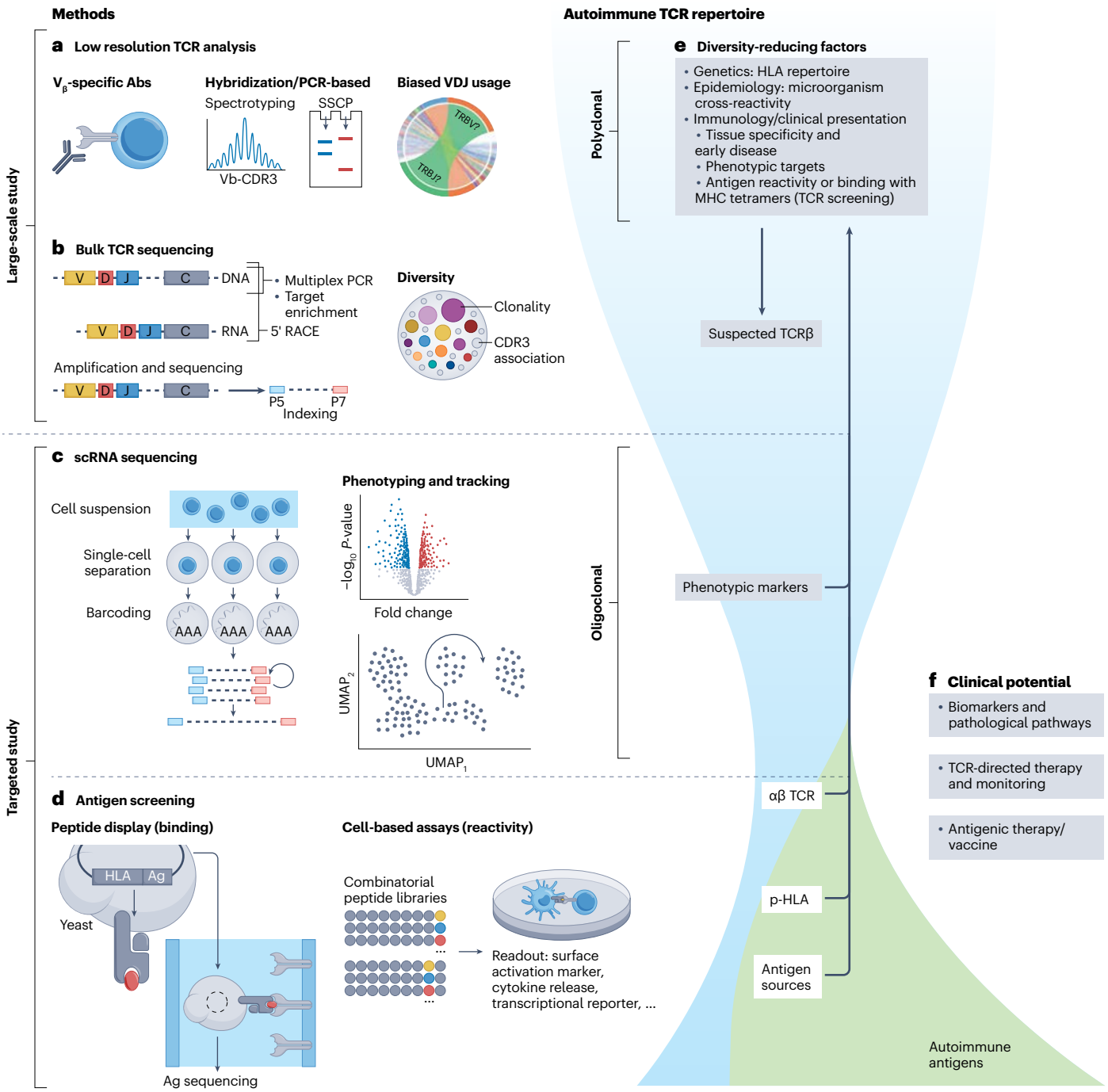
There is strong genetic and immunological evidence for the involvement of CD4$^+$ T helper 1 (T_H1), T_H17 and IL-17-secreting CD8$^+$ cytotoxic T (T_C17) cells in PsA pathogenesis[48]. In one study, activated memory CD8$^+$ T cells were found to constitute the majority of lymphocytes infiltrating the PsA synovium[50], and these cells were shown to display increased clonality compared with circulating CD8$^+$ T cells[51] (Supplementary Table 1). T cells from the synovial fluid and skin lesions of patients with PsA were reported to share TCRβ sequences[52,53], indicative of shared antigen-specificity across tissue types. However, the low power and resolution of the techniques available at the time that these studies were performed hindered the identification of clonotype associations. It was not until later that AS-associated TRBV9 clones were also found in the synovial fluid and peripheral blood of HLA-B27$^+$ patients with PsA[39]. HLA-B38-driven clonotype associations were also reported, and this is of relevance given the reported association of this HLA class I allele with PsA[39]. Most of the clonotype associations involved CD8$^+$ T cells, indicative of interactions with HLA class I antigens. This contrasts with the predominance of CD4$^+$ T cell expansions seen in RA (see below), a disease with primarily HLA class II associations. However, both CD8$^+$ and CD4$^+$ TCR repertoires in the synovial fluid of patients with PsA showed lower diversity than TCR repertoires in the blood of the same patients[39], raising the possibility that HLA class II antigen presentation is also involved is PsA pathogenesis.

Regarding antigenicity, studies in psoriasis found T cell cross-reactivity to streptococcal M peptides and human epidermal keratins[54] and over-representation of TRBV genes involved in recognition of streptococcal superantigens, supporting the hypothesis of streptococcal cross-reactivity within psoriatic conditions. Virus-reactive TCR sequences have also been detected in synovial fluid of patients with PsA[55], but no mechanistic association has so far been established.

Phenotypic characterization of PsA-associated clonotypes

Lymphocytes infiltrating the inflamed skin lesions and joint synovium in PsA express the collagen receptor VLA1 (ref. 56). Expression of CD49a, the α1 integrin of VLA1, was found to differentiate two oligoclonal CD8$^+$ T_{RM} cell populations in healthy human skin, with CD49a$^+$CD8$^+$ T_{RM} cells being cytotoxic and CD49a$^-$CD8$^+$ T_{RM} cells producing IL-17 (ref. 57). The CD49a$^-$ inflammatory T_{RM} cell population promoted local inflammation in psoriatic skin[57]. This suggests that the specificity of tissue inflammation was not determined by recruitment of these cells driven by expression of the CD49a adhesion molecule, but probably other mechanisms such as their local priming due to local antigen presentation or chemokine-mediated retention.

Similar pro-inflammatory CD8$^+$ T_{RM} cell populations have been identified by scRNA-seq analyses of the synovium of patients with PsA or other rheumatic conditions[58–60], where they appear to drive flares and chronic joint inflammation. These synovial pro-inflammatory CD8$^+$ T_{RM} cell populations show signs of clonal expansion, biased V gene usage, and clonal convergence[60], indicative of local antigenic exposure, as well as phenotypes overlapping with those of CD49a$^-$ epidermal skin CD8 T_{RM} cells[57,59]. Their phenotypic features, such as IL-17 production and HLA-DR$^+$ expression, and specific patterns of tissue-homing chemokine receptors, such as CXCR6 (ref. 57), CXCR3 (ref. 59) or CCR6



(ref. 60) (Supplementary Table 1), might explain their tissue location and contribute to the spectrum of PsA clinical presentations.

In summary, apart from the finding that the AS-associated TRBV9 clonotypes are also expanded in patients with PsA, other shared TCR clones have not been consistently reported in this condition. PsA is clinically and genetically heterogeneous with psoriatic axSpA and psoriatic peripheral arthritis not only being clinically distinct but also having different genetic associations[61]. Studies to date in this disease have generally not controlled for these important and relevant differences, and have generally involved late-phase established disease, when immunological dysfunction is likely to be more diverse and complex. Narrowing down the study cohort and investigating phenotypic signatures is likely to improve reproducibility.

TCR repertoire findings in RA

The presence of anti-citrullinated protein antibodies (ACPA) and rheumatoid factor preceding disease onset, as well as the efficacy of B cell targeting therapies support a major role for humoral autoimmunity in RA[62,63]. However, T cell immunity has also been shown to contribute to RA pathology. TCR profiling studies in patients with RA have

Fig. 2 | TCR profiling methodologies and strategic study flow design to address the vast complexity of autoimmune TCR and antigenic repertoires in order to reach findings of clinical potential. a, Early low-resolution T cell receptor (TCR) profiling methods studied V-J usage using either specific antibodies (by flow cytometry), or probes for hybridization. The latter include PCR amplification and spectratyping measurement of CDR3 length and detection of single-strand conformation polymorphisms (SSCP). **b**, Bulk sequencing uses either DNA or RNA as starting material and specific primers for library preparation, generally based on a single TCR β-chain. When applied to pooled immune populations, bulk sequencing allows comprehensive diversity assessment. In properly controlled large-scale studies, it is empowered to address complex polyclonal and rare autoimmune associations. **c**, Single-cell sequencing methods apply a process of barcoding to transcripts derived from each cell during the library construction before bulk library sequencing, allowing subsequent single-cell annotation. This enables paired TCRαβ definition and clonotype phenotyping and evolutive tracking. Single-cell approaches are best suited to studies implementing targeted strategies to enrich and/or focus on a particular autoimmune population, as the number of cells profiled using most current methods is typically lower than in bulk sequencing approaches, and to optimize the use of precious tissue samples. **d**, The resolution of disease-associated TCR–peptide–HLA interactions requires further validation such as by antigen screening methods based on high-throughput peptide display assays that measure antigen binding by T cells or in vitro T cell reactivity assays. **e**, The detection of shared oligoclonal responses among otherwise polyclonal autoimmune profiles of rheumatic diseases requires the consideration of several factors during study design. Both clinical variables and insights from previous TCR repertoire studies can be considered in a continuous feedback loop, to break down autoimmune diversity. An opposite approach, termed TCR screening, is also possible, starting with the identification of autoimmune antigenic ligands (peptide–HLA complexes) for a defined TCRαβ, followed by validation of the identified TCR reactivity in the general patient population. TCR screening approaches often use peptide–HLA tetramer technology. **f**, Insights from TCR profiling studies can enable biomarker discovery for autoimmune phenotypic markers, TCRs or antigens, and development of therapeutics that, for example, target autoimmune clonotypes or induce tolerance through vaccination. Ab, antibody; Ag, antigen; scRNA, single-cell RNA.

demonstrated evidence of antigenic selection and clonal expansion among synovial CD4$^+$ T cells[64–66], and these findings are consistent with the strong HLA class II association of the disease[67]. Compared with circulating CD4$^+$ T cells, synovial CD4$^+$ T cells exhibit increased clonotype expansion[64,68–73] and biased V gene usage[64,69,74–83] (Supplementary Table 2). Unlike the situation in axSpA though, these studies did not reach concordant conclusions regarding any RA-specific clonotypes.

Patients with RA have reduced TCR diversity, both in peripheral blood and inflamed synovial tissue[84,85], particularly among CD4$^+$ T cells[76,86–90]. Analysis of peripheral blood samples from 206 patients with RA, 877 patients with SLE and 439 healthy individuals, associated 53 TCRs with RA and 198 TCRs with SLE, including clones shared between the two diseases[88]. Shared clones were also found in healthy individuals, but had higher frequencies in patients with RA and SLE, thus representing a shared autoimmune TCR signature. Earlier studies in RA using the low-resolution methods of flow cytometry and CDR3 spectratyping showed that abatacept treatment can partially normalize disease-associated reductions in CD4$^+$ TCR diversity[91]. Repertoire correlations with disease activity or treatment resistance have also been found by NGS analysis[86,88,92–94]. Although no specific clonotypes have been identified as being expanded in RA so far, the reported reduction in TCR repertoire diversity is consistent with the presence of disease-specific clonotypes, and supports further studies aiming to identify them.

Tissue specificity and longitudinal studies

Studies tracking TCRs across tissues[72], over time[95] and within specific T cell subsets[96] have facilitated the search for phenotypic signatures, though without producing robustly replicated, definitive, findings to date. T_H17 cell clonal expansions contributing to reduced TCR diversity in RA have been reported[93]. The frequency of expanded T_H17 cell clones correlated with the levels of ACPA and IgA[93], in line with the identification of citrullinated antigen-specific T_H17 cells in RA[97]. Greater oligoclonality and more shared clonotypes were observed in inflamed synovial tissue than in peripheral blood[76,85,88,93,98,99]. Repertoire sharing has been shown to be high across joints of the same patients[64,87], reinforcing the idea of tissue specificity, as well as the representativeness of single-site biopsies. In a study of T cells from patients with PsA or RA, clonal expansion was more evident in inflamed synovial tissues than in synovial fluid or peripheral blood. The most expanded clones were also those that showed the greatest enrichment in inflamed joints[100]. This homing of expanded T cell clonotypes to inflamed tissues suggests that these clonotypes react to and expand locally, or are drawn to or by, inflammation-associated factors in these tissues.

Regarding repertoire dynamics, substantial persistence of expanded clonotypes has been observed over time[68,69]. Profiling studies have demonstrated that in synovium from patients with recent-onset RA, a small number of highly expanded T cell clones dominate, whereas a more diverse range of expansions have been found in established RA[72,101]. Thus, TCR repertoire studies using samples from patients with early disease might be more successful at pinpointing true autoimmune drivers than studies using samples from patients with established disease, who are likely to have TCR repertoires enriched in autoimmune TCRs resulting from epitope-spreading effects.

T cell subsets

scRNA-seq is a powerful method to identify phenotypic markers, although the number of cells that can be studied per experiment currently is far lower than typically studied in bulk RNA-seq studies. In addition to CD4$^+$ T helper cells, other immune cell populations have been shown to be involved in RA by scRNA-seq studies using paired synovial tissue and peripheral blood samples[102]. The highest clonal expansion was observed among synovial CD8$^+$ T cells, which, based on their transcriptome, appear to contribute to synovial inflammation through inflammatory cytokine production rather than cytotoxicity. Expansion of CD8$^+$ T cells in RA has been previously described[103–106], and shows similar or even higher clonality than the CD4$^+$ T cell population. MAIT and γδ T cells have also been identified in the synovial compartment[102], with signs of activation and selective enrichment for $V_\delta1$ γδ T cells[102]. Reduced natural killer T (NKT) cell frequency in RA and other autoimmune conditions has also been described, and their function correlates with differential TCR affinity for the CD1d[107] (Box 1). The invariant NKT (iNKT) TCR repertoire displays a shift in early RA, with loss of high-affinity iNKT clones, reduced proliferative capacity, and a skewed cytokine response[108]. Steroids and antirheumatic drug treatment increases the proportion of T_H2-like iNKT cells while reducing the numbers of T_H1-like iNKTs[108]. This correlation with disease activity aligns with prior observations[109–111] and is consistent with a regulatory

and protective role of T_H2-like iNKT cells bearing high-affinity TCRs, which may be lost upon RA development.

TCR repertoire studies have provided considerable evidence for a role for T peripheral helper (T_{PH}) cells as leading autoimmune drivers in RA. T_{PH} cells are one of the most clonally expanded populations in RA, clonally related to T follicular helper cells[102,112]. Circulating T_{PH} cells show clonal overlap with both tissue-resident and proliferating synovial T_{PH} cells. A common pathway was identified from which effector $CD4^+$ T cells can differentiate from either of these two synovial T_{PH} cell subsets[96]. T_{PH} cells have been shown to display among the strongest activation signatures and signs of local expansion in RA[113], and are endowed with inflammatory T_H1-like or T_H17-like phenotypes and cytotoxic properties[102] in addition to their classic B cell helper function. Pathologically expanded synovial T_{PH} cells drive B cell responses in seropositive RA[112]. This confirms at least one mechanism for autoimmune T cell–B cell crosstalk in RA, which is likely to drive the generation of tertiary lymphoid structures within sites of autoreactivity[114]. Whether T_{PH} cell antigenicity is causally involved in RA is still unclear, however.

Some hypotheses locate the onset of RA autoimmunity outside the joints, with evidence of reactivity to post-translationally modified autoantigens in mucosal organs, such as the lung, periodontium or gut, years before the first signs of joint autoimmunity and inflammation[115]. As in other autoimmune conditions, cross-reactivity with infectious microorganisms has been proposed. Pathogen-reactive T cells were found to be enriched in the circulation[88] and synovium[102,116,117] of patients with RA, but devoid of signs of expansion or activation that would suggest an active role in disease pathogenesis. At this point, therefore, T cell profiling study findings do not provide robust support for RA autoimmunity being driven by cross-reactivity with microorganisms, though they do not rule out a role for this mechanism, particularly in disease induction. Overall, the diversity of TCR profiling findings in RA is likely to reflect the diversity of study designs used, as well as the complexity and dynamic nature of histocompatibility and immunological disturbance in the disease. Targeted study designs, discussed below, will be required to properly address these challenges.

TCR repertoire studies in SLE

Autoantigens from the cell nucleus are key drivers of dysregulated lymphocyte activation in SLE, characterized by an interferon-stimulated gene (ISG) signature[118], autoantibody production and subsequent tissue damage in joints, skin, kidneys and nervous system. Besides the primary role of B cells, T cells also infiltrate affected SLE tissues and mediate autoantibody production[119–121], presenting shared TCR sequences[120]. Therefore, TCR repertoire profiling has been widely used in SLE research.

Diverse low-throughput techniques have provided preliminary evidence of an altered TCR repertoire in SLE[70,120–128] (Supplementary Table 3). Subsequent use of NGS found decreased TCR diversity in the circulation of patients with SLE, with biased VDJ usage[88,122,126,129–136], suggesting a potential autoimmune role for dominant TCRs. Consistent with such a role, SLE-associated clonotypes have been found to be enriched in affected tissues, such as the skin[121] and kidney[125,126,137,138]. Laser capture microdissection and TCRβ sequencing in kidney biopsies demonstrated an oligoclonal TCR repertoire in lupus nephritis. In addition, the observation of clonal expansions among $CD4^+$ T cells is consistent with the hypothesis that SLE is a HLA class II-driven autoimmune disease. Such expansions were also observed in periglobular $CD8^+$ T cells, and this feature was shown to correlate with the progression of tubulitis[126].

Repertoire correlations with disease activity or treatment response were studied comparing inactive versus active SLE[88,122,123,128,131,135,139]. Skewed V-J usage and a set of disease-associated clonotypes were better correlated with clinical and immunological measures of disease activity than overall TCR diversity measures[88]. SLE-associated clonotypes showed shorter CDR3 sequences than clonotypes found in patients with RA, and this has been suggested to favour autoimmune TCR interactions[88]. Various studies have also highlighted recurrent motifs within associated CDR3 sequences, and in peripheral blood[122], kidney[125] or skin[121], with frequent involvement of polar residues. These CDR3 motifs are involved in recognition of charged autoantigens, such as nucleosomes, potentially representing T_H cells that promote the formation of anti-double-stranded DNA antibodies[120,140]. Subsets of $CD8^+$ T cells expressing granzymes have been identified in patients with SLE, with TCR profiling demonstrating clonal expansion particularly in these subsets, suggesting a pathogenic role[141]. This is in line with observations in renal tissues[126] and activation of cytotoxic $CD8^+$ T cells are generated by dendritic cells cultured in the presence of patient serum[142], suggestive of a pathogenic role for CD8 T cells in SLE.

As with each of the diseases discussed here, the lymphocyte population that predominantly drives autoimmunity in SLE is not clear, or indeed if, as seems likely, multiple lymphocyte subsets are involved. In addition to the data indicating roles for $CD4^+$ and $CD8^+$ T cells discussed above, there is evidence for involvement of iNKT and γδ T cells. Patients with SLE show suboptimal iNKT function that correlates with defective CD1d antigen presentation[110,143]. However, the iNKT TCR repertoire or CD1d binding affinity have not been assessed. Regarding γδ T cells, recruitment and activation of TRDV2-positive lymphocytes have been implicated in SLE[144,145], in line with the T_H1 cell-specific and ISG-specific signature of the disease, and contrasting with the predominance of TRDV1-positive lymphocytes in SpA and RA. T cell clonotypes known to target influenza, Epstein–Barr virus and tuberculosis were found to be enriched in patients with SLE. In addition, some clonotypes that are enriched in SLE have also been reported to be expanded in multiple sclerosis, allergy and cancer[88], which altogether seems to indicate a state of global immune dysregulation.

Overall, these studies further confirm the diversity of autoimmune alterations in SLE, which is likely to involve polyclonal T cell populations. As yet, the hypothesis that defined clonally expanded T cells have a major pathogenic role in the disease has not been definitively confirmed. Studies controlling for genetic and clinical diversity, polyclonal response to common antigens and focusing on specific T cell subsets are required to confirm the existence and potential role of T cell autoimmunity in SLE.

TCR repertoire studies in Sjögren syndrome

Sjögren syndrome is characterized by autoimmunity towards secretory glands, mainly lacrimal and salivary glands. The presence of immune foci of T cells and B cells with frequent formation of tertiary germinal centres in salivary glands from patients with Sjögren syndrome are indicative of antigen-driven responses[146]. Whether autoimmune repertoires differ between primary and secondary Sjögren syndrome, or demonstrate joint–gland cross-reactivity, remains unclear given that available scientific evidence mostly refers to primary Sjögren syndrome (pSS) (Supplementary Table 4).

Sjögren syndrome autoimmunity involves autoantibodies to nuclear antigens (anti-SSA/SSB), the presence of which correlates with an earlier disease onset and more severe lymphocytic infiltration in salivary glands[147]. Initial studies using peripheral blood, salivary glands

or renal tissue provided evidence of T cell clonal expansion, highlighting biased V gene usage and CDR3 associations[148–159], although with low consistency across studies.

A well-controlled study in 260 patients with pSS, including detailed medical history and excluding patients with concurrent RA, SLE or myositis, used multiplex PCR and NGS to analyse the circulating TCR repertoire at three different time points, and identified 18 pSS-associated T cell clones[160]. Although the identified TCR motifs did not overlap with those previously reported in patients with Sjögren syndrome[151,154,158,161], they matched clonotypes previously reported in patients with RA and SLE[88], suggestive of shared autoimmunity. The observed TCR abnormalities including V-J usage and clonotype expansions did not reverse with treatment[88,160]. However, the extent of clonal expansions correlated with some clinical measures[160], as well as with reduced saliva production and increased fibrosis of salivary glands[161,162], supporting their role in pathogenesis.

Further analyses using scRNA-seq identified identical TCR clonotypes in the peripheral blood and salivary glands within patients[161]. Moreover, memory CD4$^+$ T cells exhibited lower TCR diversity and clonotypic expansions in salivary glands compared with peripheral blood, and preferential VDJ usage and clonotype frequency in these T cells correlated with oral pathology. Similarities among the expanded CDR3 sequences of patients carrying the HLA-DR3/DQ2 (*DRB1*0301/DQB1*0201*) risk haplotype were also observed[161]. These findings suggest that shared T cell clonotypes across HLA-DR3/DQ2-positive patients probably react to a restricted antigenic peptide presented by the known Sjögren syndrome HLA risk alleles.

Regarding TCR reactivity, conserved CDR3 motifs in tissue-infiltrating T cells have been frequently reported following bulk[151,154,158] or single-cell TCR sequencing[159,161]. Studies have suggested that some of these conserved motifs, bearing hydrophobic amino acids at positions 6 and 7 in CDR3β, promote TCR self-reactivity[163]. Similarly, expression of a second TCR α-chain makes T cells with this property more likely to escape thymic deletion, and therefore infers an increased risk of autoimmunity[164]. An increased frequency of T cells bearing a second TCR α-chain has been observed in patients with pSS, supporting this as a potential mechanism for the development of autoimmunity in pSS[165]. Independent confirmation of these findings is awaited.

Preferential *TRBV* and *TRAV* gene usage and some CDR3β associations have been reported in both T_H1 and T_H17 cells in the salivary glands from patients with pSS[162]. Two pSS-unique motifs were identified, 'VVSDTVLETAGE' from TRAV8-2/J5 and 'LSTD*E' from varying CDR3α[162], adding to the limited evidence of TCR α-chain signatures in Sjögren syndrome[155,157,159]. Again, though, there is no consistency between studies in findings of V gene usage or CDR3 associations.

Effector T cell phenotypes

Some expanded CD4$^+$ T cells exhibit cytotoxic functions, similar to CD8$^+$ CTLs[166]. Such a cytotoxic CD4$^+$ population was initially described in both the peripheral blood and tissue lesions of patients with pSS[167], and is likely to respond to the activation of interferon pathways[168]. However, the clonal relationship between these circulating cytotoxic CD4 T cells and cytotoxic CD4 T cells found in salivary glands has not yet been determined, and it remains unclear whether they exert cytotoxic activity via MHC-II. It will, thus, be interesting to investigate whether these cells are expanded in response to pSS autoantigens or TCR-independent activation mechanisms.

In summary, TCR repertoire studies in patients with Sjögren syndrome have shown, at most, modest overlap, with no robustly reproduced findings. This suggests that either Sjögren syndrome pathogenesis does not involve shared T cell clonotypes, or that experimental designs have not been appropriate to identify them. Future approaches that may be more productive in identifying and characterizing clonal expansions in Sjögren syndrome are discussed below.

TCR–peptide–HLA confirmation studies

Defining autoimmune TCR–peptide–HLA pairs is a key step for disease understanding and clinical translation into diagnostic and therapeutic applications. The methodological approaches to resolve this association involve antigen screening studies (Fig. 2d), when progressing from identified TCRαβ sequences, or TCR screening studies, when investigating TCR reactivity to defined antigenic peptides or proteins, as reviewed in depth elsewhere[1]. As shared full-length autoimmune TCR clonotypes have been confirmed only for AS and no other major rheumatic diseases, antigen-screening study has only been possible in this condition[41].

If the antigen driving an immune-mediated disease is suspected, as in Sjögren syndrome, SLE or RA, TCR sequencing can be coupled with antigen screening to obtain a targeted strategy for autoimmune TCR identification (Fig. 2e). Antigen reactivity has been traditionally performed by detection of T cell activation in pooled lymphocyte populations. Increased throughput and multiplexing can be achieved using soluble MHC tetramers loaded with the antigen[169]. Different labelling methods allow the identification of TCR binding by flow cytometry (fluorescent tags)[170], cytometry by time of flight detection (metal tags)[171] or single-cell sequencing (DNA tags)[172].

Approaches to the detection of antigen-driven T cell reactivity have been applied in rheumatology, either as stand-alone readout of autoimmunity or coupled to TCR analysis. In Sjögren syndrome, T cell reactivity to the Ro(SSA) antigen was detected using lymphocyte populations and T cell clones from labial salivary glands[173], and this reactivity was attributed to CD4$^+$ T cells carrying $V_\beta2$ and $V_\beta13$ chains and using conserved CDR3 motifs[174]. In addition, in a study in ten patients with Sjögren syndrome, half of them showed T cell reactivity against the M3 muscarinic acetylcholine receptor (M3R) in ELISpot assays and mainly against peptide 83–95 of M3R. Furthermore, circulating M3R-specific T cells displayed a T_H17 cell phenotype, and their presence correlated with higher titres of anti-M3R antibodies and a trend for higher disease activity[175]. In SLE, T cells have shown reactivity against the chromosomal HMG protein or nucleosomal histone autoantigens[120]. Similarly, proliferative lymphocyte responses have indicated T cell reactivity against U1 small nuclear ribonucleoprotein A (U1 snRNPA) in patients with SLE that were positive for anti-snRNP autoantibodies, and the TCR and antibody recognition sites on snRNPs were found to overlap[176,177]. In RA, numerous autoantigens have been suggested, such as citrullinated peptides derived from extracellular matrix proteins, collagen and tenascin-C[178,179], or cartilage proteoglycan[180]. Molecular mimicry between peptides from proteoglycan and the Yop protein of *Yersinia* has been suggested based on sequence homology and shared reactivity in RA and osteoarthritis[180]. Moreover, TCR clones that are reactive towards an HLA-DRB1*10:01-restricted citrullinated-type II collagen (Cit-CII) peptide have been identified in individuals with Cit-CII-specific autoantibodies[181]. This aligns with RA risk polymorphisms affecting the peptide binding groove of HLA-DRβ1 (ref. 182) and its interaction with citrullinated peptides[183]. However, expanded T cell clones showed negligible reactivity against citrullinated peptides in HLA-DRB1*04:01-positive patients with RA who had ACPA positivity, despite the preferential TRBV20-1 usage[116].

TCR profiling in HLA-DR4 transgenic mice identified clonal expansions of TRAV6$^+$ T cells that also displayed reactivity against citrullinated vimentin and enolase[184]. This extended a previous observation of TRAV26-1 biased gene usage in T cells found in Indigenous North American patients with RA who carried a citrullinated vimentin epitope presenting HLA-DRB1*1402 (ref. 185). This is a rare example of similar T cell subsets being identified in different studies in the same rheumatic disease. Both studies used peptide–MHC tetramers to enrich samples for TCR profiling, suggesting that this method is particularly effective for identifying low prevalence clonotypes.

While these studies provide valuable information about reactivity to antigens potentially involved in autoimmune T cell expansions, there is a paucity of independent replication studies and of interdisciplinary validation of TCR profiling results by antigen screening studies and vice versa. This is required for the field to determine whether the findings are robust, and the extent to which these autoimmune associations are shared among patients. We discuss below lessons to improve study design and the chances of successful identification of expanded T cell subsets, assuming these exist and are shared by substantial proportions of patients or react with common antigenic sources.

Lessons from TCR repertoire studies

This Review demonstrates that, with the exception of axSpA, there is a paucity of reproduced findings of clonotype associations with major immune-mediated rheumatic diseases. This is despite the fact that these diseases have strong HLA associations and are widely thought to involve adaptive immune reactions and expansions to HLA-presented peptides, as an important part of disease pathogenesis. While this hypothesis may be wrong, and multiple cell types are additionally involved in the mechanisms underpinning IMIDs, we propose that the lack of reproducible findings relates to: the diversity of the TCR–HLA repertoires; aetiopathogenic and clinical heterogeneity within disease cohorts; and the spread of immune system disturbances and range of antigens involved with increasing disease duration, which might cloud initial disease-causative effects.

Future studies need to better consider these factors in experimental designs for a successful application of unbiased or targeted methodologies and to reach conclusive results. Based on our experience and the above-described studies, we propose a workflow with sequential implementation of these methodological approaches for best results (Fig. 2) and we discuss important study variables below.

Genetic susceptibility

Several hundred loci are associated with autoimmunity in rheumatic pathologies[186–188]. Strong associations have been found for antigen processing and presentation pathways, particularly with MHC loci and with genes involved in T cell activation and differentiation. To our surprise, few studies to date have adequately controlled for the HLA associations of rheumatic diseases, let alone for the non-MHC associations, which have also been shown to influence TCR usage diversity[189].

The need for integrative analyses with HLA typing is well reflected by the success of TCR profiling studies in axSpA to identify disease-associated clonotypes. The highly homogeneous HLA background for axSpA development is likely to have facilitated the identification of disease-associated T cell clonotypes. These findings strongly support the arthritogenic peptide hypothesis[1], pinpointing a causal mechanism and a target population, and making axSpA a model disease for TCR repertoire studies. The success from initial TCR findings in ReA[33,34] to confirmation in HLA-B27$^+$ SpA[35,36,38], and phenotype and peptide screening upon paired TCRαβ characterization[41,43], will hopefully encourage better controlled studies in rheumatology. Despite higher HLA heterogeneity in other conditions, HLA-controlled studies might facilitate the identification of sets of autoimmune TCRs and their subsequent antigenic protein targets. If these are disease-relevant proteins, they could also be an antigenic source for other HLA alleles, amenable to antigen reactivity validation studies (Fig. 2e).

Non-hereditary factors

Most autoimmune diseases appear in adult age and exhibit considerable sex dimorphism, with generally a higher incidence in women[190]. Female hormones affect immunological reactivity[191] and their different levels in men might explain why men require a greater cumulative genetic risk for disease development[192]. In line with this, stronger repertoire alterations were observed among men with RA[193]. Similarly, disease diagnosis at a young age generally implies a more severe pathology and genetic risk. As autoimmune diseases progress, cumulative T cell repertoire expansions develop and accumulate through the impact of epitope spreading. This effect is likely to obscure the identification of driver autoimmune clones, favoured when studying early disease, as observed in ReA[33,34], juvenile idiopathic arthritis[86,87,194–196] and RA[64,72,90,108,197–199]. This scenario is favoured by conditions such as infections, in the case of ReA, where infection-triggered alterations prompt an acute development of cross-reactive autoimmune TCRs, thus speeding up repertoire findings[33,34].

Exposure to novel antigens and cross-reactivity

Autoimmunity arises from the 'leakage' of autoreactive clones. This might occur owing to failures of central or peripheral tolerance. However, infection, tissue damage and somatic mutation can expose the immune system to unseen antigens. TCR cross-reactivity or molecular mimicry of endogenous peptides trigger autoimmunity in conjunction with an appropriate costimulatory environment[200]. This concept lies behind the numerous associations of rheumatic conditions with infections and gut dysbiosis[201,202]. It might also explain the co-morbidities among rheumatic conditions such as psoriasis and PsA, and lupus nephritis in SLE, and with other IMIDs, due to cross-tissue autoimmunity. Chronic exposure to tissue-specific self-antigens with negligible presentation under homeostatic conditions[203] following their release from peripheral tissues, might also trigger and maintain autoimmunity. This is the concept of 'damage-induced epitope spreading', and includes protein modifications favoured in the inflammatory microenvironment[204], as observed with antigen citrullination in RA[71,97,115,205,206]. This is influenced by the immune system itself[106] and bacterial infection, directly promoting citrullination of self-antigens[207] or inducing citrullinating enzymes in neutrophils[208]. These factors not only support the use of early disease as a homogenization factor for the study population, but given the tissue-specificity of these events, analysis of tissue-infiltrating lymphocyte samples might be particularly informative for detection of autoimmune clonotype associations.

Altered microenvironment and autoimmune priming

Infection and tissue damage also shift the inhibitory balance that keeps autoimmunity under control. This is evidenced by upregulation of co-stimulatory molecules and by the development of rheumatological conditions upon therapy with immune checkpoint blockers in RA[209–211] and SLE[212], with frequent implication of tissue CD8$^+$ T cells. We believe that TCR repertoire studies focused on these rheumatic events are of interest and could provide information on rheumatic autoimmune

mechanisms despite their differential aetiology. In addition to infection-derived stimuli, sensing of self-nucleic acids from damaged tissues seems to be a major mediator of autoimmunity, as appreciated first in SLE[213]. Thus far, disease has mostly been associated with expansion of pathogenic TCRs rather than loss of protective ones, with the exception of reduced numbers of high-affinity NKT cells in RA[108–111]

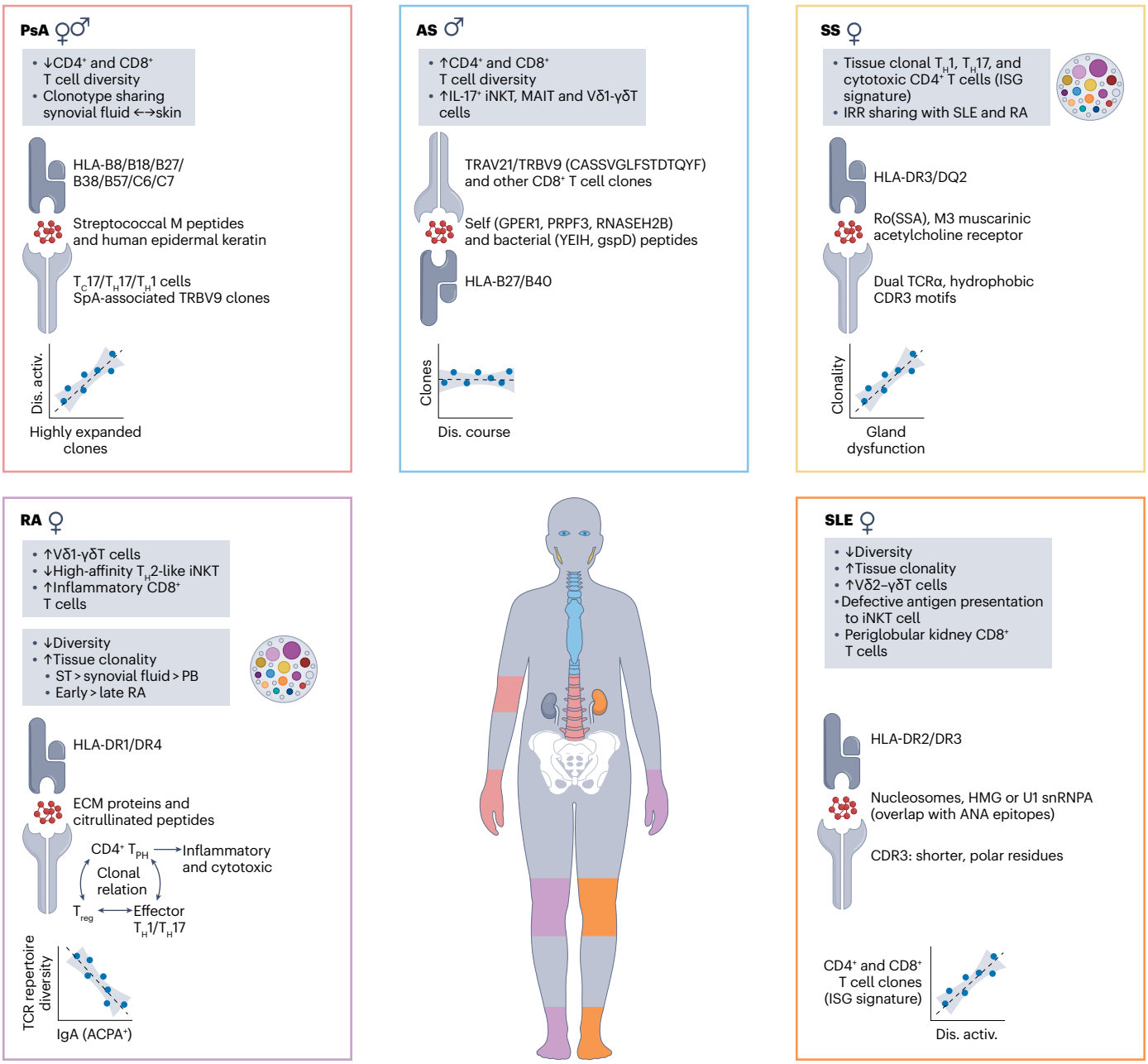


Fig. 3 | Main factors influencing T cell receptor repertoire in common major HLA-associated rheumatic diseases. Dominant sex influence, immunological responses and preferential tissue locations might differ across HLA-associated rheumatic diseases. Sjögren syndrome involves lacrimal and salivary glands; psoriatic arthritis (PsA) involves axial and peripheral joints and skin; ankylosing spondylitis (AS) affects spinal and sacroiliac joints, and eyes; systemic lupus erythematosus (SLE) affects peripheral joints and kidney; rheumatoid arthritis (RA) involves peripheral joints. For each condition, overall findings of HLA associations, suggested antigenic sources and T cell receptor (TCR) motifs are summarized, as well as repertoire diversity and clonality (bubbled circle), and correlations (graph) and implications of particular lymphocyte populations. ♂, male; ♀, female; ACPA, anti-citrullinated protein antibody; ANA, antinuclear antibody; Dis. activ., disease activity; ECM, extracellular matrix; iNKT, invariant natural killer T; IRR, incident rate ratio; ISG, interferon-stimulated gene; MAIT, mucosal-associated invariant T; PB, peripheral blood; RA, rheumatoid arthritis; snRNPA, small nuclear ribonucleoprotein A; SpA, spondyloarthritis; SS, Sjögren syndrome; ST, synovial tissue; T_C17, IL-17-secreting CD8⁺ cytotoxic T; T_H, T helper; T_{PH}, T peripheral helper; T_{reg}, regulatory T.

and SLE[110,143]. This is probably explained by the common challenges of studying homeostatic mechanisms compared with pathogenically activated pathways, including the constrained diversity of arthritogenic antigens while antigenic tissue tolerance expands through all the individual's HLA repertoire driving overlapping polyclonal coverage. Genetic alterations favouring autoimmunity and autoinflammation may also favour a phenotype switch among pre-existing tolerogenic tissue immunity, over time contributing to antigen spreading effects and overlap among repertoires of effector and regulatory subsets. Study designs involving affected tissue sampling, particularly in early disease, are likely to be more informative because of this. We think that studies of immunological factors associated with tolerance and reduction of excessive inflammatory responses are also of great value because they offer the potential to inform design of novel therapeutics, particularly in conditions where defined pathogenic clonotypes are elusive, as in SLE and Sjögren syndrome.

Clinical translation

The final goal of TCR repertoire studies is to improve our knowledge of autoimmune T cell dynamics in rheumatic conditions and reach clinical applications. The latter requires the definition of a small set of targetable features, such as TCR sequences, antigenic peptides or proteins and phenotypic markers (Fig. 2f). The recent progress in axSpA is a clear example of how to 'climb down' the complexity ladder of the TCR repertoire to translational opportunities, opening the door for selective depletion or suppression of self-reactive T cells. A case report study in AS set the 'first stone' in that direction testing an anti-TRBV9-depleting antibody (BCD-180) for targeted clonotype elimination without associated toxicities[214]. Following successful evaluation in non-human primates, treatment of a patient with established AS with BCD-180 led to a complete remission that had persisted for 4 years up to the publication date. This breakthrough trial offers a new generation of curative therapies for an autoimmune disease that are potentially applicable to other HLA class I-associated and *ERAP1*-associated diseases, such as psoriasis, anterior uveitis, Behçet's disease and birdshot retinopathy. The potential of this approach is such that it strongly supports the need for better clonotype characterization in these diseases, with validation of their disease involvement, and downstream research.

The presence of expanded T cell clonotypes may also have diagnostic value. In axSpA, the >90% sensitivity and high specificity of the presence of AS-associated CDR3 expansions suggests a potential for these clonotypes as biomarkers to distinguish the minority of patients meeting current classification criteria for the disease who have true inflammatory disease from those with other conditions. Investigation of the performance of expanded T cell clonotypes as a screening test to identify the 1–5% of HLA-B27 carriers who subsequently develop axSpA is also warranted, as this could potentially allow presymptomatic approaches to completely prevent development of the disease.

To date, TCR findings in other major rheumatic conditions (summarized in Fig. 3) have not yet succeeded in resolving consistent TCR associations, limiting translation to the clinic, apart from the potential for immune monitoring of treatment responses[215]. However, applying the lessons learned thus far, and using the recent advances in NGS and scRNA-seq approaches, the tools now exist to rapidly progress this promising field. In particular, future studies should focus on affected and control individuals carrying specific disease-associated HLA groups, analysing adequate sizes of tissue samples at early stages of disease to address the heterogeneity involved. A more precise characterization of autoimmune clones might help to identify phenotypic signatures of shared cellular biomarkers among polyclonal responses, as accomplished with the identification of $CD4^+$ cytotoxic and T_{PH} cells in RA[71,86,96,102,112,113]. We propose that this new knowledge would allow targeted enrichment strategies for research purposes, and the development of therapies informed by underlying disease mechanisms (Fig. 2). Recruitment receptors expressed by autoimmune clones in PsA[57,59,60] explain their tissue tropism and could also become therapeutic targets. Although immune cell activation and recruitment have been extensively studied in rheumatology, there has been little research combining these studies with TCR profiling to determine if specific clonotypes are involved.

The use of expanded TCRs to identify the antigenic drivers and their sources would have great translational potential, including in early diagnosis evaluating antigen reactivity as well as tolerogenic therapeutic approaches. Cross-reactivity with antigens from infectious microorganisms raises the possibility of specific antimicrobial therapies to manage or prevent the disease. In AS, the consistent finding of a highly restricted set of TCRs has enabled a peptide library screening study and the identification of their cognate antigens among relevant bacteria and human proteins[41]. This example shows the potential of this approach to resolve basic pathogenic mechanisms. Although not identified starting from disease–TCR associations, in RA, knowledge of potentially immunopathogenic citrullinated peptides, antibodies to which are present 15 years before the onset of clinical symptoms, has revolutionized diagnosis[205] and opened the door for development of peptide-specific therapies[216,217]. For SLE, nuclear proteins are key drivers of immunity. This raises the therapeutic possibility of inactivating an antigen-specific TCR response against them by targeting the antigen to steady-state dendritic cells, which in turn regulate antigen-specific memory and effector T cell populations[218–221]. Alternatively, by TCR gene transfer, the target of primary regulatory T cells could be redirected, and adoptive therapy used to induce antigen-specific suppression of the pathology[222]. As these strategies would be specific to the condition, we hypothesize that they could avoid off-target toxicity and achieve long-term remission effects[214].

Conclusions

There is great value in reaching a more complete understanding of the autoimmune TCRs in rheumatic conditions. The identification of disease-specific and tissue-specific dominant clonotypes might help to establish a set of reliable biomarkers and to develop personalized immune therapy. The challenge remains in finding consistent and reliable dominant TCR clonotypes and in targeting autoimmune T cell populations through shared antigenic or phenotypic signatures. Improved methodology and study design have shown that this is surmountable, although it requires interdisciplinary efforts of a larger scale than so far undertaken. The strategies informed by repertoire studies have enabled the prospect of preventative or early treatment for axSpA with potential for prolonged disease remissions. With better definition of the T cell clonotypes potentially involved in other HLA-associated rheumatic diseases, similar therapeutic approaches might become possible in these conditions.

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Competing interests

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Perspective

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An international perspective on the future of systemic sclerosis research

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Abstract

Systemic sclerosis (SSc) remains a challenging and enigmatic systemic autoimmune disease, owing to its complex pathogenesis, clinical and molecular heterogeneity, and the lack of effective disease-modifying treatments. Despite a century of research in SSc, the interconnections among microvascular dysfunction, autoimmune phenomena and tissue fibrosis in SSc remain unclear. The absence of validated biomarkers and reliable animal models complicates diagnosis and treatment, contributing to high morbidity and mortality. Advances in the past 5 years, such as single-cell RNA sequencing, next-generation sequencing, spatial biology, transcriptomics, genomics, proteomics, metabolomics, microbiome profiling and artificial intelligence, offer new avenues for identifying the early pathogenetic events that, once treated, could change the clinical history of SSc. Collaborative global efforts to integrate these approaches are crucial to developing a comprehensive, mechanistic understanding and enabling personalized therapies. Challenges include disease classification, clinical heterogeneity and the establishment of robust biomarkers for disease activity and progression. Innovative clinical trial designs and patient-centred approaches are essential for developing effective treatments. Emerging therapies, including cell-based and fibroblast-targeting treatments, show promise. Global cooperation, standardized protocols and interdisciplinary research are vital for advancing SSc research and improving patient outcomes. The integration of advanced research techniques holds the potential for important breakthroughs in the diagnosis, treatment and care of individuals with SSc.

Sections

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Introduction

Sir Winston Churchill's definition of the Soviet Union as "a riddle, wrapped in a mystery, inside an enigma" could also apply to systemic sclerosis (SSc, also known as scleroderma). The pathogenesis of SSc, clinical heterogeneity, absence of validated biomarkers, difficult classification, problematic monitoring of treatment response and lack of well-designed randomized control trials contribute to the complexity of SSc[1,2]. Consequently, diagnosis is often delayed, the assessment of disease activity is challenging, there is a lack of reliable disease-modifying drugs, and morbidity and mortality are high, resulting in a substantial burden on patients and the health care system[3,4]. Despite more than a century of clinical and experimental investigations into SSc since the first descriptions of the disease[5,6], the precise relationship among the hallmark features of SSc — microvascular dysfunction, autoimmune phenomena and pathological tissue fibrosis — remains elusive. It is unclear whether these events are interlinked, triggered by the same or different agents, or occur as a cascade. Additionally, the heterogeneous nature of SSc raises questions about whether it is one disease with distinct subsets and temporal stages or a collection of closely related diseases with similar symptoms (Fig. 1).

As no animal model encompasses all features of SSc, insights gained from animal studies remain limited[7]. Most evidence suggests that vascular abnormalities or immunological dysregulation occur at early stages of the disease and precede fibrosis. This progressive model of pathogenesis, however, has not translated into therapeutic advances. Most treatment strategies target single-organ manifestations, often improving quality of life and survival but falling short of being disease-modifying therapy. Unclear definitions, variability in patient subsets, small and underpowered studies and difficulties in obtaining patient samples contribute to these challenges. Examples of these clinical trials in SSc include studies with tocilizumab (anti-IL-6 receptor therapy), abatacept (CTLA-4 co-stimulation blocker) and pan-PPARγ agonists, which all failed to meet the primary endpoints[8–10]. Although the anti-fibrotic agent nintedanib was shown to have beneficial effects for patients with interstitial lung disease (ILD)[11], and some studies with TGFβ inhibitor therapy showed promising effects[12,13], much remains to be done to effectively manage all the manifestations of SSc.

There has been substantial progress in molecular and cell biology and cellular immunology, coupled with technological breakthroughs in next-generation sequencing, single-cell RNA sequencing, spatial transcriptomics, proteomics and metabolomics. Integrating these research technologies into SSc research requires computational biology, statistical and artificial intelligence (AI) to properly analyse the data generated in studies using them and to link them to clinical information. These unprecedented developments in the past few years now provide the field with a unique opportunity to integrate these new approaches into SSc research in a systematic fashion to generate a more detailed mechanistic understanding of disease pathogenesis and to make personalized medicine in SSc a reality through the identification of specific and effective treatments tailored to individual patients. Organized global cooperation is a prerequisite for the success of such an endeavour. With this aim, an international consensus workshop was held in Portonovo (Ancona) Italy in October 2023. The principal conclusions from this workshop represent the basis for this Perspective. The goals of the workshop were to provide a foundation for bringing the international SSc research community together, to develop a common understanding of the disease pathobiology, to explore innovation in clinical trial designs and outcome measures, as well as to educate health care professionals, patients, the general public, regulators, industry leaders and policymakers (Fig. 2). Our vision for the workshop was to advance research in SSc by exploring disease complexity and organ-specific complications and to highlight major research hurdles and how these can be overcome by leveraging novel technological innovations for biomarker discovery and emerging therapies and translational opportunities aimed at enhancing the design of more effective and impactful clinical studies and overall patient benefit.

The complexity of systemic sclerosis and organ-based complications

SSc is an acquired autoimmune disease of unknown cause with worldwide distribution and a strong female sex bias, and is characterized by vascular damage and immunological abnormalities that lead to immune dysfunction, autoantibody production and the development of skin and internal organ fibrosis[1–3]. Its complexity arises from disease heterogeneity, the involvement of multiple organ systems and a blend of inherited, environmental and lifestyle factors. Research over the past few years deploying advanced omics[14] and analytic approaches has unveiled novel pathways, cell types, circuits and mechanisms involved in SSc, offering new therapeutic targets and insights into disease pathogenesis. SSc is highly heterogeneous and can be readily separated into two major disease subsets, limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc), according to the pattern of skin involvement[1–3] (Fig. 1). Other aspects of disease diversity include variations in clinical manifestations, disease progression, organ involvement, treatment response and molecular heterogeneity[14], thus emphasizing the need for personalized treatment approaches.

Advances in genomic and proteomic platform technologies have accelerated the identification of molecular pathways underlying SSc pathogenesis. In addition to well-known signalling pathways (such as the TGFβ, CCN, platelet-derived growth factor, fibroblast growth factor, insulin-like growth factor binding protein, IL-6 and IL-31 signalling pathways), novel mechanisms have been implicated, such as Notch, Hedgehog, Wnt–β-Catenin, Hippo, CXCL4 and various extracellular matrix (ECM) remodelling pathways[15–19]. Understanding the interconnections between these pathways is critical for identifying new therapies. The pathology of SSc is traditionally defined by vascular dysfunction, inflammation, autoimmunity and hyperactivation of myofibroblasts. Novel approaches, including single-cell and spatial analysis, have extended the knowledge of the cell types that are involved in SSc and the communication between these cells and with the ECM; the ECM is known to be a major contributor to disease[20–22]. Researchers have uncovered novel aspects of fibroblasts and myofibroblasts that highlight the plasticity of these cells. The fibrotic tissue in patients with SSc contains multiple transcriptionally distinct fibroblast subsets[23], some of which have critical roles in the initiation, progression and persistence of pathology (Fig. 3). Other studies have also focused on the pathogenetic role of the microbiota in the skin, lungs and gut, reporting that the intestinal microbiome of patients with SSc differs from that of healthy individuals; however, establishing the effects of microbial dysbiosis in different organs on the initiation and progression of SSc features remains challenging, and the pathogenic role of gut dysbiosis, along with the potential mechanisms involved, remains completely unknown and merits further study[24–30].

Genomic studies have identified nearly 30 loci associated with SSc, highlighting the contribution of immune cell activation, type I interferon signatures, cytokine signalling, inflammation, apoptosis

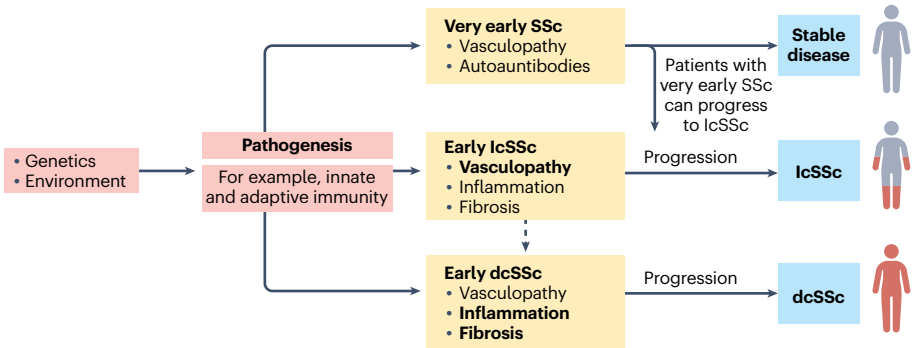


Fig. 1 | Steps in the pathogenesis of systemic sclerosis phenotypes. Genetic (both HLA and non-HLA loci) and environmental (both infectious and non-infectious) factors can promote systemic sclerosis (SSc), although it is not clear whether the initial involvement always concerns a single organ or multiple organs and this can vary from patient to patient. The extent and type of immune response that is activated and the associated molecular pathways in an individual with SSc can lead to the stratification of patients who meet the criteria for very early diagnosis of SSc (an early phase of disease that is associated with vasculopathy and the presence of autoantibodies, and is an opportunity for targeted intervention), limited cutaneous SSc (lcSSc, which accounts for 60–80% of SSc cases with disease restricted to the extremities and that is associated with vasculopathy and limited fibrosis; localization of fibrosis is indicated in red in the middle silhouette) and prevalent vascular involvement or diffuse cutaneous SSc (dcSSc, which accounts for 20–40% of SSc cases and is characterized by extensive skin fibrosis, which is associated with vasculopathy and internal organ fibrosis, indicated in red in the bottom silhouette). The pathophysiological alterations that occur in the different organs (lung, gastrointestinal tract and skin) can then contribute to the aggravation and progression of the disease.

and autophagy to the disease process[31]; these studies also provide insight into vascular and ECM fibrotic pathology. The assessment of shared genetic factors and cross-phenotype genome-wide association studies have uncovered new pathways by identifying shared genetic factors between SSc and other autoimmune diseases[32]. This overlap provides opportunities to extend applications for specific drugs, potentially accelerating the development of effective therapies for SSc. Future directions in SSc genomics include trans-ethnic genome-wide association studies, whole-genome sequencing, studying structural and non-coding RNA variants and epigenetic studies. The integration of genomic data with epigenomic, transcriptomic and proteomic data can help to elucidate the functional effect of genetic variants[33,34]. Deciphering the interplay between genetic predisposition, environmental exposures and social factors will increase the understanding of SSc pathogenesis. Environmental factors, such as occupational exposures, infections and lifestyle factors can increase disease risk and could trigger disease onset and influence the progression of SSc[35,36].

Besides skin involvement and Raynaud phenomenon, organ-based complications are a key feature in SSc and can involve several internal organs, including the kidney, gastrointestinal tract and cardiopulmonary system[1]. The underlying pathophysiology in the affected organs in SSc seem similar, although there are some organ-specific pathogenic mechanism(s). Skin involvement in SSc can cause substantial morbidity; management of skin manifestations consists of topical therapy, vasodilators and anti-inflammatory approaches[1]. Gastrointestinal tract involvement is common and represents an important unmet clinical need, with underlying molecular and cellular alterations still being investigated[37]. SSc-associated pulmonary complications (pulmonary arterial hypertension (PAH) and ILD) are complex and life-threatening and require early recognition, accurate diagnosis and comprehensive management[38]. Research into pulmonary vascular disease has provided genetic insights and new concepts regarding the pathophysiology of SSc-associated PAH[39,40]. Around 5% of patients with SSc have pathological coding variants in PAH-related genes. Advances in the treatment of PAH focus on vasodilation and targeting the underlying pathobiology of the disease. The development of molecular therapeutic agents, such as sotatercept, an activin signalling inhibitor, show promise but raise concerns about adverse effects, such as vascular malformations. Other potential treatments include small molecule tyrosine kinase inhibitors, poly (ADP-ribose) polymerase inhibitors, bromodomain-containing protein 4 inhibitors, senolytics, elastase inhibition, stem cell therapy, autologous haematopoietic stem cell transplantation (HSCT) and chimeric antigen receptor (CAR) T cell therapy[41–44].

In SSc-associated ILD (SSc-ILD), inflammation and fibrosis of the lungs can substantially impair lung function and, hence, quality of life, and is associated with high mortality[1–3]. The course of SSc-ILD is highly variable and predicting individual disease progression is problematic owing to the lack of reliable biomarkers, which hinders the implementation of personalized therapy[1–3]. Current treatments for SSc-ILD focus on slowing disease progression, managing symptoms and improving patient outcomes. Typical treatments include immunosuppressants (mycophenolate mofetil, cyclophosphamide and rituximab) and new anti-fibrotic agents (nintedanib)[11,41–45]. Additionally, in a subset of patients with SSc, treatment with tocilizumab can help to manage symptoms and improve functional capacity[10,46]. Ongoing research is driving the discovery of new therapeutic strategies, with a focus on identifying early pathogenic events in SSc-ILD and developing innovative approaches to mitigate the effects of lung disease in patients with SSc-ILD. The use of the 2019 classification criteria[47] for the early identification of scleroderma renal crisis should facilitate early treatment with angiotensin-converting enzyme inhibitors and improve the prognosis of this rare but severe complication. Vasodilators used in PAH (endothelin receptor antagonists and prostacyclin analogues) have not demonstrated efficacy in this setting but could, in the future, along with complement inhibitors (eculizumab) help to improve the prognosis of renal crisis in SSc. Heart involvement is the third leading cause of death related to organ involvement in SSc[48]; heart involvement in SSc represents an unmet clinical need as only symptomatic non-specific treatments are proposed for these patients. This cardiac involvement remains an understudied area of research in SSc[49]. Patients with SSc can undergo organ transplantation, particularly for the lungs, heart

and, in some cases, the kidneys. Organ transplantation is considered for patients with severe, end-stage organ involvement that is refractory to other treatments[1–3].

The association of SSc with biomarkers of aging provides fresh insights into its pathogenesis[50–53]. Biological clocks indicate an apparent acceleration of aging in individuals with SSc, and cellular senescence is greatly augmented in affected organs[54]. Senescent cells, which are characterized by irreversible cell-cycle arrest and the senescence-associated secretory phenotype, increase in abundance with age and correlate with chronic inflammation[55]. Senolytics and senomorphic therapies are aimed at eliminating or reducing the effect of senescent cells, but the reparative role of senescent fibroblasts in tissue repair requires careful investigation[56]. Accurately measuring biological ageing and cellular senescence in SSc and understanding their contributions to pathogenesis can provide new therapeutic targets.

A compelling need exists to better understand the natural history of SSc and the various subtypes of this disease. Long-term, multicentre and multinational longitudinal studies are crucial for capturing disease progression, identifying biomarkers and evaluating therapies. Although these studies present challenges, such as patient recruitment and standardizing data collection, the potential benefits outweigh these obstacles, offering improved patient outcomes and a deeper understanding of SSc.

Overcoming major barriers for research in systemic sclerosis

Research in SSc faces several hurdles that hinder progress towards effective therapies; for example, the difficulties in defining and classifying SSc, the relative rarity and clinical heterogeneity of the disease, the complex heritability and elusive aetiology and perhaps, most notably, the incomplete understanding of underlying molecular mechanisms. These factors and changes in outcome assessment impede efforts to generate definitive evidence for altering clinical practice. Many of these issues are interconnected. Untangling the complex and dynamic temporal and pathogenic relationships linking fibrosis, vascular injury and immune activation remains elusive. A more complete understanding of the pathogenesis of SSc will probably emerge from a combination of both unbiased and hypothesis-driven approaches that address all three hallmark features: vascular, immunological and fibrotic changes.

The striking female bias in SSc, similar to many other autoimmune diseases, remains unexplained[57], with multiple competing, although not mutually exclusive, proposed underlying mechanisms. Exciting findings suggest a potential role for X chromosome inactivation escape and Xist ribonucleoprotein-driven autoimmmunity in sex bias[58]. As already noted, current animal models of SSc fail to capture all aspects of human disease. Alternate preclinical model systems, such as precision-cut skin slices and 3D organ cultures that are populated with

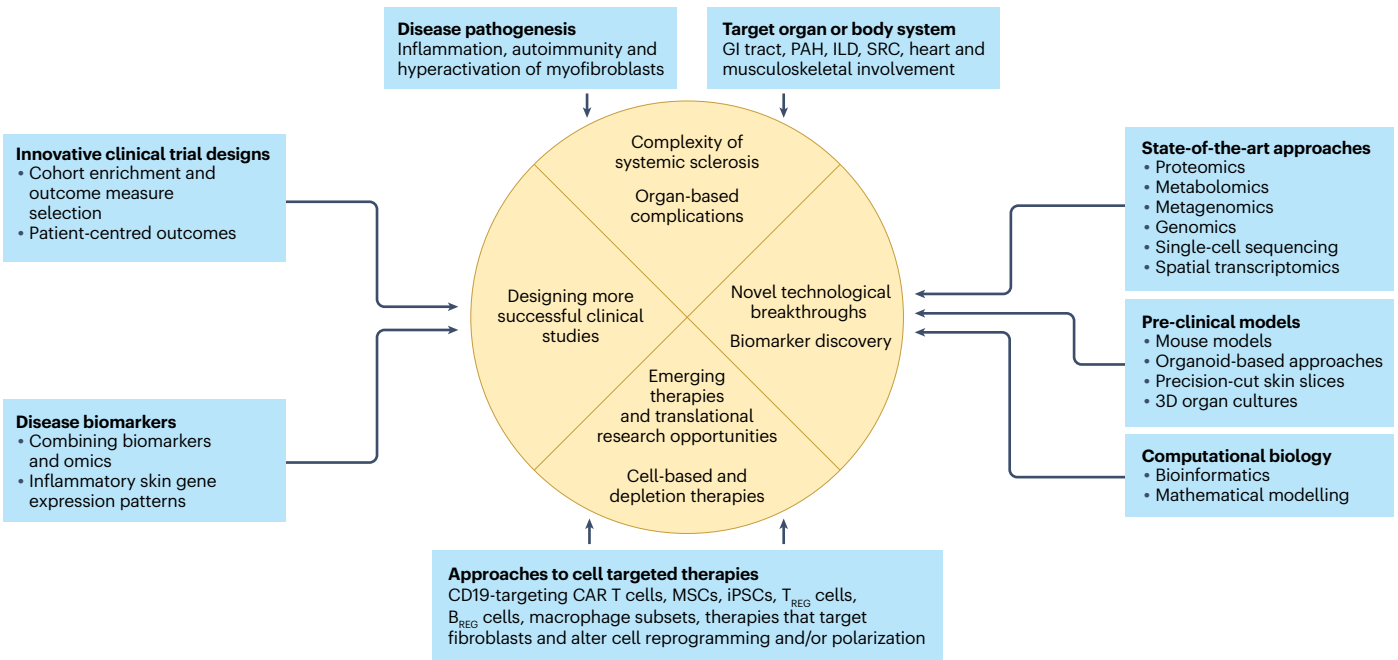
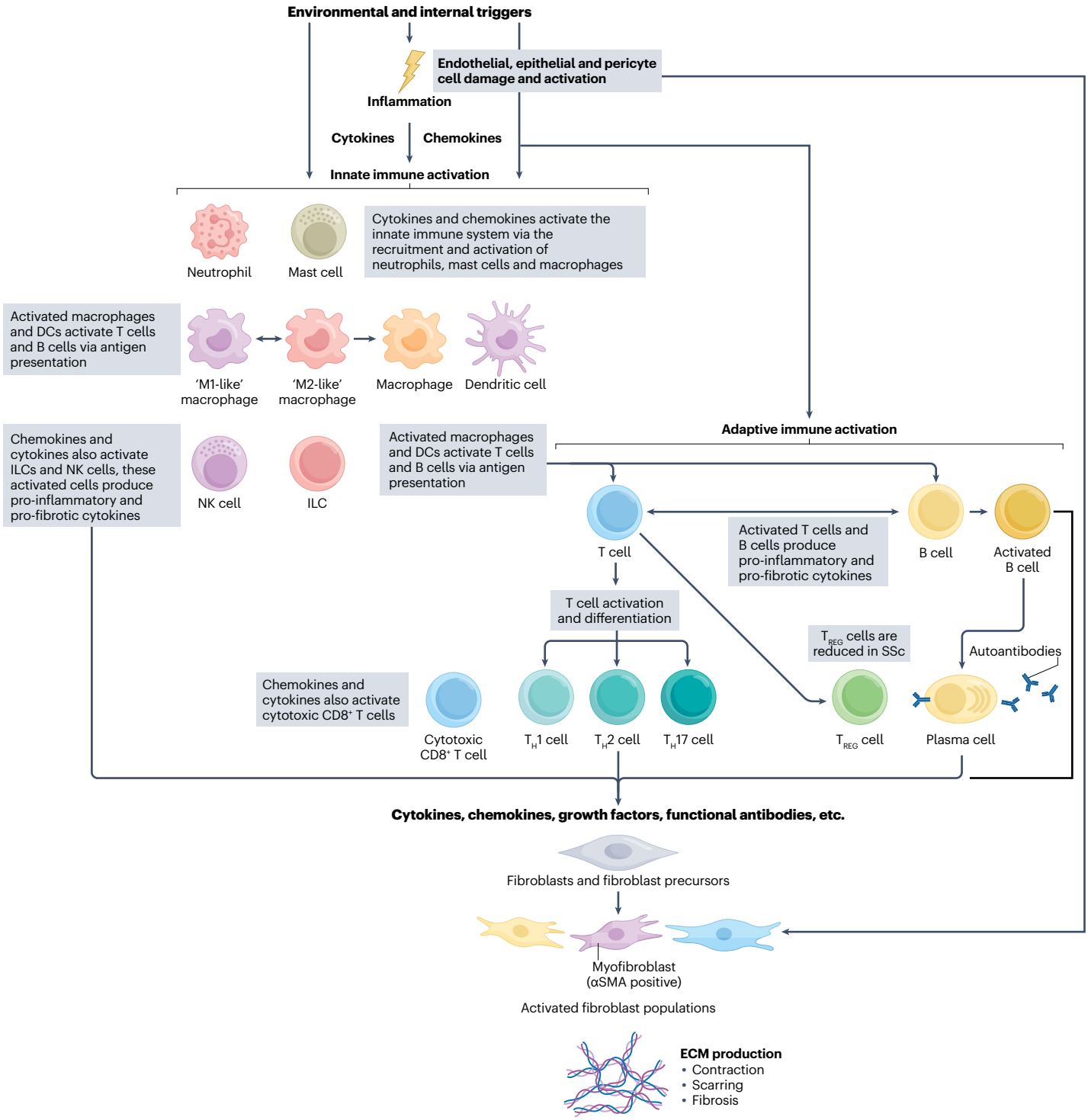


Fig. 2 | The future of research in systemic sclerosis. The future research strategy for systemic sclerosis (SSc) is shaped by four overarching themes (shown in the yellow circle in this figure), which are intricately connected to a variety of advanced approaches and emerging opportunities (shown in the outer blue boxes in the figure). The first theme, the complexity of SSc and organ-based complications, highlights the multifactorial nature of the disease and its widespread effect on various organs. The second theme focuses on novel technological breakthroughs and biomarker discovery, which are driving innovations such as proteomics for identifying biomarkers, metabolomics for understanding disease mechanisms and metagenomics for exploring microbial communities. The third theme, emerging therapies and translational research opportunities, emphasizes the importance of translational research, leveraging preclinical models such as genetically modified mouse models, organoid-based approaches, skin equivalents and precision tissue slices to study disease pathogenesis and test new therapies. Finally, the fourth theme, designing more successful clinical studies, underscores the need to improve clinical trial design and outcomes. These four themes collectively guide the research strategy for SSc, which will advance understanding of this disease and pave the way for more effective treatments. B_{REG} cells, regulatory B cells; CAR T cells, chimeric antigen receptor T cells; ILD, interstitial lung disease; iPSCs, induced pluripotent stem cells; MSCs, mesenchymal stem cells; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; T_{REG} cells, regulatory T cells.



multiple cell types (such as monocytes, fibroblasts or endothelial cells), might aid the efforts to better understand pathogenesis and for pre-clinical drug testing[59–64]. It is widely appreciated now that improved disease models are essential for assessing the efficacy of new therapies and understanding drug interactions in clinical trials. These models are promising, especially when leveraging emerging technologies with single-cell level resolution, such as single-cell RNA sequencing, single-cell ATAC-sequencing and spatial transcriptomics[22,23,65–67]. Relating the novel findings from emerging technologies to potential environmental exposures and acquired genetic and/or epigenetic changes[68–70] are expected to yield novel insights into SSc pathogenesis and enable more predictive determination of the efficacy of

Fig. 3 | Overview of the pathogenesis of systemic sclerosis. The pathogenesis of systemic sclerosis (SSc) is complex and involves interplay between environmental factors or exposures, host genetics, target organ injury, immune activation and fibrosis. Environmental and internal damages and triggers that lead to endothelial and/or epithelial cell activation are likely to be prominent mechanisms underlying early disease in SSc (although these damages and triggers can also directly activate the innate immune system), leading to the release of cytokines and chemokines. These factors promote innate immune system activation, by attracting and activating neutrophils, mast cells and macrophages. Macrophage activation results in cell polarization into 'M1-like' or 'M2-like' phenotypes, contributing to inflammation and further production of cytokines and growth factors. External stimuli or internal tissue damage cause antigen release and presentation via antigen-presenting cells such as dendritic cells (DCs) and macrophages to the adaptive immune system. Both T cells and B cells initiate the adaptive immune responses. Activated T cells (T helper 1 (T_H1), T_H2 and T_H17 cells) and B cells produce inflammatory and pro-fibrotic cytokines. Activated B cells differentiate into plasma cells and produce autoantibodies that further perpetuate immune responses and tissue damage, contributing to inflammation autoimmunity and fibrosis. Chemokines and cytokines also activate natural killer (NK) cells, cytotoxic $CD8^+$ T cells and resident innate lymphoid cells (ILCs), exacerbating tissue injury and the release of potent pro-inflammatory and pro-fibrotic cytokines and growth factors. Potent cytokines and growth factors stimulate the activation of fibroblasts and their subsequent differentiation to myofibroblasts (which are αSMA positive). These cells produce excessive extracellular matrix (ECM) proteins, which leads to tissue contraction, scarring and fibrosis, which is typically observed in SSc. This multi-faceted process underscores the complexity of SSc pathogenesis, involving both innate and adaptive immune systems, leading to fibrosis and clinical manifestations of the disease. Regulatory T (T_{REG}) cells are a specialized subset of $CD4^+$ T cells that have a critical role in maintaining immune tolerance, preventing autoimmunity and modulating inflammation. In SSc, circulating T_{REG} cells are reduced and their suppressive function impaired, contributing to disease pathogenesis, including autoimmunity, chronic inflammation and fibrosis.

therapeutics. In addition, emerging interest in enhancing endogenous anti-fibrotic pathways offers new opportunities for boosting blunted or suppressed anti-fibrotic responses in SSc and other fibrosing diseases[71,72].

Detecting early-stage SSc remains an important challenge as this stage of disease could be the most responsive to disease-modifying therapies (Fig. 1). The Very Early Diagnosis of Systemic Sclerosis (VEDOSS) classification criteria help to identify disease risk, but studies[73–75] show that autoantibodies often appear years before the onset of overt disease. The levels of antibodies that target Ro52, Ro60 and CENP-A are elevated decades before disease manifests and remain high, whereas anti-RNA polymerase III and anti-topoisomerase I antibodies increase progressively and more rapidly as clinical diagnosis approaches[73]. Interestingly, both Ro52 and Ro60 proteins are linked to interferon, and a type I interferon signature in monocytes has been found during the earliest phases of SSc before overt fibrosis, suggesting that it is also an early event in the pathogenesis of the disease[76–78]. The relationship between these autoantibodies and the interferon signature should be explored in more depth as it can provide hints as to what triggers the disease. The data obtained thus far suggest that early biomarkers (such as autoantibodies and a type I interferon profile) could be crucial for understanding and predicting disease onset; however, reliable biomarkers for disease activity and progression are still lacking. Deploying powerful new technologies in the framework of collaborative networks to share biological samples from well-characterized and diverse patient cohorts is crucial. Research should also focus on unique populations with high disease prevalence or specific genotypes, and twin studies that can offer insights into the complex interplay and relative roles of genetics and environmental factors that affect the epigenome are key to understanding the disease[79,80]. Promoting interdisciplinary research, international collaboration and standardized data collection will help to identify new biomarkers. Integrating multi-omics data and refining preclinical models will advance SSc research and facilitate patient selection for clinical trials.

Novel technological breakthroughs and biomarker discovery

Clinical progress often results from technological breakthroughs; for example, the discovery of the patch clamp technology, the invention of cryo-electron microscopy, the development of state-of-the-art light microscopy, the advancement of genomic technologies and the improvements in computational methods. Technological advances have had a crucial influence on SSc research. Innovations such as next-generation sequencing have accelerated analyses of genome sequence variation, gene expression and epigenetic markers. Next-generation sequencing has demonstrated that messenger RNA expression varies with clinical subsets of SSc, and with progression of the disease[14,81–85]. Molecular classifications have been used to stratify patients in clinical trials such as for abatacept (as a post hoc analysis)[86], and in HSCT. In many cases, it is clear that only a subset of patients respond to a specific treatment. Machine-learning algorithms can use gene expression data for molecular classification in clinical trials, and gene expression could help to predict outcomes, such as the modified Rodnan Skin Score (mRSS)[87–89]. As the volume of data increases, AI methods will become increasingly important for data analysis.

Sequencing individual cell transcriptomes from skin sample biopsies has permitted detailed characterization of the cell types that are present, including fibroblasts, macrophages, lymphocytes, endothelial cells and keratinocytes. Advances in bioinformatics and computational methods have provided insights into spatial interactions between different cell types and provided a basis for hypotheses about their role in the disease[90–92]. The advent of spatial transcriptomics, which enables gene expression to be imaged at a single-cell resolution in histological sections, has provided further insight into active gene programs and into interactions among different cell types involved in disease development[66,93,94]. Single-cell-resolved spatial protein analysis has also progressed, including techniques that can pinpoint activity states in cellular signalling cascades by reflecting dynamic protein interactions and post-translational modifications[95]. Automated detection of numerous proteins in situ with advanced bioinformatics enables comparison of data across laboratories, which is crucial for research into rare diseases[96]. Standardization of procedures and the development of novel in vitro systems, such as organoid models and tissue slices, could enhance drug testing and understanding of SSc[97].

Methods have also become available that permit the levels of thousands of proteins to be measured in minute amounts of sample, such as blood plasma and tissue lysates. These methods use DNA aptamers[98] or pairs of oligonucleotide-conjugated antibodies[99] to detect specific proteins in the blood. Thanks to the development of AI-based machine

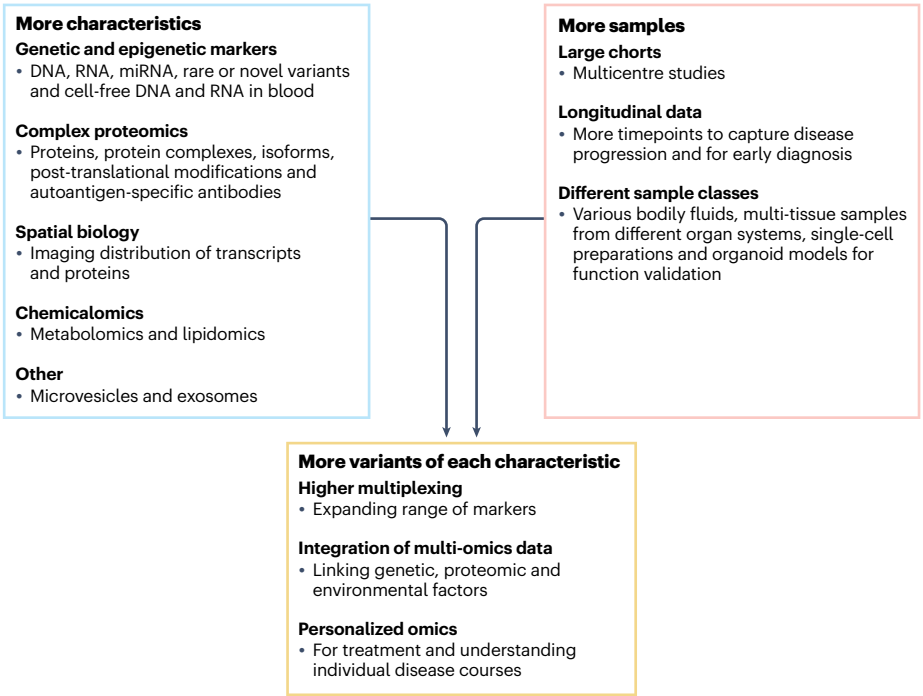


Fig. 4 | Omics for disease differentiation and precision medicine in systemic sclerosis. Omics technologies can be used for the analysis of samples from patients with systemic sclerosis. Comprehensive molecular profiling includes genetic profiling of DNA, RNA, epigenetic signatures, microRNA (miRNA) and rare variants, alongside proteomics that focus on proteins, immune system markers, post-translational modifications and rare protein isoforms. Additionally, metabolomics and lipidomics provide deeper insights into disease mechanisms. There is an emphasis on collaboration across groups to assemble large patient numbers, the use of rare disease and highly stratified cohorts and longitudinal data collected at multiple time points. Tissue biopsies, single-cell analysis and multi-tissue sampling facilitate a comprehensive understanding of the disease across different tissues and organ systems. Higher multiplexing technologies enrich the detection of multiple genetic and protein variations, and the integration of multi-omics data (genomics, proteomics and metabolomics) paves the way for the deployment of personalized treatment approaches that are based on the unique molecular profile of each patient.

analyses, progress in the analysis of protein expression patterns with diagnostic and prognostic value can be anticipated. The analyses can be applied to blood samples collected by patients via finger prick that can be sent and stored in a dry state for future analysis[100]. This technology paves the way for convenient measurements of protein levels after repeated sampling, sensitively reflecting disease processes and/or response to therapy. Profiling of blood protein levels serves as a diagnostic tool in SSc[101], as has been reported for many forms of cancer[102]. Similar methods can also be used for comprehensive measurements of autoantibody repertoires in individual patients.

To exploit technological breakthroughs in SSc research it will be important to build biobanks with samples that are compatible with these emerging technologies. Specifically, fresh-frozen or formalin-fixed tissue sections are required to take advantage of spatial transcriptomics. In patients with ILD, meaningful information can be provided by lung tissue obtained via cryobiopsy, which serves as an alternative to surgical lung biopsy, when performed by experienced hands using standardized protocols[103]. Similarly, blood samples, consecutively collected from large groups of individuals and inexpensively stored in a dry state, will enable monitoring of disease progress and responses to therapy. Such samples will also be crucial to the identification of blood biomarkers and other robust biomarkers, such as collagen-derived peptides, which are indicative of ECM formation or degradation, the altered expression of which could be used to predict onset of disease. Protein biomarkers will assume increasing importance if and when methods become available to guide early treatment and avert disease progression. Repeated blood sampling and molecular imaging (such as fibroblast-activation protein quantification with PET-CT) offers a way of monitoring disease-relevant events. Combining these advances with systematic research approaches will help to identify new therapeutic targets and biomarkers, paving the way for improved clinical trials and treatment strategies (Fig. 4).

Emerging therapies and translational research opportunities

Numerous novel treatment strategies with a wide variety of distinct mechanisms are being explored in clinical trials of SSc (Table 1). The current pharmacological and non-pharmacological approaches, such as organ transplantation, and their clinical development phases have been reviewed elsewhere[41,103,104]. Thus far, no therapy that targets a single cell, pathway or molecule has been shown to induce long-term drug-free full remission of any autoimmune disease. Cell-based and targeted cellular depletion therapies are emerging as options for selectively modulating the immune response, mitigating vascular damage and the symptoms of Raynaud phenomenon, and also slowing or reducing fibrosis in skin and other organs, and promoting tissue repair (Table 2). Important developments include the use of CAR T cells, particularly CD19-targeting CAR T cells, which were shown to have clinical efficacy and relative safety in patients with SSc, along with other autoimmune diseases[105–107]. Although data are still limited, early evidence suggests that treatment with anti-CD19 CAR T cells might be better tolerated than autologous HSCT. This therapy might offer a more complete depletion of $CD19^+$ cells than B cell-depleting antibodies such as rituximab[108], potentially resetting the immune system. Despite the tremendous promise of this treatment and the surrounding great excitement, careful longitudinal studies are needed to confirm these findings, optimize the treatment protocols and patient selection criteria and explore the persistence of antibodies against nuclear antigens. Autologous HSCT, which 'resets' the immune system, has demonstrated substantial clinical benefit in SSc, particularly in improving skin manifestations, vascular changes and lung function[109–111]; however, HSCT carries a risk of adverse effects[112], but advances in the past 2 years have improved these shortcomings[113]. The use of mesenchymal stem cells and harnessing the immunomodulatory and anti-inflammatory properties of these cells have shown promise in

early clinical trials. Ongoing research is focused on optimizing delivery methods, optimal therapeutic range, frequency of administration and understanding the long-term effects of MSC-based therapy. Finally, randomized controlled trials are required to provide definitive evidence of the efficacy of MSC-based therapy and differences from HSCT[114,115]. Research into the use of induced pluripotent stem cells, which remains at the preclinical stage, focuses on the potential of induced pluripotent stem cells to regenerate damaged tissues and

Table 1 | Summary of recently completed placebo-controlled clinical trials in systemic sclerosis (SSc)

Trial	Trial design	Drug	Outcomes assessed	Summary of trial results
faSScinate[46]	48 weeks (*n*=48)	Tocilizumab	mRSS[b] FVC HAQ-DI PtGA PhGA	The primary endpoint was mRSS at 24 weeks, with a trend favouring tocilizumab reported at 24 and 48 weeks (strong trend at 48 weeks). Exploratory analysis of FVC was highly statistically significant at 24 and 48 weeks. Post hoc CRISS analysis was significant (all nominal)
focuSSced[10]	48 weeks (*n*=212)	Tocilizumab	mRSS[b] FVC[b] HAQ-DI PtGA PhGA	Primary endpoint of mRSS at 48 weeks, with a beneficial trend reported. Highly statistically significant (nominal) and clinically meaningful benefit in key secondary end point of FVC. Post hoc analysis of SSc-ILD subgroup supported regulatory approval of tocilizumab for SSc-ILD
ASSET[131]	52 weeks (*n*=88)	Abatacept	mRSS[b] HAQ-DI FVC CRISS PtGA PhGA	Primary endpoint of mRSS at 52 weeks, with a beneficial trend favouring abatacept reported. Secondary endpoint of HAQ-DI showed significant benefit for abatacept (nominal). Post hoc analysis supports selective benefit for skin in inflammatory intrinsic molecular subset
RISE-SSc[130]	52 weeks *n*=121	Riociguat	mRSS[b] CRISS HAQ-DI FVC PtGA PhGA	Primary endpoint of mRSS at 52 weeks with a beneficial trend favouring riociguat reported. Subgroup analysis confirmed significant benefit in anti-RNA polymerase antibody-positive subgroup (nominal). Group-level improvement in placebo arm less than other studies reflecting enrichment for early disease and low mRSS
JBT-101-SSc[137]	38 weeks (*n*=38)[a]	Lenabasum	CRISS[b] mRSS SSPRO FVC	The overall results of this study were promising. CRISS responder rate significantly favoured lenabasum with trends of benefit in components of this composite score
RESOLVE-1 (ref. 138)	52 weeks (*n*=365)[a]	Lenabasum	CRISS[b] mRSS[b] FVC	CRISS response rate was excellent but similar across all study groups consistent with effective background immunosuppression, particularly MMF. Possible minor treatment effect from lenabasum in post hoc subgroup analysis
FASST[8]	48 weeks (*n*=145)[a]	Lanifibranor	mRSS[b] FVC PtGA	No benefit in active treatment arms for mRSS or other endpoints. Lanifibranor is currently under evaluation in liver fibrosis
SENSCIS[11]	52 weeks (*n*=580)[a]	Nintedanib	FVC[b] mRSS[b] SGRQ[b]	The overall results of this study were promising. Primary endpoint of FVC decline at 52 weeks favoured nintedanib. Numerically greater effect when combined with MMF. No apparent benefit for mRSS or SGRQ
SAR156597-dcSSc[139].	24 weeks (*n*=97)[a]	Romilkimab	mRSS[b] FVC HAQ-DI EQ-5D-5L	The overall results of this study were promising. Primary endpoint of mRSS favoured active treatment, especially in participants with more severe baseline skin disease. Benefits were also reported in some exploratory endpoints. This study provides a feasibility template for phase II trials in dcSSc
NOVESA[140]	24 weeks (*n*=33)[a]	Ziritaxestat	mRSS[b]	The overall results of this study were promising for mRSS at 26 weeks, but the difference observed was below the minimal clinical significance for skin
DESIRES[141]	24 weeks (*n*=56)	Rituximab	mRSS[b] FVC	The overall results of this study were promising with benefit for skin (mRSS) and lung (FVC) reported. Supported regulatory approval for SSc in Japan
CERTA[142]	12 weeks (*n*=30)[a]	FT011 (asengeprast)	CRISS[b] mRSS	The overall results of this small phase II trial were promising and showed benefit for CRISS and some components of CRISS, including FVC and HAQ-DI

[a]Trial participants could have background immunosuppressive treatments. [b]Primary and key secondary endpoints of each trial. CRISS, Composite Response Index in Systemic Sclerosis; dcSSc, diffuse cutaneous SSc; EQ-5D-5L, Euroqol generic health status measure; FVC, forced vital capacity; HAQ-DI, Health Assessment Questionnaire Disability Index; ILD, interstitial lung disease; MMF, mycophenolate mofetil; mRSS, modified Rodnan skin score; PhGA, physician global assessment of disease status; PtGA, patient global assessment of disease status; SGRQ, St. George's Respiratory Questionnaire; SSPRO, Scleroderma Skin Patient-Reported Outcome.

Table 2 | Approaches to cell-based therapies in systemic sclerosis

Cells that could be targeted with cellular therapy	Approach to cell-based therapy and effects
B cells	Cell ablation to reduce B cells and pro-inflammatory and pro-fibrotic cytokines
Monocytes	Cell ablation to reduced pro-inflammatory cells
Macrophages	Differential cell polarization (from 'M1-like' to 'M2-like') to reduce inflammation
NK cells	Modulation of differential NK function
Fibroblasts	Pathogenic cell subset ablation
DCs	Cell ablation to suppress the production of pro-inflammatory cytokines
T cells ($CD4^+$, $CD8^+$, T_H1, T_H2, T_H17 cells)	Approaches include T cell depletion, checkpoint inhibition, inhibition or modulation of pro-inflammatory cytokines, growth factors and co-receptors
Innate lymphoid cells (ILC2)	Modulating the function of ILCs by targeting pro-inflammatory cytokines, cell signalling and ILC-related cytokines
Cells that could be used for cellular therapy	
B_{REG} cells	Immunosuppressive (via the release of IL-10), maintain tolerance and regulate autoreactive B cells and autoantibodies
Monocytes	Specific cell polarisation to induce tissue repair
T_{REG} cells	Restore T_{REG} cell function (via administration of IL-2), adoptive transfer or immune suppression (pro-inflammatory cytokines)
MSCs	Immune modulation and tissue repair
iPSCs	Regeneration and repair of tissues
MDSCs	Cell-based therapeutics
HSCs	Cell replacement and immune reset
CAR T cells	Selective immune cell ablation

B_{REG} cells, regulatory B cells; CAR T cells, chimeric antigen receptor T cells; DCs, dendritic cells; HSCs, haematopoietic stem cells; ILCs, innate lymphoid cells; iPSCs, induced pluripotent stem cells; MDSCs, myeloid-derived suppressor cells; MSCs, mesenchymal stem cells; NK cells, natural killer cells; T_{REG} cells, regulatory T cells.

the development of patient-specific therapies with a reduced risk of immune rejection compared with allogeneic stem cells or other transplantation methods requiring immunosuppression. Other cell-based therapies under investigation include modulation of regulatory T cells that can restore immune tolerance; preclinical studies and early clinical trials have shown the potential of this therapeutic approach[116]. The role of regulatory B cells has been investigated in in vivo models of SSc in order to test the ability of these cells to modulate autoimmunity and fibrosis[117,118]. Moreover, several other immune cell types are also under investigation, such as monocyte and macrophage (M1-like (classically activated) and M2-like (alternatively activated)) subsets, for which direct cell reprogramming and metabolism (via CD38) and polarization strategies to effect tissue repair have been the focus. Modulating the activity of both dendritic cells (and other antigen-presenting cells) and natural killer cells is also under investigation; these approaches focus on regulating the immune responses and harnessing cell cytotoxic potential to treat autoimmune disease[119].

Another promising area involves fibroblast-targeting therapies; in this setting the design of non-viral vectors for nucleic acid delivery represents a promising perspective. These approaches are aimed at selectively modulating disease-relevant pathways in fibroblasts[120], potentially minimizing adverse effects on other cell types. Cell-surface markers, such as fibroblast-activated protein, are being investigated for drug targeting and liposomal carrier coating[121]. A particularly fast-moving area of research focuses on the identification and characterization of distinct fibroblast subpopulations. Such cellular heterogeneity, only uncovered with the advent of single-cell transcriptomics, indicates that not all fibroblasts in lesional tissue are the same, with some subpopulations being relevant to disease progression and associated with specific cell-surface markers[65,66,122]. Cell-based therapies offer hope for more effective and targeted treatments for SSc, although further research is needed to establish their safety, efficacy and long-term outcomes.

Advances in the development of in vitro models include precision-cut skin and lung slices, which, when combined with omics techniques and bioinformatic methods, enable the testing of new therapies and the personalization of treatments. These precision-cut skin slices retain cellular niches, but unlike ex vivo skin biopsies, this method ensures that the entire specimen receives adequate oxygen and nutrition supply via diffusion. Furthermore, multiple slices can be created from a single biopsy, enabling a direct comparison of different therapies across slices from the same sample. The changes in these skin slices in response to test compounds or therapies can be analysed in an unbiased manner through the use of omics approaches. Precision-cut skin slices can be utilized as an ex vivo trial approach for human SSc skin, but they can also be used to select optimal treatments for individual patients on the basis of molecular responses[20,123,124]. Promising preliminary observations indicate that the molecular response to currently used therapies in precision-cut skin slices faithfully predicts clinical responses. This approach could thus be used to guide individualized treatment selection. Synthetic 3D skin-like tissues provide another in vitro model for studying SSc[125,126]. These tissues are constructed using cells from individuals with SSc and have been shown to recapitulate key features of SSc skin, including increased tissue thickness, stiffness, fibrosis and the activation of immune and fibrotic pathways. These in vitro tissues enable the study of cell–cell and cell–matrix interactions that are not captured in 2D culture and provide an alternative preclinical testing model for potential therapeutics.

Designing more successful clinical studies

As already noted, no approved disease-modifying treatment exists for SSc, partly owing to challenges in conducting effective clinical trials. The heterogeneous nature of SSc, the absence of reliable biomarkers for disease monitoring, the lack of validated outcome measures and variable clinical course complicate clinical trials[127]. To overcome these obstacles, the SSc community has collaborated to propose innovative trial designs and updated protocols that are based on emerging knowledge (Table 1); novel strategies have focused on cohort enrichment and outcome measure selection[127–129]. For example, the RISE-SSc trial using riociguat targeted patients with dcSSc at a high risk of skin fibrosis progression[130]. The trial did not meet its primary endpoint, as it showed no improvement in mRSS in the treatment group; however, some patients did show rapid mRSS improvement, which highlights the limitations of current patient selection approaches[130]. The ASSET trial that evaluated abatacept used a definition of 'active disease' for cohort enrichment; however, mRSS improvement was reported in both

the active treatment and placebo groups, indicating that active disease alone was insufficient for cohort enrichment[131,132]. In the same study, gene expression profiling of biopsy-obtained skin samples and stratification by molecular subset revealed differences in mRSS progression and treatment response. One subgroup of patients, termed the 'inflammatory subset', showed strong activation of the CD28 co-stimulatory pathway, which is the target pathway of abatacept[131]. The focuSSced trial applied cohort enrichment on the basis of active disease and inflammatory markers but did not meet its primary endpoint[10]. Nevertheless, this trial highlighted the effectiveness of tocilizumab in preventing the decline of forced vital capacity in patients with SSc-ILD, leading to FDA approval for SSc-ILD[9] (Table 1).

Key observations from these clinical trials are that early disease (duration <18 months) and mild skin thickening are useful for cohort enrichment[129]. Molecular measures of heterogeneity, such as gene expression in skin or blood can clearly be used as secondary endpoints, as demonstrated in the ASSET clinical trial. Combining autoantibody information, such as excluding patients who are anti-centromere antibody-positive, can be informative. Disease subtype alone is insufficient for cohort enrichment for those with skin progression. Combining biomarkers and omics data could improve cohort enrichment and treatment-response prediction. Inflammatory gene expression patterns in skin predict subsequent skin thickening and responses to certain treatments but this method is not yet viable for patient selection[83].

The RESOLVE-1 trial using lenabasum, which had minimal cohort enrichment, used the ACR-CRISS (ACR Composite Response Index in Systemic Sclerosis), but the high score in the placebo group indicated that background immunosuppressive treatments might have influenced results[114]. The SENSCIS trial, by contrast, successfully demonstrated the efficacy of nintedanib in SSc-ILD, with a significant reduction in the decline of forced vital capacity over 52 weeks and enrolled a broad range of patients with SSc-ILD[11] (Table 1). Challenges remain in using mRSS as a primary endpoint owing to its tendency to improve over time, necessitating cohort enrichment. Composite measures such as ACR-CRISS have shown varied results[133], and new endpoints such as revised CRISS-25 and wearable devices could offer future solutions[134]. In the future, the use of omics data to identify reliable biomarkers and rapid skin gene expression profiling will be essential for selecting patients likely to respond to a given therapy and for evaluating therapeutic efficacy. The platform clinical trial adopted by CONQUEST could be an alternative trial design that accommodates sample-size reduction and robust patient participation, as the study allows multiple therapies to be evaluated under a common trial infrastructure with a common control arm[135]. In this trial setting, innovative designs such as digital twins (that is, virtual versions of real-world objects), connected devices, AI and mathematical modelling could be proposed to test or validate personalized therapeutic strategies in groups of patients stratified according to specific biomarkers. Patient-centred trial design that involves patient organizations is crucial for addressing real-world medical needs.

Future perspectives

Studying rare diseases such as SSc and designing therapeutic trials requires a specific approach. International cooperation on a global level has been successful in the collection of sufficient data from well-characterized cohorts of patients. The desirability of this approach to research has already been recognized by the scientific community, which is reflected in the many publications for several other disease entities, which list several specialized centres and research groups as co-authors.

There was, therefore, a clear consensus that emerged at this symposium that these developments need to be strengthened and brought from national and regional levels to a global level. The long-term goal should be creating a common database that combines clinical investigations involving regular follow-up of patients with the molecular and cellular analysis of biopsy-obtained skin samples and blood samples. This approach will require complex organization of multiple centres taking into consideration all legal and ethical aspects of data collection and transmission within and between networks on national, regional and intercontinental levels.

However, the rapid developments of methods of molecular and cellular analyses combined with computational methods, including AI and detailed clinical investigations, now offer a unique opportunity to better understand this complex, heterogeneous disease and to develop personalized therapeutic interventions. Determining a common definition of SSc and the development of a unified classification for all disease subtypes is essential. A consensus for common protocols for clinical trials (encompassing both inclusion criteria and treatment regimens) is required, and systematic cooperation with industrial partners early on in clinical trials will be mandatory. Biopsies and blood samples should be collected from all patients under standardized conditions and analysed using state-of-the-art technologies, including single-cell RNA sequencing, as well as proteomic and metabolomic approaches, to generate an interactive digital atlas for SSc. The atlas would enable users to generate a geographic map of SSc rates, risk factors, screening statistics and other biological, genomic and epigenomic data to contribute to a more uniform approach and consensus guide for clinical trials and scientific research.

There is also a need for consensus regarding the selection of animal models and other cell and organoid-based in vitro models for testing novel hypotheses developed from basic research studies and for screening new compounds, which again needs to be achieved in cooperation with industrial partners. Animal models, although widely used to study SSc, need to be improved in terms of their initiation, response to injury, organ involvement and outcome to better reflect the complex pathogenesis of SSc.

Large data sets obtained from next-generation DNA sequencing techniques (including analysis of non-coding sequences, somatic mutations and methylation) need to be correlated with clinical, genetic and additional molecular data to identify very rare mutations in selected patients and their families as this information could have important implications for understanding SSc in general. Selecting populations and/or phenotypes that provide the largest signal-to-noise ratio (that is, to ensure phenotypic homogeneity within groups to enable the study to detect meaningful biological differences while avoiding confounding factors or variability) is critical to success. An excellent example is the GRASP study[136], which has focused on African Americans with SSc who have a more severe disease and poorer outcomes than reported in cohorts of predominantly European ancestry. Based on large cohorts of very well-characterized patients, international consortia (including the scleroderma clinical trials consortium and the EULAR Scleroderma Trials and Research) will be able to rapidly carry out specific trials and evaluate distinct, currently unanswered, questions. Examples of studies that are needed include studies that determine the value of short-period pilot studies, studies that evaluate the highly efficient elimination of B cells as a therapeutic advantage and studies that evaluate whether treatment of patients who meet the VEDOSS criteria for very early SSc might prevent further development of the disease. Precision-medicine approaches that could enrich studies for

populations that are most likely to improve on specific treatments could dramatically increase the success rate of trials and ensure that patients get the most effective treatment.

The discussion during the workshop demonstrated that the international SSc research community is maturing and coalescing. Several examples of how generating networks between centres can work effectively and patient cohorts have been generated in the USA, Europe, Canada, Australia and Japan. Several of these activities have initially been funded by national governments but often only for a short initial period. The participants of the workshop agreed that it is now time to join such individual activities to raise awareness for SSc in the medical community and the public to facilitate better and earlier diagnosis and treatment and to collaborate on different levels to secure national and international funding. In particular, the cooperation of the scientific community with industrial partners and patient organizations should advance research on SSc even further for the benefit of the patients.

Conclusions

The integration of cutting-edge and emerging research techniques into SSc studies is poised to transform the understanding of this complex disease. Omics analyses, including bulk and single-cell analyses, spatial transcriptomics, proteomics, epigenetics and comprehensive cell and protein atlases, coupled with computational analyses for pathways, cell types and genetic variations, offer unprecedented insights into the molecular and cellular underpinnings of SSc. As these technologies continue to evolve, they will have a crucial role in advancing precision medicine, identifying novel therapeutic targets and ultimately improving the lives of patients with SSc. The future of SSc research is bright, with these innovative approaches paving the way for important breakthroughs in diagnosis, treatment and patient care.

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