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# Research highlights

## Inflammation

### Blocking CDK7 attenuates inflammatory arthritis

RNA polymerase II pausing during the transcription cycle regulates the expression of numerous genes in macrophages, but how it influences inflammation has remained unclear. New research suggests that interfering with the transcription cycle by targeting the transcription kinase CDK7 could offer a way to treat rheumatoid arthritis (RA) and other inflammatory diseases.

The researchers found that inhibition of CDK7 can disrupt RNA polymerase II promoter-proximal pausing, and that this disruption has a profound anti-inflammatory effect. “This effect is rapid, transcriptional and quite unexpected,” notes Inez Rogatsky, corresponding author of the study published in *Science Translational Medicine*. “It is exciting that we were able to reverse a pre-established and ongoing inflammatory programme, not just ‘prevent’ it.”

Initial experiments in bone marrow-derived macrophages from mice showed that myeloid-specific genetic ablation of NELF, an important component of the RNA polymerase II pausing complex, led to increased expression of anti-inflammatory genes in response to stimulation with lipopolysaccharide. NELF ablation in macrophages also attenuated disease severity in mice with K/BxN serum transfer arthritis, a model of acute, macrophage-dependent arthritis.

The researchers then showed that RNA polymerase II pausing could be blocked pharmacologically in mouse and human macrophages using the CDK7 inhibitors THZ1 and YKL-5-124. Similar to genetic loss of NELF, both inhibitors suppressed the lipopolysaccharide-induced upregulation of pro-inflammatory genes and

downregulation of homeostatic and anti-inflammatory genes in mouse macrophages. CDK7 inhibition with YKL-5-124 was also able to disrupt RNA polymerase pausing and reprogramme the inflammatory transcriptome in human macrophages that had been incubated with TNF and IFN $\gamma$  for 24 h to induce inflammatory polarization.

In vivo, CDK7 inhibition reduced disease severity and duration in mice with K/BxN serum transfer arthritis and also inhibited inflammation and joint destruction in human TNF transgenic mice, a model of chronic progressive inflammatory arthritis. Finally, in synovial cells isolated from patients with RA, treatment with YKL-5-124 ex vivo suppressed the expression of genes and pathways related to inflammation and upregulated anti-inflammatory genes.

Notably, CDK7 has emerged as a promising therapeutic target in cancer, and CDK7 inhibitors have been well tolerated in early clinical trials in that setting. The present study suggests that targeting CDK7 also warrants attention as a potential treatment for RA and possibly other inflammatory diseases, although some questions remain. “It is not clear why pro-inflammatory genes and pathways are downregulated whereas those that signify a non-inflammatory, ‘homeostatic’ macrophage state are not, or are even upregulated,” notes Rogatsky, adding that further work is also needed to understand how CDK7 inhibition affects the many other cell types involved in the pathogenesis of arthritis.

**Sarah Onuora**

**Original article:** Chen, X. et al. Disrupting the RNA polymerase II transcription cycle through CDK7 inhibition ameliorates inflammatory arthritis. *Sci. Transl. Med.* **16**, eadq5091 (2024)

## Clinical trials

### Inebilizumab shows promise for IgG4-RD

Results from the phase III MITIGATE trial demonstrate the efficacy of CD19-targeted B cell depletion with inebilizumab for the treatment of IgG4-related disease (IgG4-RD), a condition for which there are currently no approved pharmacotherapies.

In the parallel-cohort, double-blind trial, conducted at 80 sites across 22 countries, 135 adults with active IgG4-RD were randomly assigned to receive treatment with inebilizumab or placebo; for all participants, glucocorticoid treatment was tapered over 8 weeks.

Over the 52-week treatment period, inebilizumab treatment reduced the risk of IgG4-RD flare by 87% compared with placebo; 10% of participants in the inebilizumab group and 60% of those in the placebo group experienced  $\geq 1$  disease flare. More participants in the inebilizumab group achieved flare-free, treatment-free complete remission (57% versus 22%) at 52 weeks. During the treatment period, 90% of the inebilizumab group discontinued glucocorticoid treatment entirely, compared with 37% of the placebo group.

The rate of adverse events was similar across both groups, although serious adverse events were more frequent among those who received inebilizumab (19% versus 9%). Long-term studies are needed to confirm the safety and efficacy of inebilizumab treatment for IgG4-RD.

**Sarah Onuora**

**Original article:** Stone, J. H. et al. Inebilizumab for treatment of IgG4-related disease. *N. Engl. J. Med.* <https://doi.org/10.1056/NEJMoa2409712> (2024)

**Related article:** Perugino, C. A. & Stone, J. H. IgG4-related disease: an update on pathophysiology and implications for clinical care. *Nat. Rev. Rheumatol.* **16**, 702–714 (2020)

## Myositis

### Abatacept for myositis

Encouraging results from case reports and early trials suggested that abatacept could be an effective treatment for idiopathic inflammatory myopathy (IIM). Now reported in *Arthritis & Rheumatology*, a phase III clinical trial of abatacept for IIM failed to meet its primary endpoint, but analysis of the results suggests that abatacept could have benefits in some subtypes of IIM.

In the multicentre, international trial, 149 adults with active, treatment-refractory IIM were randomly allocated to receive subcutaneous abatacept (125 mg weekly) or placebo, in combination with standard treatment. At 24 weeks, 56.0% of the abatacept group and 42.5% of the placebo group met the IMACS definition of improvement. In additional analysis, no between-treatment differences were observed among patients with dermatomyositis, but among those with polymyositis or immune-mediated necrotizing myopathy response rates were higher in the abatacept group than in the placebo group (57.1% versus 32.3%).

Following a further 24-week open-label period, 69.8% of those who continued abatacept and 69.0% of those who switched to abatacept from placebo met the IMACS definition of improvement, suggesting a sustained benefit with abatacept up to 1 year. The addition of abatacept to standard treatment was safe and generally well tolerated in the trial.

**Sarah Onuora**

**Original article:** Aggarwal, R. et al. Efficacy and safety of subcutaneous abatacept + standard treatment for active idiopathic inflammatory myopathy: phase III randomised controlled trial. *Arthritis Rheumatol.* <https://doi.org/10.1002/art.43066> (2024)

## Rheumatoid arthritis

# Synovial dendritic cell subsets in RA

Dendritic cell (DC) phenotypes determine the tolerogenic or pro-inflammatory outcome of an immune response. A study published in *Immunity* “sought to explore the role of synovial DC subsets in shaping tissue niches and regulating immune tolerance versus autoimmunity in rheumatoid arthritis (RA),” as Mariola Kurowska-Stolarska, co-corresponding author, explains. “This huge piece of work leverages multiple technologies to detail the subtypes of synovial DCs in health and active RA, their differentiation pathways from blood and their interactions with CD4<sup>+</sup> T cells, giving new insight into the development of synovial niches in active RA as well as into how remission is initiated or lost,” notes Ranjeny Thomas, researcher of DC biology in RA who was not involved in the study.

The most dominant DC subset in the healthy synovium, located primarily beneath the macrophage-populated lining layer, was a DC2 population that expressed the immune checkpoint AXL and genes implicated in tolerance or tissue homeostasis. AXL<sup>+</sup> DC2s appeared to develop from blood-derived KLF4<sup>+</sup> DC2s via a KLF4<sup>+</sup> ATF3<sup>+</sup> intermediate.

Tissue-infiltrating KLF4<sup>+</sup> DC2s also appeared to give rise to a CCR7<sup>+</sup> LAMP3<sup>+</sup> DC2 subset in the sub-lining layer of the synovium

of patients with active RA. This so-called mReg subset required expression of microRNA-155 for its differentiation and was associated with both an immunogenic and a regulatory gene signature, indicating context-dependent function. In addition, the most dominant synovial DC phenotypes in active RA were an ALDOA<sup>+</sup> DC3 population that expressed the type I interferon receptor and a FABP5<sup>+</sup> inflammatory DC3 (iDC3) subset that expressed the receptors for the cytokines IL-6 and TNF.

All pro-inflammatory DC subsets populated the hyperplastic lining layer of the synovium of patients with active RA but were found to be downregulated during remission. However, the tolerogenic AXL<sup>pos</sup> DC2 subset was not restored in patients with RA in remission, although its KLF4<sup>pos</sup> DC2 and KLF4<sup>pos</sup> ATF3<sup>pos</sup> DC2 precursors accounted for some anti-inflammatory gene expression.

mReg cells localized within ectopic germinal centres of the RA synovium where they interacted mainly with naive T cells. “This indicates that mReg cells are likely to fuel the generation of autoreactive T cells in tissue,” comments Kurowska-Stolarska. By contrast, DC3s and iDC3s were localized in myeloid-rich niches and were required for the activation of CCL5<sup>+</sup> T effector memory cells and their

differentiation into CCL5<sup>+</sup> T peripheral helper cells. Further supporting a key role for iDC3s in the perpetuation of pathogenic T cell responses, analyses of blood DC subsets in patients with RA in remission associated RA flares with the upregulation of activation-associated genes, such as integrins, pattern recognition receptors and alarmins, in circulating iDC3s.

“It now remains to be seen whether DCs in [the synovium of] patients remaining in drug-free remission return to the status of healthy [synovium],” notes Thomas, adding that it will also be important to delineate the antigens being presented by remission- and flare-associated DCs in RA synovial tissue. In the meantime, “therapeutic strategies to block the pathogenic functions of the iDC3s and their blood predecessors and reinstating the tolerogenic functions of the AXL<sup>+</sup> DC2 cluster might be the necessary step-change for the resolution of synovitis and transition from remission into self-maintained tolerance,” suggests Stefano Alivernini, co-corresponding author of the study.

**Maria Papatriantafyllou**

**Original article:** MacDonald, L. et al. Synovial tissue myeloid dendritic cell subsets exhibit distinct tissue-niche localization and function in health and rheumatoid arthritis. *Immunity* **57**, 2843–2862.e12 (2024)

# The emergence of SLE-causing UNC93B1 variants in 2024

George C. Tsokos

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During the past year, four studies have reported ten mutations in *UNC93B1*, which encodes the Toll-like receptor (TLR) chaperone protein UNC93B1. All variants increased TLR7 and TLR8 signalling and caused systemic lupus erythematosus in young individuals, and highlight the therapeutic potential of targeting TLR7 and TLR8 in this disease.

Genetic factors are major contributors to the pathogenesis of systemic lupus erythematosus (SLE). Genome-wide association studies have revealed numerous loci linked to an increased risk of SLE which, in most cases, are not in gene expression or regulatory sequences. In the past 5 years, whole-genome sequencing studies have identified variants of several genes involved in apoptosis, nucleic acid degradation and sensing, regulation of the interferon pathway, adaptive immune tolerance and metabolism<sup>1</sup>. In 2024, four publications reported ten mutations in *UNC93B1* (which is found on chromosome 11 (q13.2) and encodes the Toll-like receptor (TLR) chaperone protein UNC93B1) that cause SLE, mostly in young individuals<sup>2–5</sup> (Table 1).

TLRs were the first pattern-recognition receptors for pathogen-associated molecular patterns to be recognized and are the most studied molecules of this class. Among TLR family members, the single-stranded RNA-sensing TLR7 and TLR8 and the single-stranded DNA-sensing TLR9 have been shown to contribute to autoimmune pathology in both mice and humans, and a gain-of-function mutation in TLR7 is known to cause SLE<sup>6</sup>. TLRs are synthesized in the endoplasmic reticulum and subsequently transported to plasma or endosomal membranes. TLR7 and TLR9 are transported via an association with the chaperone protein UNC93B1. UNC93B1 is a 597-amino-acid, 12-fold transmembrane protein that is vital for the folding, stability and function of TLRs in addition to their proper localization<sup>7</sup>. UNC93B1 has several domains, each with a distinct function.

UNC93B1 remains associated with TLRs that have been delivered to endosomes and has an active regulatory role there. One such role is the dampening of TLR7 signalling that occurs via the recruitment of the cytosolic protein syntenin-1. In addition, the regulated release of TLR9 from UNC93B1 is necessary for the binding of TLR9 to its ligand CpG and subsequent signalling. Mutations generated in vitro that inhibit the release of UNC93B1 from TLR9 result in defective TLR9 signalling. Notably, distinct mutations in *UNC93B1* have differential effects on TLR9 signalling and TLR7–TLR8 signalling, which suggests that similar mutations in humans could contribute to the diversity of SLE clinical phenotypes<sup>8</sup>.

In the first of the four reports of new *UNC93B1* variants reported in 2024, Wolf et al.<sup>2</sup> reported two UNC93B1 variants in four patients with

early onset SLE – E92G in two siblings and R336L in a father and son. Both variants caused the production of high amounts of TNF and IL-6 after stimulation with TLR7 and TLR8 agonists but not with TLR9 agonists. A subsequent study by Rael et al.<sup>3</sup> reported an UNC93B1 variant that lead to tumid lupus in three siblings (two female and one male) and their father, and another variant that lead to juvenile idiopathic arthritis in one female patient. The first mutation, T93I, is located in the intraluminal loop that connects the first and second transmembrane pass of UNC93B1, and the other, R336C, is in the cytosolic loop that connects the sixth and seventh transmembrane pass of UNC93B1. Both mutations caused increased TLR7 and TLR8 responses without affecting TLR9 responses. It is unclear whether the location of these mutations determines the clinical phenotype or whether these variants contribute to the effects of other pathogenetic factors. Moreover, Al-Azab et al.<sup>4</sup> presented one patient with a T314A mutation and seven patients with a V117L mutation, all of whom presented with childhood-onset SLE. Interestingly, several family members carrying the same mutations were asymptomatic, whereas insertion of the V117L mutation in mice caused lupus-like pathology. Finally, David et al.<sup>5</sup> reported UNC93B1 variants in five families, each with a distinct mutation that caused SLE or chilblain lupus. SLE associated with these mutations was inherited as either an autosomal dominant or a recessive trait. Two variants (I317M and G325C) caused gain-of-function of TLR7 and to a lesser extent of TLR8 and presented clinically as SLE, whereas three variants (L330R, R466S and R525P) caused increased TLR8 activity and presented as chilblain lupus. Four out of five of these mutations were heterozygous and led to the development of disease; only one of these genes required homozygosity for disease to develop. Notably, some of the heterozygous mutations were inherited from asymptomatic parents.

In almost all presented cases of human UNC93B1 variants, TLR7 activity was enhanced whereas TLR9 responses were unaffected, indicating that the various domains of UNC93B1 have diverse functions that

## Key advances

- The UNC93B1 variants E92G and R336L have been identified in four patients with early onset systemic lupus erythematosus (SLE) and were found to cause disease via TLR7 hyperactivation<sup>2</sup>
- The UNC93B1 variants T93I and R336C were found in two families; these variants promote tumid lupus and juvenile idiopathic arthritis, respectively, via enhanced TLR7 and TLR8 responses<sup>3</sup>
- The UNC93B1 variants T314A and V117L were reported in a cohort of East Asian individuals with childhood-onset SLE; these variants promoted disease via exaggerated TLR7 and TLR8 responses<sup>4</sup>
- Five UNC93B1 variants identified in five families caused SLE or chilblain lupus depending on enhancement of TLR7 or TLR8 signalling<sup>5</sup>

**Table 1 | UNC93B1 variants that cause SLE and inflammatory disease**

Study	Variant(s)	Zygoty	Clinical phenotypes	Mechanism	Expression of variants in mice
Wolf et al. <sup>2</sup>	E92G	Homozygous	Early onset SLE in two siblings (one female, one male), both parents were heterozygous and healthy	Pro-inflammatory cytokine production (TNF and IL-6) upon stimulation with TLR7 or TLR8 agonist, but not with TLR3 or TLR9 agonists	Not performed
	R336L	Heterozygous	Early onset SLE in a male and his father		
Rael et al. <sup>3</sup>	T93I	Heterozygous	Cutaneous tumid lupus with early childhood-onset in three siblings (two female, one male) and their father	Enhanced TLR7 and TLR8 responses	Mice that expressed T93I developed systemic autoimmune pathology
	R336C	Heterozygous	Juvenile idiopathic arthritis in one female		
Al-Azab et al. <sup>4</sup>	T314A	Heterozygous	Childhood-onset SLE in one female	Increased expression of NF- $\kappa$ B-dependent cytokines and enhanced responses to TLR7 and TLR8 but not TLR3 and TLR9 stimulation	Not performed
	V117L	Heterozygous	Childhood-onset SLE in seven females		
David et al. <sup>5</sup>	I317M	Homozygous	SLE in one female	Gain of TLR7 and TLR8 activity and normal TLR3 and TLR9 activity	Not performed
	G325C	Heterozygous	SLE in one female		
	L330R	Heterozygous	Chilblain lupus in one male		
	R466S	Heterozygous	Chilblain lupus in one female		
	R525P	Heterozygous	Chilblain lupus in two males and two females		

determine how this protein interacts with TLRs. Indeed, large-scale mutagenesis analysis of *UNC93B1* revealed mutations that could increase or decrease the response of TLR3, TLR7 and TLR9, albeit very few led to increased TLR9 responses<sup>3</sup>. These findings suggest that more mutations will be identified as whole-genome sequencing approaches expand, and that these mutations will probably be associated with diverse clinical phenotypes. Incomplete activation of the X chromosome, where the *TLR7* and *TLR8* genes reside, could possibly account for some of the clinical manifestations observed in SLE while additional genetic variants and epigenetic changes could account for the reported clinical heterogeneity.

TLR signalling is complex. In endosomes, TLRs are cleaved at the leucine-rich region by cathepsins (the two fragments remain together), a process that is necessary for the dimerization and subsequent binding to their ligands. Downstream signalling involves the formation of supramolecular organizing centres around MYD88 or TRIF<sup>7</sup>. In addition, the availability of single-stranded nucleic acid ligands, which is controlled by sufficient endonuclease activity<sup>1</sup>, contributes to TLR signalling. This complexity suggests that each mutation in *UNC93B1* should be examined in the context of all molecules involved in TLR signalling (including endonucleases, TLRs, syntenin-1 and molecules involved in downstream signalling) to explain why some individuals were homozygous whereas most were heterozygous and why heterozygous family members remained unaffected.

Do *UNC93B1* mutations represent a cause of monogenic SLE or a strong genetic contributor in the pathogenesis of SLE? Existing evidence has yet to settle this question. The V117L variant reported by Al-Azab et al.<sup>4</sup> is prevalent in East Asian populations and is present in the patients with SLE they described; however, this variant was also present in asymptomatic family members, suggesting that it is not a monogenic SLE-causing variant but instead a contributing risk factor (odds ratio of 17.9)<sup>4</sup>. In the study by David et al.<sup>5</sup>, *UNC93B1* variants were reported in 5 out of 63 individuals in their cohort of French patients, documenting the important genetic contribution of this chaperone molecule to the development of SLE.

Finally, it should be recalled that *UNC93B1* deficiency in two children has been linked to herpes virus encephalitis owing to lack of interferon production<sup>9</sup>. This finding illustrates a continuous spectrum between immunodeficiency and autoimmunity<sup>10</sup> and nicely shows the opposing roles of interferons in protecting against infections and promoting autoimmunity. As whole-genome sequencing becomes increasingly available, we will undoubtedly learn how new *UNC93B1* variants affect immunity in the context of other gene variants in an individual's genome and how these variants interact and communicate to cause or not cause pathology.

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## Competing interests

The author declares no competing interests.

# The quest for targetable pain mechanisms in 2024

Neil Basu

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Studies published in 2024 suggest that although the repurposing of established rheumatology drugs seems to deliver incremental benefits for pain management, greater benefits could be gained in the future by targeting newly discovered pain mechanisms.

In 2024, pain continues to dominate patient–rheumatologist conversations regardless of the underlying musculoskeletal diagnosis. This year, an interesting example of transferring knowledge between diseases was reported with the successful analgesic testing of methotrexate (the anchor drug for classical inflammatory arthritis) in patients with knee osteoarthritis (OA; classically defined as a non-inflammatory arthritis)<sup>1</sup>. However, although the attribution of various inflammatory mechanisms across the spectrum of musculoskeletal disease broadens, rheumatologists must resist the temptation to assume inflammation is the source of all burden in their patient populations. In the context of pain, intriguing non-inflammatory mechanisms, both peripherally and centrally based, were reported this year<sup>2,3</sup>.

There have been few clinical advances in the pharmacological management of knee OA this century. No disease-modifying OA drugs have been approved and NSAIDs and paracetamol remain first-line treatments for pain relief<sup>4</sup>. The idea that the synovitic component of OA could contribute to the pain experience and be modified by DMARDs already widely used in diseases such as rheumatoid arthritis (RA) is not new; however, previous randomized controlled trials (RCTs) have been generally underpowered and underdosed. The PROMOTE study by Kingsbury et al.<sup>1</sup> was a double-blinded placebo-controlled RCT that sought to evaluate the effect of methotrexate in addition to usual analgesia in people with symptomatic knee OA. The study met its primary endpoint of average knee pain (numerical rating scale) at 6 months. Those who received methotrexate ( $n = 77$ ) reported a greater reduction in pain than those who received placebo ( $n = 78$ ); the mean between-group difference was 0.79 (95% confidence interval (CI) 0.06–1.51) in favour of methotrexate, representing a standardized effect size (0.34) equivalent to that typically observed in trials of NSAIDs for OA<sup>1</sup>. In the context of the negative results from previous trials of methotrexate trials for OA, this study is notable for its more aggressive target dose of 25 mg per week and aligns with another positive trial of methotrexate in hand OA from 2023, which also adopted a high-dose regimen<sup>5</sup>. Ultimately, most rheumatologists would consider the reported size of the clinical effect to be modest at best; however, the trial reports some notable insights. Specifically, there was no association between baseline knee synovitis, as measured by MRI, and pain at 6 months. By contrast, there was a significant relationship between the baseline level of high-sensitivity C-reactive protein and pain at 6 months.

These tentative observations could help to inform future treatment strategies and indicate that the mechanism of the analgesic action of methotrexate in OA could be mediated by alternative (non-synovial) pathways, such as the neuroimmune and/or metabolic pain axes.

Characterizing alternative pain pathways in the synovium was the focus of a 2024 transcriptome study in RA. Persistence of pain despite attainment of disease remission represents one of the greatest burdens in RA care. The long-held assumption that RA pain is entirely related to the extent of inflammation in the synovium has been challenged by several observational studies, but the precise mechanisms for this disconnect are unknown. Bai et al.<sup>2</sup> extracted bulk RNA from the synovial tissue of 39 patients with established RA who were undergoing arthroplasty. Patient-reported pain failed to differentiate between synovial tissue samples, which were categorized histologically as ‘high inflammatory’ or ‘low inflammatory’. No single gene was associated with pain after sequencing, and the researchers then developed an innovative machine learning algorithm (graph-based gene expression module identification). This algorithm subsequently identified an 815-gene module that significantly correlated with pain in individuals classified as having ‘low inflammatory’ synovium. This correlation was externally replicated in a larger ( $n = 87$ ) early RA cohort, although it is important to stress that a significant relationship, albeit weaker, also existed with the ‘high inflammatory’ category. Next, single-cell RNA sequencing of the RA synovium was used to interrogate the cellular source(s) of this genetic pain signature. Interestingly, compared with immune cells, the pain-associated genes were highly expressed by fibroblasts, especially CD55<sup>+</sup> synovial lining fibroblasts. A point of emphasis is that these classically non-immune cells do not exclusively account for pain in this key sub-group of patients classified as ‘low inflammatory’. The authors do, however, report an *in vitro* functional study in which supernatants from human synovial lining fibroblasts propagated the growth of injured mouse dorsal root ganglion nociceptors (which were CGRP<sup>+</sup>). In addition, CD55<sup>+</sup> fibroblasts and CGRP<sup>+</sup> axons co-localized with papillary outgrowths in ‘low inflammatory’ state human RA synovium.

## Key advances

- In a randomized double-blinded placebo-controlled study, high doses of methotrexate reduced pain in knee osteoarthritis<sup>1</sup>
- Gene expression in synovial fibroblasts was linked to pain in patients with rheumatoid arthritis with limited synovial inflammation<sup>2</sup>
- In a mouse model, a neural circuit comprising the rostral anterior cingulate cortex and the pontine nucleus seemed to subservise placebo analgesia<sup>3</sup>

The specificity of this model must now be examined and challenged with several confounding factors, such as central sensitization, which contribute to the multi-dimensional experience of pain<sup>2</sup>.

Overcoming confounding effects from co-existing pain mechanisms is a challenge faced by all pain researchers. As the brain is the final common pathway for most, if not all, pain mechanisms, neurobiological models of pain might aid in addressing this challenge while also delineating generic pathways that are of relevance across the clinical spectrum of pain. Placebo analgesia is an exemplar generic construct, the potency of which has undermined the hopes for countless analgesics. Understanding the mechanisms involved in placebo analgesia to inform the development of therapeutics has been a long-held ambition. Functional MRI studies are successfully mapping the neurobiological network of placebo analgesia at the macroscale<sup>6</sup>. The precise neural circuits that characterize placebo analgesia and could subsequently be the future targets of pharmacological and/or neuromodulatory approaches still need to be identified. Earlier this year, Chen et al.<sup>3</sup> pinpointed populations of neurons within the rostral anterior cingulate cortex (rACC) and their projection to the pontine nucleus as key mediators of placebo analgesia. Their series of elegant mouse experiments began with the robust development of a behaviour assay that mimicked human analgesic expectation, using a cage with differentially heated floors. Calcium imaging in conditioned mice delineated the evocation of rACC to pontine nucleus neural activity, a communication that was then validated with complementary electrophysiological and optogenetic experiments. Thereafter, activation and inhibition of the rACC–pontine nucleus circuit, via *in vivo* photomanipulation, increased and reduced pain thresholds, respectively. The pontine nucleus, located within the brainstem, is a key relay station to the cerebellum, but neither the pontine nucleus nor the cerebellum are usually implicated with pain; however, the authors provided transcriptional evidence that opioid receptors were prevalent in the pontine nucleus and also linked the activation of cerebellar Purkinje cells to the rACC–pontine nucleus circuit. This compilation of high-resolution technology-based experiments, which are impossible in humans,

represents one of the strongest attempts to describe the biology of placebo analgesia. It is not feasible to comprehensively model the human experience on studies carried out in mice and it is also highly unlikely that the reported rACC–pontine nucleus circuit represents the only mechanism of placebo analgesia. However, the decision by the authors to inform their mouse experiments with learnings from clinical research (for example, the selection of rACC as the initial region of interest based on human functional MRI studies) offers hope for future forward translation<sup>3</sup>.

Although the described advances in knowledge will not take the topic of pain off the rheumatology clinic agenda, it is encouraging that leading technologies are being intelligently applied and are beginning to identify therapeutic targets for the long term, while clinical trialists continue to test the possibilities of drug repurposing to discover solutions in the shorter term.

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## Competing interests

The author declares no competing interests.

# Insights into IVDD pathogenesis in 2024

Daisuke Sakai

 Check for updates

Emerging research in intervertebral disc degeneration in 2024 highlights microbial, immune and inflammatory mechanisms that drive chronic low back pain. These insights pave the way for potential transformative therapies that address the root causes of intervertebral disc degeneration and could improve patient outcomes.

Intervertebral disc degeneration (IVDD) continues to be a substantial contributor to chronic low back pain, affecting millions of people globally and placing an enormous burden on healthcare systems and economies<sup>1,2</sup>. Traditional treatments for IVDD typically focus on pain management but fail to address the root causes of the degeneration. In 2024, new research has provided transformative insights into the pathogenesis of IVDD, emphasizing the role of microbial pathophysiology, immune-mediated inflammation and changes to specific degenerative nucleus pulposus cell populations and identifying potential strategies for innovative treatments<sup>3–5</sup>.

Building on previous findings, one of the most compelling observations in 2024 further challenges the long-standing notion that the intervertebral disc is a sterile environment<sup>3</sup>. Emerging evidence now suggests that microbial dysbiosis might be implicated in type I Modic changes, a common IVDD feature that is linked to inflammation and pain. Mengis et al.<sup>3</sup> identified a distinct microbial profile within degenerative intervertebral discs that was associated with Modic type I changes, including an abundance of Gram-negative bacteria and the consistent presence of the Gram-positive bacterium *Cutibacterium acnes*. These microbial communities seem to drive inflammatory changes within the disc, potentially triggering or exacerbating immune responses that contribute to IVDD.

Despite these findings, previous microbiome studies have often produced inconsistent results<sup>3,6,7</sup> and have created uncertainty around the role of microorganisms in the pathogenesis of IVDD. The 2024 study by Mengis et al.<sup>3</sup>, which utilized sophisticated techniques to identify bacterial genera, underlines the need for standardized sampling and analysis techniques to ensure reliable, reproducible data. Future research that further validates microbial involvement could redefine IVDD management by supporting the development of adjunct therapies, such as targeted antibiotics or anti-inflammatory treatments, for patients with Modic changes. This new perspective could pave the way for targeted interventions that address specific bacterial profiles and transform both the diagnosis and the treatment of microorganism-driven IVDD-associated low back pain.

Although microbial dysbiosis presents a potential target for therapeutic intervention, advances in immune-mediated inflammation research provide another promising path for IVDD therapy. Another pivotal 2024 study by Burt et al.<sup>4</sup> demonstrated the role of prolonged activation of the NF- $\kappa$ B pathway in the exacerbation of IVDD in which

pro-inflammatory immune cells, particularly macrophages, are recruited into the disc. This chronic activation induces an inflammatory environment in which pro-inflammatory ‘M1-type’ macrophages release a myriad of cytokines, such as TNF and IL-1 $\beta$ , thereby accelerating the breakdown of extracellular matrix and cellular damage<sup>8</sup>. By contrast, the reparative functions of inflammation-resolving ‘M2-type’ macrophages, which also reside in the disc, are compromised by persistent, overwhelming pro-inflammatory signals.

**“advances in immune-mediated inflammation research provide another promising path for IVDD therapy”**

Burt et al.<sup>4</sup> further emphasize the crucial role of macrophages in disease progression and highlight these cells as potential therapeutic targets. Targeting immune pathways to promote the transition from pro-inflammatory ‘M1-type’ macrophages to anti-inflammatory ‘M2-type’ macrophages, or by directly inhibiting NF- $\kappa$ B, represents promising therapeutic strategies to reduce or slow IVDD progression. This approach suggests a paradigm shift from symptomatic pain management to immunomodulatory therapies aimed at the underlying inflammatory processes. Given the intricate balance between immune activation and resolution, further research into the timing and type of immune modulation could optimize outcomes for patients at different stages of IVDD.

Another important study in 2024 by Chen et al.<sup>5</sup> highlighted the crucial role of serglycin (SRGN), a proteoglycan linked to inflammation and tissue degradation, in the maintenance of nucleus pulposus cell integrity and further emphasized the role of macrophage recruitment in IVDD progression. Advances in single-cell RNA sequencing enabled the authors to identify a specific subpopulation of late-stage nucleus pulposus cells with high SRGN expression. Chen et al.<sup>5</sup> found

## Key advances

- Microbial dysbiosis, including the presence of *Cutibacterium acnes*, is linked to Modic type 1 changes, which suggests that microorganisms have an important role in intervertebral disc degeneration (IVDD)<sup>3</sup>.
- Chronic NF- $\kappa$ B expression is linked to the recruitment of pro-inflammatory macrophages, which promote inflammation and extracellular matrix breakdown in IVDD<sup>4</sup>.
- Specific end-stage IVDD nucleus pulposus cell populations have high expression of serglycin, which drives macrophage recruitment and inflammation. Serglycin inhibition strongly reduced disc degeneration by limiting macrophage recruitment<sup>5</sup>.

that increased SRGN levels in these cells promoted the release of pro-inflammatory cytokines, which was in turn responsible for macrophage recruitment and disc degeneration.

Targeting SRGN with the known SRGN inhibitor daphnetin was effective and resulted in a significant reduction in inflammatory markers and macrophage influx in mouse models of IVDD. By downregulating SRGN, daphnetin effectively mitigated inflammation and slowed disc degeneration; the findings thus highlight SRGN as a promising therapeutic target and biomarker for late-stage IVDD. This discovery paves the way for the development of SRGN inhibitors as targeted therapies with the aim of preserving nucleus pulposus cell function and reducing inflammation-induced damage within the disc, an approach that has the potential as a new IVDD therapeutic.

## “Collectively, these 2024 findings offer a broader understanding of IVDD”

Collectively, these 2024 findings offer a broader understanding of IVDD and underscore its complex, multifactorial nature, with microbial, immune and inflammatory mechanisms driving degeneration. Addressing microbial imbalances, modulating immune responses and preserving nucleus pulposus cell function could herald a new era in IVDD treatment, marking a shift from pain management to targeted, disease-modifying interventions. These advances suggest a future in which IVDD therapies are tailored to underlying immune-related and inflammation-related pathophysiological processes, offering hope for a more comprehensive

and effective approach to the management of chronic low back pain, and ultimately, improved quality of life for patients.

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### Competing interests

D.S. serves as a scientific advisor for TUNZ Pharma.

# Molecular profiling for advancing precision rheumatology

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Here, we highlight three publications in 2024 that have advanced the field of molecular and immunological profiling, for the diagnosis, prognosis and treatment of patients with systemic autoimmune rheumatic diseases.

Recent flow cytometry-based immune-phenotyping studies have substantiated the concept that autoimmune rheumatic diseases (ARDs) can be grouped according to their immune profile, regardless of disease classification<sup>1,2</sup>. One study identified 5 across-disease clusters in a cohort of >400 patients with 15 different autoimmune or autoinflammatory diseases<sup>1</sup>, whereas the analysis of 1,088 patients representing 11 ARDs identified multiple across-disease groups with distinct immune and clinical phenotypes<sup>2</sup>. However, the development of robust biomarkers for personalized approaches is still lacking and is complicated by the complexity of high-dimensional datasets and differences in cell-specific molecular profiles. This also requires validation both within and across established disease phenotypes, while accounting for age, sex and race or ethnicity<sup>3</sup>.

Here we highlight three publications in 2024 that have combined in-depth analysis of molecular or immune profiles with novel computational analysis techniques to stratify patients across traditional disease boundaries and provide an opportunity to discover biomarkers for improved prediction, diagnosis and prognosis of complex diseases. The use of large-scale biobanks such as the UK Biobank, which currently features data from 500,000 individuals, enables such research by making available omic data (including proteomics, metabolomics, genetic sequencing and imaging) with continuously updated electronic health records and standard blood test results<sup>4</sup>.

Garg et al.<sup>5</sup> highlight the power of the UK Biobank to drive predictive analysis using a powerful machine-learning platform (MILTON) that enabled case-control studies across five ancestries. This model identified disease-specific omic signatures from already diagnosed patients (using international classification of disease 10 (ICD10) codes) and predicted potential 'new' cases within the control cohort with relatively high predictive power for many disease phenotypes (area under the curve (AUC) > 0.7 for 1,596 ICD10 disease codes). This approach was able to predict new disease before onset and outperform some known polygenic risk scores. The authors also performed a phenome-wide association using matched plasma proteomics data, which further improved prediction for some diseases ( $\Delta\text{AUC} \geq 0.1$  for 52 diseases). These results were validated using the FinnGen biobank (Fig. 1a). Not all disease phenotypes could be predicted using this model; this could be due to limitations in the ICD10 coding system or the need for more biomarker datasets. However, this approach might inform data collection in future biobanks, provide mechanistic insight into many disease pathologies and have implications for future strategies for prevention and early detection of disease. Usefully, the open access

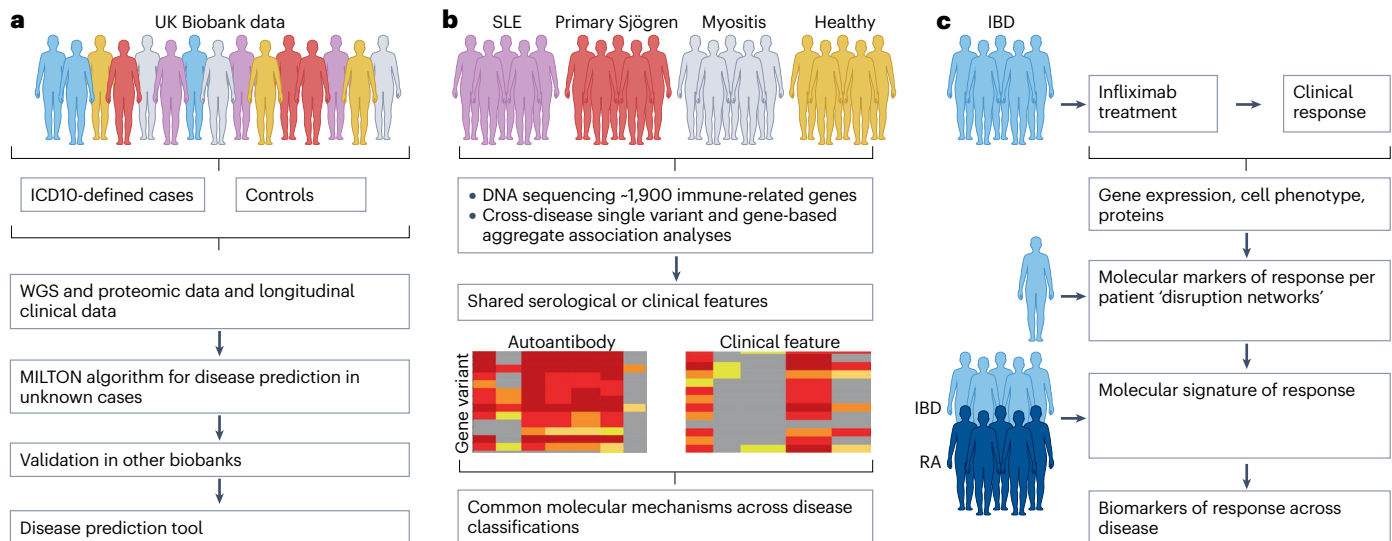
MILTON platform enables researchers to explore information about ARDs using ICD10 codes.

Bianchi et al.<sup>6</sup> investigated common molecular mechanisms in patients with systemic lupus erythematosus (SLE), primary Sjögren syndrome and myositis. These conditions are characterized by overlapping clinical and serological features, with diagnostic and therapeutic implications, as well as high comorbid associations pertaining to individuals and families affected by more than one autoimmune condition, all suggestive of shared risk factors. The major histocompatibility complex (MHC) genes have been identified by genome-wide association studies (GWAS), targeted genotyping studies and meta-analyses as the strongest single genetic cause of many systemic ARDs<sup>7</sup>. However, a converse approach, focused on investigating the impact of the full spectrum of genetic variation, including genes with lower allele frequency, is required to comprehensively evaluate genetic heritability. This strategy is important to counterbalance the limitations of GWAS analyses leading to the identification of common gene variants broadly spread across the genome with limited pathogenic or therapeutic implications, as advocated by the 'omnigenic model'<sup>8</sup>. Bianchi et al.<sup>6</sup> implemented this model using next-generation targeted DNA sequencing of regulatory and coding regions in a large cross-sectional cohort study including 2,292 individuals with SLE, primary Sjögren syndrome or myositis, as well as 1,252 matched healthy individuals recruited from Scandinavian countries, aiming to identify genetic profiles of individuals with shared clinical and laboratory features, with implications for personalized medicine strategies (Fig. 1b).

The single variant analysis confirmed MHC, the interferon pathway and reactive oxygen species metabolism as the key genetic contributors to systemic ARDs. The gene-based aggregate testing confirmed both MHC (*C2*, *HLA-C*, *MSH5* and *TNXB*) and non-MHC (*IRF5* and *YDJC*) associations, and reported associations with new genetic variants of *MAP3K6*, *SLCSA6* and *CGREF1*, which are all related to type I interferon pathway activation. In addition to facilitating the discovery of genetic variants, this study identified common genetic associations of individuals with shared serological features across disease phenotypes. Rheumatoid factor, SSA-Ro52, SSA-Ro60 or SSB-La autoantibody positivity was strongly associated with MHC region variants, whereas double-stranded

## Key advances

- Use of multiomic data from population-wide biobanks has the potential to accelerate the drive towards personalized medicine approaches for autoimmune rheumatic diseases (ARDs)<sup>5</sup>.
- Systemic ARDs have distinct full-spectrum genetic associations identifying shared serological and clinical phenotypes<sup>6</sup>.
- Blood-based biomarkers can predict drug response across immune-mediated diseases<sup>9</sup>.



**Fig. 1 | Approaches for molecular and immunological profiling in autoimmune rheumatic diseases.** **a**, Using the longitudinal health record and omic data from participants in the UK Biobank and a new ensemble machine-learning framework (MILTON), a range of biomarkers to predict 3,213 diseases was developed. **b**, Targeted DNA sequencing of coding and regulatory regions in a large well-characterized cohort of patients with systemic lupus erythematosus (SLE), primary Sjögren syndrome or myositis as well as healthy individuals, combined with knowledge of autoantibody profiles and clinical features, identified

subgroups with unique genetic profiles. **c**, Longitudinal high-dimensional whole-blood data from patients with inflammatory bowel disease (IBD) undergoing treatment with infliximab combined with a computational approach to examine individual response to therapy (disruption networks) was able to identify cell-centred individual-level molecular networks, predictive of treatment response. This molecular pattern of response was validated in publicly available data from patients with rheumatoid arthritis (RA). ICD10, international classification of disease 10; WGS, whole-genome sequencing.

DNA-specific autoantibody positivity was significantly associated with non-MHC genes, suggesting a role for environmental triggers on susceptible genetic backgrounds. Antinuclear antibody positivity had weak associations with both MHC and non-MHC genes, supporting previous observations related to the molecular heterogeneity of antinuclear antibody-positive individuals. Furthermore, certain clinical features were linked with specific genetic profiles; arthritis was associated with genetic variants of protein kinase C $\zeta$  and skin involvement was associated with dual specificity phosphatase, a finding confirmed at the functional level and externally validated in eczema and psoriasis studies.

Finally, Gerassy-Vainberg et al.<sup>9</sup> used across-disease analysis looking for biomarkers of response to treatment. Immune features were profiled over time and in association with treatment responses to the TNF inhibitor infliximab in patients with inflammatory bowel disease (IBD). High-dimensional data from patients with IBD responding or not responding to infliximab were assessed at baseline, week 2 and week 14 post treatment. Variation in individual patient response to drug over time was evaluated using a 'disruption network' model, which indicated changes in immune and molecular regulation. By assessing the effect of every non-responding patient to a pre-defined reference response network, the level of deviance from treatment response was calculated. This enabled disruptions in cell-specific functional modules and networks to be identified that would otherwise not be detected using conventional analyses (Fig. 1c). Using this approach, cytoskeleton organization and VEGF receptor signalling pathways in monocytes were most regulated in response to treatment at week 2. Furthermore, baseline monocytic expression of genes of the RAC1-PAK1 axis was predictive of infliximab response in patients with IBD and was validated in publicly available datasets from patients with rheumatoid arthritis. This approach supports earlier findings assessing secondary non-response to TNF inhibitors in a study using complex computational analysis of non-response due to development of anti-drug antibodies across disease phenotypes<sup>10</sup>.

In conclusion, the three highlighted studies provide evidence for the increased relevance of molecular diagnosis and its potential role in guiding patient diagnosis, stratification and targeted therapeutic approaches across established disease classification criteria in

rheumatology. However, several challenges remain, including validation of identified signatures in large cohorts, integrating molecular omic signatures to provide a global picture of underlying pathogenesis in disease subsets, and establishing robust and cost-effective tests for identified biomarkers that can be translated for routine use. An important goal is to improve the effectiveness of clinical trials in rheumatology by the inclusion of patients based on their molecular signatures.

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## Competing interests

The authors declare no competing interests.

# Advances in the calculation of minimal important change estimates for patient-reported outcome measures

Ewa M. Roos

 Check for updates

In 2024, studies using more advanced methods to calculate the minimal important change have described how different methods and timings of estimating minimal important changes can affect the estimates.

Patient-reported outcome measures (PROMs) are questionnaires that are used to evaluate pain, function and quality of life in patients treated for and living with osteoarthritis (OA). Common condition-specific PROMs used in knee OA include the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the Knee Injury and Osteoarthritis Outcome Score (KOOS), and the Oxford Knee Score (OKS). PROMs must show good reliability, validity and responsiveness, but the use of robust PROM score estimates for the clinical interpretation of study results is less well understood.

PROM score interpretation thresholds are used to evaluate the clinical meaningfulness of study results and can be useful when comparing and deciding on treatments in clinical practice. Other important aspects to be considered in treatment decisions include cost, risks and accessibility. PROM interpretation thresholds usually consider longitudinal change scores (calculated by subtracting 'before treatment' scores from 'after treatment' scores). As a change in PROM score does not have a clear meaning, estimates of minimal important change (MIC) (which is the smallest within-individual change in score that patients on average perceive as important) are used to interpret the clinical meaningfulness of changes in PROM scores. More than 80 variations of the MIC concept are available, with many combinations of methods, definitions, terms and abbreviations used.

A PROM result can be presented as one total or several subscale scores. Often, scores are normalized and range from 0 to 100; however, as an example, the WOMAC subscale function is presented as a score from 0 to 68. Confusingly, a higher score can represent a worse outcome (typical for PROMS developed in rheumatology) or a better outcome (typical for PROMS developed in orthopaedics). Published interpretation estimates for any given PROM vary greatly<sup>1</sup>, with suggested reasons largely related to study heterogeneity, including the use of a range of methodologies to calculate estimates. As patient and study characteristics can influence PROM scores, users are recommended to apply an estimate from a study of a population and a similar treatment to their own study, but less attention has been paid to the importance of the different methodologies applied to calculate the interpretation thresholds.

In 2024, methodological advances were made that could help to guide users to choose more appropriate and robust MIC estimates to interpret OA study results, and help in presenting treatment alternatives to patients with OA. Terluin et al.<sup>2</sup> introduced longitudinal

confirmatory factor analysis as a method to estimate MIC thresholds and compared different methods of calculating MIC estimates to identify which methods provide the least biased and most precise MIC estimates<sup>3</sup>. A study by Harris et al.<sup>4</sup> identified the impact of time elapsed from total joint replacement on MIC estimates using a contemporary and more advanced calculation method.

A strength of longitudinal item response theory<sup>5</sup> and longitudinal confirmatory factor analysis<sup>2</sup> is that PROM data used for MIC calculation does not have to be normally distributed (treatments that show great improvement such as joint replacement are not normally distributed). Advantages of longitudinal confirmatory factor analysis over longitudinal item response theory include that it is less computationally intense, takes a fraction of the time to run, and is more familiar to researchers<sup>2</sup>. Terluin et al.<sup>2</sup> found that results from the longitudinal confirmatory factor analysis method and the longitudinal item response theory method were comparable, but that longitudinal confirmatory factor analyses were more than 50 times quicker<sup>2</sup>.

An inherent weakness of all longitudinal anchor-based methods is that responding to a global rate of change (or transition) item after treatment, with response options such as 'much worse', 'worse', 'no change', 'a little better' and 'much better', requires patients to remember and recall what their status was before treatment. Memory is well known to be susceptible to bias<sup>3</sup>. Typically, the result is that change ratings are more influenced by the patient's post-treatment state than by their baseline state. This common phenomenon is known as 'present state bias' and methods have been developed to estimate the degree of present state bias in real data<sup>3</sup>.

## Key advances

- Longitudinal item response theory (LIRT) and longitudinal confirmatory factor analysis (LCFA) are recently introduced methods to estimate minimal important change (MIC) estimates<sup>2</sup>.
- Adjusted predictive modelling (APM), longitudinal item response theory (LIRT) and longitudinal confirmatory factor analysis (LCFA) provide more precise and bias-free minimal important change (MIC) estimates than older methods (mean change and receiver operating characteristic (ROC) methods)<sup>3</sup>.
- MIC estimates change with time to follow-up after surgery. Patients who undergo hip or knee replacements appreciate any improvement early after surgery, but between 3 months and 12 months they raise their expectations for what they perceive as a minimal important improvement; after 12 months, patients do not anticipate further improvement<sup>4</sup>.

## Box 1 | Anchor-based methods for establishing minimal important change estimates compared by Terluin et al.<sup>3</sup>

### Mean change method (1989)

Uses the mean change score of the minimally importantly changed subgroup as the minimal important change (MIC).

### Receiver operating characteristic (ROC) method (1986)

Aims to classify improved and not-improved patients with the least misclassification.

### Predictive modelling (2015)<sup>6</sup>

Based on logistic regression using the dichotomized anchor-based transitioning ratings as the outcome and the PROM change score as the determinant. The change score of interest is the one with a likelihood ratio of 1 (that is, the change score that is equally likely to occur in the improved and the not-improved groups). Predictive modelling identifies approximately the same MIC threshold as the ROC method but is more precise.

### Adjusted predictive modelling (APM; 2017 and improved in 2022)<sup>7,8</sup>

Adjusts for proportion improved other than 50% (as is commonly the case in individuals with OA) and the reliability of the transition anchor.

### Longitudinal item response theory (LIRT; 2023)<sup>5</sup>

The LIRT-based MIC is based on a LIRT model of the PROM items, with the dichotomized transition item serving as an indicator of both time factors.

### Longitudinal confirmatory factor analysis (LCFA; 2024)<sup>2</sup>

The LCFA-based MIC is based on a LCFA model for ordinal indicators with the PROM items before and after treatment loading on the latent factors before and after treatment, respectively.

In their 2024 simulation study, Terluin et al.<sup>3</sup> tested the ability of different anchor-based methodologies (Box 1) for establishing true (bias-free) MIC estimates and the precision of the estimates. They found that when the mean change method, described in 1989, was used, the presence of present state bias on average slightly overestimated the true MIC, with the precision of the estimate decreasing with increasing present state bias. When they used the receiver operating characteristic (ROC) method, developed in 1986, the presence of present state bias slightly underestimated the true MIC, with imprecise estimates across all degrees of present state bias. The more recent and complex approaches yielded better results. Predictive modelling<sup>6</sup> (developed in 2015) estimated the true MIC but with considerable imprecision; adjusted predictive modelling (developed in 2017 (ref. 7) and improved in 2022 (ref. 8)) estimated the true MIC with much better precision. Longitudinal item response theory<sup>5</sup> (developed in 2023) and longitudinal confirmatory factor analysis<sup>2</sup> (described in 2024) also provided good results. On the basis of the findings, Terluin et al.<sup>3</sup> recommended that the older mean change and ROC methods are not used for establishing thresholds for interpreting changes in PROM scores.

In their 2024 study, Harris et al.<sup>4</sup> wanted to better understand whether duration after surgery is an important consideration when interpreting the outcomes from joint replacement. They used adjusted predictive modelling to study whether the time from total knee or hip replacement influenced the MIC estimate. Adjusted predictive modelling adjusts for the reliability of the global transition item and for the proportion of improved patients being other than 50%, as is the case

in joint replacement studies, in which much more than 50% of patients report at least some improvement. They found that the MIC thresholds for the Oxford Hip Scores and Oxford Knee Scores were very low at 3 months after surgery and that these thresholds increased from 3 to 12 months but not from 1 to 2 years<sup>4</sup>. These findings suggest that patients appreciate any improvement early after surgery but that over time they raise their expectations for what they perceive as a minimal important improvement; after 12 months, patients do not expect further improvement. Similar to previous studies, Harris et al.<sup>4</sup> also found that patients who had more severe symptoms before surgery needed, on average, a greater change (versus those with less severe symptoms) to feel that their change was important. Findings suggesting that MIC estimates should be personalized introduce a dilemma for users as such customization would be complex and impractical.

When evaluating changes in PROM scores, we must consider what is more important to patients: 'feeling better' (that is, the PROM score has improved with treatment) or 'feeling good' (that is, the post-treatment PROM score is acceptable)<sup>9</sup>. Post-treatment thresholds have been introduced as complementary thresholds that enhance the interpretation of clinically meaningful treatment effects. Such thresholds circumvent the need for patients to recall their pretreatment state. One of these post-treatment thresholds, the Patient Acceptable Symptom State (PASS), is the threshold above which patients will consider themselves well and satisfied with treatment. Improvement and absolute post-treatment interpretation thresholds can yield very different results. For example, at 2 years after treatment of a severe knee ligament injury, 9 out of 10 patients at high risk of OA felt better, whereas only 5 out of 10 felt that their current state was acceptable to them<sup>10</sup>. Considering both whether a patient is feeling better and whether they are feeling good can improve the interpretation of clinical study results and inform the conversation between patients and health care practitioners about treatment options for joint problems. Combining MIC estimates with post-treatment thresholds such as PASS might enhance clinical interpretation and improve shared decision making.

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### Competing interests

The author declares no competing interests.

# HLA-B27 and spondyloarthritis: at the crossroads of innate and adaptive immunity

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## Abstract

*HLA-B\*27* confers a strong risk of developing spondyloarthritis (SpA), which includes axial SpA with or without peripheral arthritis, enthesitis, acute anterior uveitis and gastrointestinal inflammation. Although no definitive mechanism has been established to explain the role of this HLA class I protein in the pathogenesis of SpA, three main hypotheses have emerged. First is the idea that self-peptides displayed by HLA-B27 resemble microorganism-derived peptides, leading to the expansion of autoreactive CD8<sup>+</sup> T cells that trigger disease. The second and third hypotheses focus on aberrant properties of HLA-B27, including its tendency to form cell-surface dimers that can activate innate killer immunoglobulin-like receptors on CD4<sup>+</sup> T helper 17 cells, triggering the production of pathogenic cytokines. HLA-B27 also misfolds in the endoplasmic reticulum, which can activate the unfolded protein response, increasing IL-23 expression and thereby promoting the production of type 17 cytokines. HLA-B27 misfolding in mesenchymal stem cells has also been linked to enhanced bone formation by mesenchymal stem cell-derived osteoblasts, which could contribute to structural damage in axial SpA. In this Review we summarize prevailing ideas about the role of HLA-B27 in SpA, discuss the latest developments as well as the gaps in current knowledge, and provide recommendations for future research to address these unmet needs.

## Sections

### Introduction

The emergence of three main hypotheses about the role of HLA-B27 in spondyloarthritis

New evidence regarding the role of HLA-B27 in spondyloarthritis

Implications for spondyloarthritis treatment

Future perspectives

Conclusions

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## Key points

- The role of HLA-B27 in the pathogenesis of spondyloarthritis is incompletely understood.
- Evidence implicates canonical HLA-B27-bound peptides as a target for CD8<sup>+</sup> T cells and aberrant forms of HLA-B27 that engage killer immunoglobulin-like receptors on T helper 17 cells in disease.
- HLA-B27 misfolding and *XBP1* splicing are implicated in the promotion of mineralization by mesenchymal stromal cell-derived osteoblasts.
- ERAP1 loss-of-function, which reduces the risk of arthritis in *HLA-B\*27*-positive individuals and animal models, alters the repertoire of peptides presented by HLA-B27 and reduces misfolding and endoplasmic reticulum stress.
- HLA-B27 might also affect TGFβ and BMP signalling by inhibiting the function of ALK2.
- Canonical and non-canonical effects of HLA-B27 suggest that it might have more than one role in disease predisposition and place it at the crossroads of innate and adaptive immunity.

## Introduction

Ankylosing spondylitis (AS) is the prototypic form of a family of diseases associated with *HLA-B\*27* and referred to as spondyloarthritis (SpA). In addition to AS, SpA includes reactive arthritis, inflammatory bowel disease-associated arthritis, juvenile idiopathic arthritis (specifically a subset known as enthesitis-related arthritis) and psoriatic arthritis. A classification system introduced in 2009 defined axial SpA (axSpA)<sup>1</sup>, encompassing radiographic axSpA (also known as AS) and non-radiographic axSpA; radiographic axSpA is increasingly used to describe AS, but it can include earlier stages of disease<sup>2</sup>. The strong association between *HLA-B\*27* and AS has been recognized since the mid-1970s<sup>3–5</sup>. *HLA-B\*27* is present in 85–90% of patients with AS, and when two copies are present (homozygosity) the risk of developing AS is increased<sup>6</sup>. Heritability of AS has been estimated to be >90%; however, susceptibility genes identified to date account for only about 24% of heritability of AS, with *HLA-B\*27* accounting for approximately 80% of the known heritability<sup>7</sup>. Nevertheless, *HLA-B\*27* is not sufficient to cause disease and only approximately 5% of individuals carrying this allele develop SpA; however, because the vast majority of individuals with AS carry *HLA-B\*27*, and overexpression of this allele (which encodes the HLA-B27 protein) in rodents can cause an inflammatory disease resembling SpA<sup>8</sup>, *HLA-B27* is at the centre of many studies aimed at elucidating disease pathogenesis. The arthritogenic peptide hypothesis<sup>9</sup> was stalled by a lack of progress in identifying CD8<sup>+</sup> autoreactive T cells and the self-peptides they target, and then by the recognition that CD8<sup>+</sup> T cells do not mediate disease in rodent models<sup>10,11</sup>. In addition, the discovery of aberrant features of the HLA-B27 heavy chain, including cell-surface dimerization<sup>12</sup> and its tendency to misfold during assembly in the endoplasmic reticulum (ER)<sup>13</sup>, suggested plausible alternatives to arthritogenic peptides. To date, the relative contribution of these mechanisms to SpA pathogenesis remains unclear, but has contributed to a robust discussion as to whether axSpA is an

autoimmune or autoinflammatory disease<sup>14</sup>. In this Review we provide a brief historical perspective on the emergence of the three main hypotheses that explain the role of HLA-B27 in SpA pathogenesis and then focus on new developments that have rekindled interest in the search for arthritogenic self-peptides and implicated HLA-B27 in aberrant bone formation. The clinical features and epidemiology of SpA, and susceptibility genes other than *HLA-B\*27* are not the focus of this Review and have been reviewed elsewhere<sup>2</sup>.

## The emergence of three main hypotheses about the role of HLA-B27 in spondyloarthritis

Since 1990 three lines of evidence have emerged, suggesting different roles for HLA-B27 in disease. To understand the development of these concepts we provide a brief historical perspective (Fig. 1). By the 1980s the key role of HLA class I molecules in the cytotoxic T cell response was well established; however, the field was revolutionized by the discovery that class I molecules present small protein fragments (known as peptides) coming from inside the cell following viral infection<sup>15</sup>. High-resolution crystal structures of class I molecules revealed their antigen-binding site as a peptide-binding groove with self-peptides presented in the absence of viral infection<sup>16,17</sup>. These findings led to the arthritogenic peptide hypothesis, which broadly suggests that *HLA-B\*27*-associated disease results from “a [CD8<sup>+</sup>] T-cell-mediated anti-self-reaction directed at an as yet unknown peptide-HLA-B27 combination”<sup>9</sup> (Fig. 2). If the presentation of a microbially derived peptide triggered subsequent recognition of a self-peptide (or peptides), the CD8<sup>+</sup> T cells would be autoreactive and cross-reactivity would constitute a true form of molecular mimicry.

Forced expression of HLA-B27 and human β<sub>2</sub>-microglobulin (hβ<sub>2</sub>m) in mice (*HLA-B\*27* transgenic mouse model) was reported in 1987 and demonstrated that rodent T cells could recognize HLA class I proteins<sup>18</sup>. However, these mice did not develop inflammatory disease. Adopting a similar approach in rats led to the discovery in 1990 that overexpression of HLA-B27 and hβ<sub>2</sub>m (but not HLA-B7 and hβ<sub>2</sub>m) can cause gut inflammation and arthritis, two key features of SpA<sup>8,19</sup>. Thus, in the early 1990s, armed with a novel hypothesis and an animal model, the field seemed poised to solve a nearly 20-year-old puzzle and define arthritogenic peptides presented by HLA-B27 that could trigger disease.

In 1993 a study of individuals with reactive arthritis (a transient form of SpA triggered by gastrointestinal and genitourinary infection) or AS demonstrated that patient-derived CD8<sup>+</sup> T cells could recognize target cells infected with triggering organisms. Importantly, some of these CD8<sup>+</sup> T cells also recognized uninfected cells, providing indirect evidence in support of arthritogenic peptide hypothesis<sup>20</sup>; however, little additional evidence of autoreactive T cells in AS or other forms of SpA accrued over the next several years.

By 1999 it was apparent that HLA-B27 exhibited aberrant properties<sup>12,13</sup>. Dimerized forms of HLA-B27 were found on the cell surface<sup>12</sup>, and newly synthesized HLA-B27 heavy chains were reported to misfold in the ER prior to the assembly of heavy chain–peptide–β<sub>2</sub>m complexes<sup>13</sup>. Previous studies had shown that HLA-B27 has a very strong preference for peptides containing arginine at position 2 from the N terminus<sup>21–23</sup> owing to the combination of residues lining the B pocket of HLA-B27, including a negatively charged glutamic acid at position 45 that interacts with the positively charged peptide position 2 arginine (Fig. 3). An unpaired cysteine is also found at position 67 of the HLA-B27 heavy chain<sup>23</sup>. Studies of misfolding and dimerization of HLA-B27 demonstrated that the B pocket, including this reactive cysteine residue, is responsible for the aberrant properties of HLA-B27

(refs. 12,13,24). Thus, the same structural features that link HLA-B27 to the binding of potentially arthritogenic peptides are responsible for its aberrant properties.

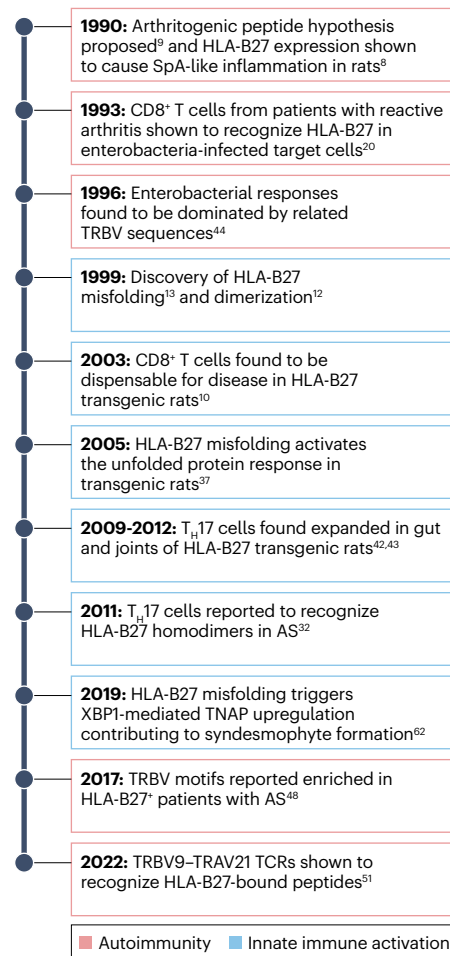
The existence of cell-surface HLA-B27 heavy chain dimers and the tendency of the heavy chain to misfold led to the investigation of other possible mechanisms, beyond arthritogenic peptides, whereby HLA-B27 might contribute to disease. In parallel, studies carried out in *HLA-B\*27* transgenic rats that emerged in the late 1990s and early 2000s implicated CD4<sup>+</sup> T cells rather than CD8<sup>+</sup> T cells in disease causation<sup>10,25</sup>, and promoted the search for these alternative mechanisms. This implication of CD4<sup>+</sup> T cells in SpA pathogenesis was further supported by reports of arthritis in *HLA-B\*27* transgenic mice lacking endogenous  $\beta_2m$ , as the absence of  $\beta_2m$  abrogates the expression of HLA class I molecules on the cell surface and virtually eliminates the development of CD8<sup>+</sup> T cells<sup>26</sup>.

Increased expression of homodimers and even misfolded multimers of HLA-B27 was reported in cell lines with defects in antigen processing and presentation apparatus. Aberrant cell-surface complexes seemed to form primarily via endosomal recycling of cell surface HLA-B27 (ref. 27) with misfolded HLA-B27 heavy chains in the ER prevented from accessing the cell surface (probably because of the cellular 'quality control' mechanisms)<sup>13,24</sup> (Fig. 4). Evidence of increased expression of non-conventional forms of HLA-B27 on peripheral blood myeloid cells, in the joints, and in the gut has been reported in HLA-B27-positive individuals with AS<sup>28,29</sup>. It was subsequently shown that recombinant HLA-B27 homodimers were able to bind to a number of innate immune receptors including killer immunoglobulin-like receptor (KIR) 3DL2 (KIR3DL2) and leukocyte immunoglobulin-like receptor (LILR) B2 (LILRB2) expressed on natural killer (NK) cells, on subsets of CD4<sup>+</sup> and CD8<sup>+</sup> T cells and on B cells<sup>30,31</sup> (Fig. 4). KIRs such as KIR3DL2 primarily deliver negative signals, but cell co-culture experiments showed that the interaction between KIR3DL2 and aberrant HLA-B27 seemed to deliver an anti-apoptotic signal resulting in the expansion of KIR3DL2-positive CD4<sup>+</sup> T cells, which were also skewed towards enhanced production of the pro-inflammatory cytokine IL-17 (ref. 32). Together, these findings suggest that HLA-B27 could trigger or promote disease via innate immune receptor recognition of aberrant cell-surface forms of HLA-B27 (Fig. 4).

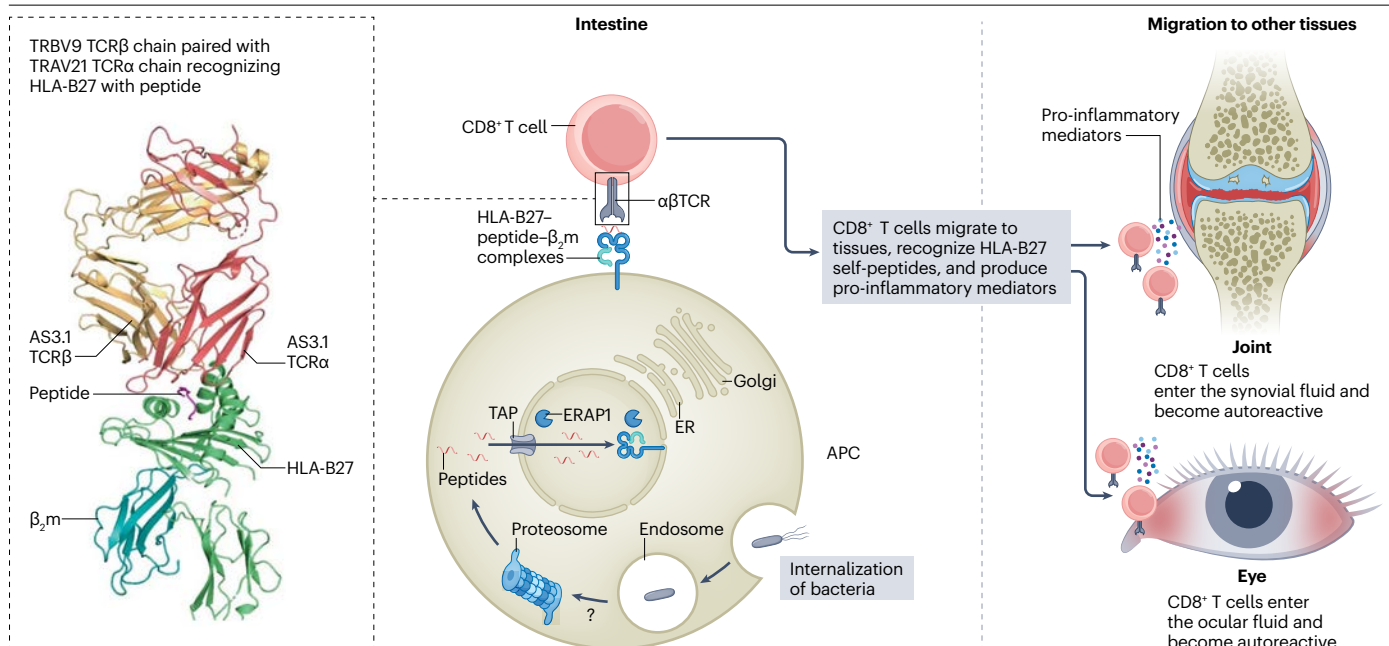
It is also important to consider that other non-*HLA-B\*27* class I alleles have been shown to contribute risk (albeit to a much lesser extent than *HLA-B\*27*) for the development of AS<sup>33,34</sup>, and there is evidence for an epistatic interaction between *HLA-B\*27* and *HLA-B\*60* whereby the presence of both substantially increases the risk of disease<sup>35</sup>. Although no unifying mechanism has been established, one possibility is that heavy chains encoded by other risk alleles can also form aberrant cell-surface multimers, the capacity for which is dependent to a large extent on the amino acid residue in position 97 (ref. 36). This potential mechanism is supported by data from studies using transfected cell lines but remains to be tested in cells naturally expressing germline-encoded alleles<sup>36</sup>. These intriguing findings require further verification but offer a potential link between several *HLA-B* alleles and pathogenic IL-17 production.

The propensity of HLA-B27 to misfold led to the discovery that exposure of myeloid cells from *HLA-B\*27* transgenic rats to cytokines that upregulate MHC class I and MHC class II protein expression led to the accumulation of HLA-B27 dimers in the ER and activation of the unfolded protein response (UPR)<sup>37,38</sup> (Fig. 5). Although HLA-B27 dimers in the ER can be degraded by quality control mechanisms such as ER-associated degradation<sup>13</sup> and autophagy<sup>39</sup>, inefficient ubiquitination

of HLA-B27 dimers seems to have a role in their accumulation<sup>39</sup>, and might help to explain why ER stress develops. UPR activation has several effects, including a reduction in the flux of proteins into the ER, and an expansion of the capacity of this organelle to fold, secrete, and/or degrade proteins<sup>40</sup>. UPR activation can also lead to synergistic upregulation of certain cytokines such as IFN $\beta$ <sup>41</sup> and IL-23 (refs. 40,42) (Fig. 5). The key role of IL-23 in the development, survival, and activation of CD4<sup>+</sup> T helper 17 cells (T<sub>H</sub>17 cells), together with earlier evidence implicating CD4<sup>+</sup> T cells in mediating the SpA-like disease in *HLA-B\*27* transgenic rats, led to the discovery that T<sub>H</sub>17 cells were major components of inflammatory infiltrates in the gut<sup>42</sup> and joints<sup>43</sup> of these rats (Fig. 1). Whether or not there is a role for IFN $\beta$  in the pathogenesis of SpA remains unknown. These observations suggested that HLA-B27



**Fig. 1 | A historical perspective of HLA-B27.** Timeline depicting major events in research on the role of HLA-B27 in spondyloarthritis (SpA) since the publication of the arthritogenic peptide hypothesis and the report of SpA-like disease in *HLA-B\*27* transgenic rats in 1990. Events shown in red boxes are related to autoimmunity, whereby HLA-B27 is targeted by adaptive immune cells, whereas events shown in blue boxes relate to alternative hypotheses, including HLA-B27 serving as a target for innate immune receptors (killer immunoglobulin-like receptors), a trigger for cytokine production, or a stimulus for mineralization and new bone formation. AS, ankylosing spondylitis; TCR, T cell receptor; T<sub>H</sub>17 cells, T helper 17 cells; TNAP, tissue non-specific alkaline phosphatase; TRAV, TCR $\alpha$ -chain variable; TRBV, TCR $\beta$ -chain variable.



**Fig. 2 | The arthritogenic peptide hypothesis.** Peptides derived from bacterial proteins in infected antigen-presenting cells (APCs) are presented by HLA class I (including HLA-B27) to generate a CD8<sup>+</sup> T cell response (middle panel). Expanded clones persist and cross react with HLA-B27 self-peptides that share similar contact residues with bacterial peptides. Autoreactive CD8<sup>+</sup> T cells can produce pro-inflammatory cytokines including IL-17A, as well as cytotoxic proteins (perforin and granzyme B) and cause inflammation in SpA target organs. Current hypotheses explaining why this is more common with HLA-B27 than with other alleles are centred on the idea that there is mimicry between

HLA-B27 self-peptides and certain bacterial peptides that does not exist for peptides presented by other HLA class I types. Left, a ribbon diagram of HLA-B27, peptide and TRBV9–TRAV21 TCR based on X-ray crystallographic studies. Right, migration of bacteria-specific CD8<sup>+</sup> T cells to the joint and ocular fluid where these cells can cross-react with self-peptides (autoreactive) on HLA-B27 to cause inflammation. ER, endoplasmic reticulum; ERAP1, ER aminopeptidase I; TAP, transporter associated with antigen processing; TCR, T cell receptor; β<sub>2</sub>m, β<sub>2</sub>-microglobulin. Adapted from ref. 51, Springer Nature Limited.

misfolding-induced ER stress, UPR activation and enhanced IL-23 production could provide a link between the *HLA-B\*27* allele and the inflammatory phenotype of SpA<sup>42</sup> (Fig. 5).

## New evidence regarding the role of HLA-B27 in spondyloarthritis

The emergence of new evidence for the role of HLA-B27 in SpA has prompted further research into identifying arthritogenic self-peptides and potential mechanisms of HLA-B27 in driving SpA pathogenesis.

### HLA-B27 and arthritogenic peptides

The arthritogenic peptide hypothesis is based on the idea that HLA-B27 is unique among HLA class I alleles in that its self-peptides resemble bacteria-derived peptides. This form of mimicry then elicits a pathogenic autoimmune CD8<sup>+</sup> T cell response. Since the initial demonstration that *Salmonella*-reactive CD8<sup>+</sup> T cells could cross-react with uninfected cells in 1993 (ref. 20), evidence of autoreactivity and arthritogenic peptides has accrued slowly. Conserved T cell receptor (TCR) β-chain variable (TRBV) usage in these cross-reactive CD8<sup>+</sup> T cells from patients with reactive arthritis provided evidence in support of specific TCR autoreactivity to a limited set of peptides<sup>44,45</sup>. Another study showed that HLA-B27 can present peptides from *Chlamydia*; however, the frequency of these CD8<sup>+</sup> T cells specific for *Chlamydia*-derived peptides was low and there was no evidence of pathogenicity or self-peptides as targets<sup>46</sup>. By using an approach that selected peptides with a potential

HLA-B27-binding motif, an increased frequency of CD8<sup>+</sup> T cell responses to a self-peptide derived from the vasoactive intestinal peptide receptor 1 protein was described in patients with AS<sup>47</sup>. This intriguing finding has not been widely replicated but deserves further attention.

Two key advances since 2017 have renewed interest in the role of arthritogenic peptides and CD8<sup>+</sup> T cells in SpA pathogenesis. First, the TRBV motif, which was first described in 2002 (ref. 45), has been confirmed in a large number of patients with axSpA using high-throughput TCR sequencing<sup>48–50</sup>. Most notably, a 2017 study included 234 patients with AS and 227 healthy individuals, including a group of healthy individuals who were *HLA-B\*27*-positive. The researchers found that the TRBV9 motif was present at a higher frequency in patients with AS than in both healthy individuals who were *HLA-B\*27* positive and those who were *HLA-B\*27* negative. These findings strongly suggest that TRBV9 T cells are enriched in AS over and above an effect of HLA-B27 alone<sup>48</sup>. Second, a 2022 study analysed the transcriptome of individual T cells from the joint and uveal fluid of patients with AS and acute anterior uveitis (AAU) using single-cell RNA sequencing<sup>51</sup>. This study confirmed the increase in TRBV9 sequences in patients with AS including the conserved CDR3 Y/FSTDQ motif, and identified TCRα-chain variable (TRAV) sequences (such as TRAV21) that frequently pair with TRBV9 in the expanded CD8<sup>+</sup> T cells in these patients (Fig. 2). The authors then expressed TCRα and TCRβ chains with the TRBV9 and TRAV21 sequences that are found in AS and/or AAU and used soluble TCRs to screen a library of peptides bound to HLA-B27. Using this approach, the

investigators identified self and microbial-derived peptides recognized by the (previously orphan) TCRs expanded in AS and AAU. Several receptors recognized a bacterial peptide (YelH.232-240), which is expressed by both commensal *Escherichia coli* and pathogenic *Salmonella* and *Shigella* spp.<sup>48</sup>. A follow-up study identified an additional expanded ocular CD8<sup>+</sup> T cell clonotype that expresses TRBV5-5 in combination with the conserved  $\beta$  chain CDR3 motif and paired with TRAV21, that recognizes YelH232.240. Interestingly, these CD8<sup>+</sup> T cells expressed a mucosal gene set consistent with antigen encounter and differentiation in the gastrointestinal tract<sup>52</sup>. It should also be noted that despite having similar antigen specificity, these ocular T cells expressed a TRBV5-4 or TRBV5-5 motif rather than TRBV9, indicating that TRBV9 is dispensable.

The authors of the follow-up study hypothesized a key role for the aforementioned T cells that were educated in the gut in the pathogenesis of AS and AAU and suggested that these cells might provide a link between the intestine and inflammation as these T cells are expanded in the eyes and joints during inflammation. However, this study<sup>52</sup> did not demonstrate the function of these clones (such as cytokine and/or cytotoxicity expression signatures), and thus additional studies will be necessary. Together, these studies<sup>48,52</sup> support a central tenet of the arthritogenic peptide hypothesis – that TCRs expressed by expanded T cells in SpA recognize HLA-B27-peptide complexes (Fig. 2). However, the nature, cell specificity, location and identity of arthritogenic self-peptides remain to be determined.

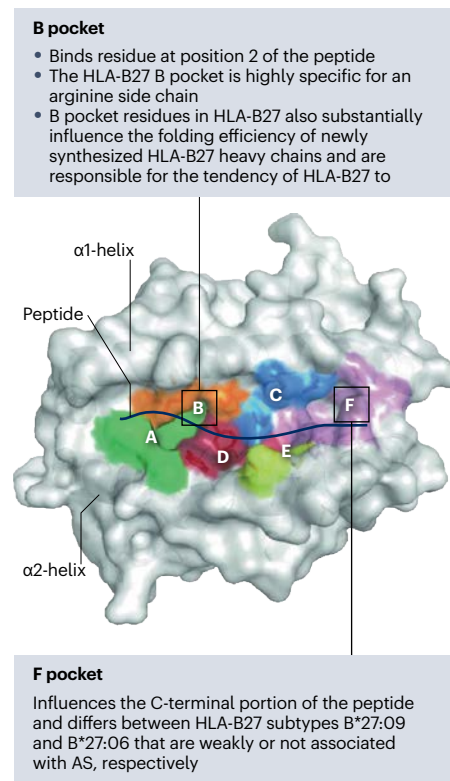
## HLA-B27 and endoplasmic reticulum stress

Although HLA-B27 overexpression in rats clearly generates ER stress and can lead to UPR activation, evidence that UPR activation occurs when HLA-B27 is expressed at physiological levels in humans is mixed<sup>53</sup>. In early studies UPR activation was not found in macrophages derived from peripheral blood even with HLA-B27 upregulation<sup>54,55</sup>; however, UPR activation has since been reported in other studies in these cell types with<sup>56</sup> and without<sup>57</sup> HLA-B27 upregulation. One challenge of measuring UPR activation is that UPR target gene expression can be transient, and the threshold for activation can be cell-type dependent. In addition, it could be that instead of a complete UPR, single components of the pathway are activated; for example, missense mutations in the TNF receptor (encoded by *TNFRSF1A*), which cause TNFR1 to misfold and accumulate intracellularly, cause *XBPI* (which encodes a UPR transcription factor) splicing without upregulation of binding immunoglobulin protein (BiP) and C/EBP homologous protein (CHOP) in cells from patients with TNF receptor-associated periodic syndrome<sup>40,58</sup>. Cells from these patients also accumulate mitochondrial reactive oxygen species, which alters pro-inflammatory cytokine production and is likely to contribute to disease pathogenesis<sup>59</sup>.

The finding that UPR induction of CHOP mediates synergistic *IL23A* upregulation after triggering Toll-like receptors (TLRs)<sup>60</sup> focused some attention on this UPR target gene in HLA-B27-mediated disease. The role of CHOP in HLA-B27-induced gut inflammation was explored using *HLA-B\*27* transgenic rats<sup>61</sup>. This study tested the hypothesis that eliminating CHOP would ameliorate gut inflammation if HLA-B27 was driving increased IL-23 expression via UPR activation. However, CHOP-deficient HLA-B27-expressing rats were found to exhibit slightly worse gut inflammation compared with *HLA-B\*27* transgenic rats with CHOP, despite a reduction in *IL23a* expression. Expression of *Il17a* and *Il17f* was not substantially reduced despite the reduction in *IL23a* expression. Enhanced expression of genes that encode cytokines such as *Tnf*, *Ifng*, *Il1a* and *Il1b* was found in the tissue in animals lacking CHOP, consistent with increased histology scores. These findings indicate that an

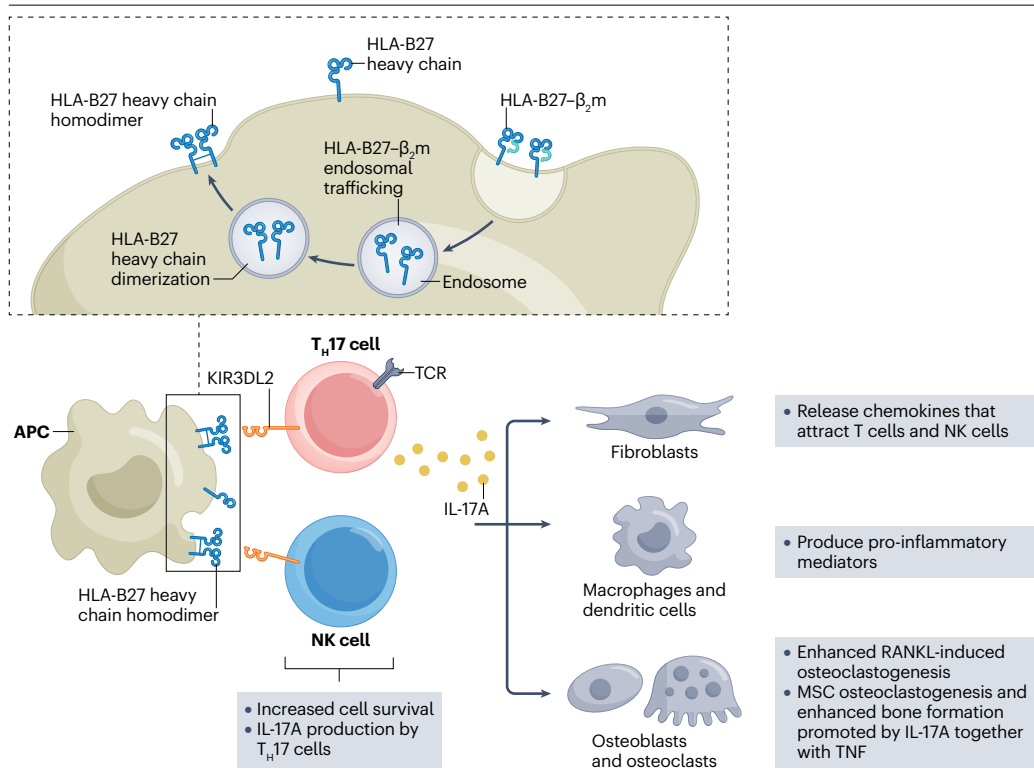
HLA-B27-induced UPR does not drive gut disease in this experimental model of SpA, but rather suggest a protective role for CHOP<sup>61</sup>.

There is compelling evidence that HLA-B27 expression could have a role in increased bone formation in patients with AS through activation of *XBPI* (ref. 62). Mesenchymal stem cells (MSCs) isolated from spinal enthesal tissue obtained during surgery from *HLA-B\*27*-positive patients with AS exhibited enhanced bone mineralization when differentiated into osteoblasts compared with cells obtained from similar sites from patients undergoing surgery for trauma. Bone-formation-pathway genes were not differentially expressed in AS cells during differentiation compared with controls, but rather there was increased expression of tissue non-specific alkaline phosphatase (TNAP; encoded by *ALPL*) in cells from patients with AS. TNAP can contribute to bone formation by increasing the pool of inorganic phosphate through pyrophosphate cleavage<sup>63</sup>. Inhibition of TNAP was sufficient to decrease the aberrant mineralization observed in cells from patients with AS. Increased TNAP levels were detected in the serum of patients with AS



**Fig. 3 | Structural features of the HLA-B27 peptide binding groove.**

Space-filling model of HLA-B27 based on a crystal structure depicting the peptide binding site shown in ‘top down’ orientation as a landing site for the T cell receptor. The HLA class I peptide binding site is shown as a series of 6 pockets (labelled A–F) that form when the heavy chain folds into a peptide-receptive state. Pockets A (green) and F (purple) contain conserved contact residues for the N terminus and C terminus of bound peptide. Amino acid residues that vary considerably between HLA alleles line the peptide-binding groove and create pockets that vary in size, shape and chemical composition, thus resulting in the binding of a vast array of peptides with substantial differences between alleles. The HLA-B27 B pocket (orange) dominates the selection of peptides that bind to HLA-B27 and has a strong preference for an arginine side chain at position 2 of the peptide. AS, ankylosing spondylitis. Adapted from ref. 108, Springer Nature Limited.



**Fig. 4 | Innate immune receptor recognition of HLA-B27 heavy chains.** A portion of cell surface HLA-B27 exists as  $\beta_2$ -microglobulin ( $\beta_2m$ )-free dimers or monomers. Cell-surface dimers form during endosomal recycling of HLA-B27 heavy chains that have lost  $\beta_2m$  and peptide (top). T helper 17 cells ( $T_H17$  cells) and natural killer (NK) cells bearing innate immune receptors (such as killer immunoglobulin-like receptor (KIR) 3DL2 (KIR3DL2) and leukocyte immunoglobulin-like receptor (LILR) B2 (LILRB2)) can bind to free HLA-B27 heavy chains, triggering survival signals and/or release of cytokines such as IL-17A (bottom). IL-17A is a potent pro-inflammatory cytokine involved in the pathogenesis of spondyloarthritis, which might occur through its effects on cells such as fibroblasts, myeloid cells, osteoclasts and osteoblasts. APC, antigen-presenting cell; MSC, mesenchymal stem cell; TCR, T cell receptor.

who exhibited more functional restriction and structural damage from their disease. Moreover, implants of these human MSCs into paraspinous areas of immunodeficient (NOD SCID) mice revealed increased ectopic bone formation by cells from patients with AS in a TNAP-dependent fashion. HLA-B27 was both necessary and sufficient for increased TNAP expression, as demonstrated by knockdown and overexpression experiments, and the effect was specific for HLA-B27, establishing a direct role for this gene product in aberrant mineralization. A link to ER stress activation was established by demonstrating misfolded forms of HLA-B27 in cells exhibiting IRE1 activation and increased *XBPI* splicing, and the investigators also showed that spliced *XBPI* could upregulate the transcription factor RARB (retinoic acid receptor- $\beta$ ), which in turn upregulates TNAP expression<sup>62</sup>. Spliced *XBPI* can also promote endochondral bone formation<sup>64</sup>, although this mechanism was not investigated in this study. Whether spliced *XBPI* promotes IFN $\beta$  production in MSCs, as has been shown in other cell types in the context of TLR stimulation<sup>41</sup>, remains to be determined. IFN $\beta$  is a potent inhibitor of osteoclast formation and thus could contribute to a relative increase in bone formation independent of its effect on osteoblasts.

**Epistasis between HLA-B\*27 and ERAP1**

There is epistasis between *HLA-B\*27* and *ERAP1* (which encodes endoplasmic reticulum aminopeptidase 1 (ERAP1)), with genetic variants in *ERAP1* conferring either risk or protection in *HLA-B\*27*-positive individuals with AS<sup>65</sup>; however, this effect also extends to *HLA-B\*40:01* in *HLA-B\*27*-negative individuals<sup>33</sup>. Although ERAP1 has more than one function<sup>66</sup>, its prominent role in trimming peptides in the ER that are eventually presented by HLA class I proteins means that this enzyme can determine the quality and/or quantity of peptides available to HLA-B27. Genetic and functional data indicate that ERAP1 variants that confer

loss-of-function or reduced expression are associated with protection from AS<sup>65,67,68</sup>. Knocking down ERAP1 expression in cell lines to mimic reduced expression and/or loss-of-function clearly affects the presence of dimers and misfolded forms of HLA-B27 as well as HLA-B27-bound peptides, although the use of different cell lines and experimental conditions has led to variable results between studies<sup>69-72</sup>. A consistent finding is that many peptides presented by HLA-B27 in the absence of ERAP1 are longer than the canonical octamers, nonamers and decamers found when ERAP1 expression is normal<sup>71,73</sup>.

Epistasis between *HLA-B\*27* and *ERAP1* has been modelled in rodents, in which eliminating ERAP1 expression protects *HLA-B\*27* transgenic rats from developing arthritis without reducing gut inflammation<sup>74</sup>. As CD8<sup>+</sup> T cell recognition of HLA-B27 does not have a role in this model, a more important finding was that in the absence of ERAP1, HLA-B27 folding was more efficient, which reduced misfolding and mitigated activation of the UPR and the increase in *Il23a* expression<sup>74</sup>. Thus, as peptide availability influences HLA-B27 folding and cell-surface stability, epistasis between *HLA-B\*27* and *ERAP1* does not imply a predominant mechanism of pathogenesis. It should be noted that epistatic interactions between *ERAP1* and HLA class I alleles are also seen with Behçet disease (*HLA-B\*51*) and psoriasis (*HLA-C*)<sup>75,76</sup>. Although loss-of-function variants of ERAP1 are associated with protection from AS, they confer risk of Behçet disease<sup>77</sup>, underscoring the complexity of the relationship between ERAP1, HLA class I and disease development.

**HLA-B27 and alterations in the gut microbiome**

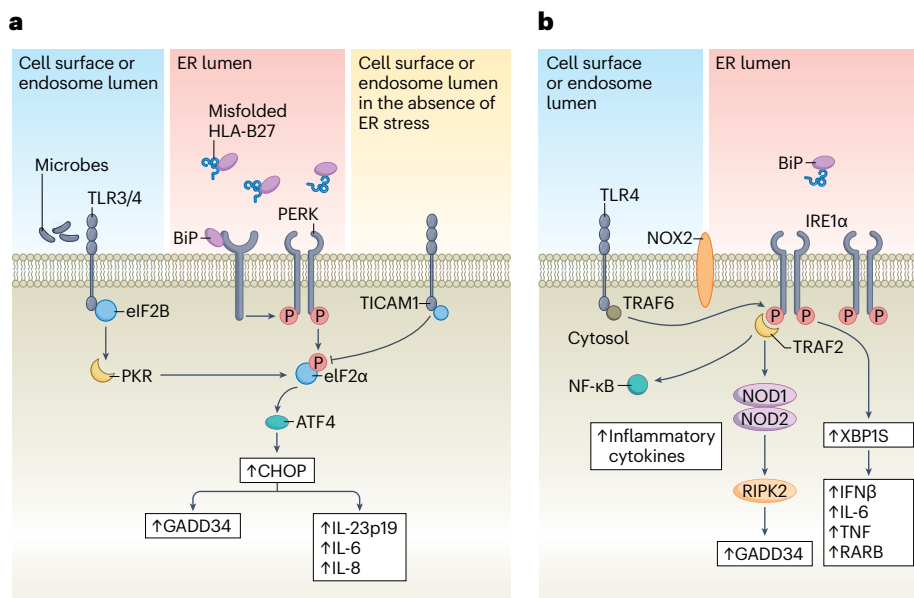
Although there is considerable evidence from animal as well as human studies that implicates gut microbial dysbiosis in the pathogenesis of SpA, the role of HLA-B27 is less clear. Pioneering studies from the 1990s demonstrated that maintaining HLA-B27 transgenic animals in

germ-free conditions could prevent the development of arthritis and gut inflammation<sup>78</sup>. HLA-B27 transgenic rats develop gut dysbiosis<sup>79</sup>; however, the characteristics of dysbiosis are highly dependent on the strain of rat (that is, the genetic background of the rat) and/or environment, with no clear evidence of increases or decreases in the abundance of specific bacteria across strains<sup>80</sup>. Furthermore, it remains unclear whether changes in microbiota precede altered cytokine expression in the gut of *HLA-B\*27* transgenic rats, or if the opposite is true. Separating out the effects of HLA-B27 in humans is even more complex owing to heterogeneity among the other HLA alleles as well as dietary and environmental differences. Nevertheless, associations between HLA and microbial composition have been observed for *HLA-B\*27* as well as other HLA alleles<sup>81</sup>. Studies have emphasized a reduction in microbial diversity in patients with SpA compared with healthy individuals, particularly in those who are *HLA-B\*27*-positive, as well as overall correlations between dysbiosis and disease activity, with reproducible increases in *Ruminococcus gnavus*<sup>82,83</sup>, which is known to produce a pro-inflammatory polysaccharide<sup>84</sup>. The presence of HLA-B27 could influence the microbiota through any or all of the three mechanisms described above. Further studies are needed to better understand the role of the microbiota in the pathogenesis of SpA.

## HLA-B27–ALK2 interactions

Ectopic expression of HLA-B27 and  $\beta_2m$  in *Drosophila melanogaster* results in an abnormal phenotype characterized by loss of crossveins in

the wings and in some cases reduced eye size<sup>85</sup>. This effect of HLA-B27 was caused by an interaction of properly folded HLA-B27– $\beta_2m$  heterodimers with the type I bone morphogenic protein (BMP) receptor 1 saxophone (Sax). The HLA-B27– $\beta_2m$  interaction with Sax seems to disrupt Sax homodimerization, removing the antagonistic effect of Sax, thereby enhancing BMP signalling and preventing crossvein formation in the wings. The investigators went on to establish the relevance of this phenomenon in humans by showing that HLA-B27 complexes exist on the cell surface in proximity to ALK2 (encoded by *ACVR1* in humans), which is the mammalian orthologue of the *Drosophila* Sax receptor. ALK2 is important for bone homeostasis, mediating BMP, TGF $\beta$  and activin signalling. At baseline and upon stimulation with activin A or TGF $\beta$ , peripheral blood T cells from patients who were *HLA-B\*27* positive exhibited increased phosphorylation of the downstream mediators SMAD2 and SMAD3 relative to T cells from healthy individuals expressing other HLA class I proteins. This finding suggests that an interaction of HLA-B27 with ALK2 might prevent its inhibitory effect on BMP signalling in SpA, possibly promoting the aberrant bone formation phenotype. This positive effect could be qualitatively similar to the effect of ALK2 mutations that reduce binding to its natural inhibitor, resulting in the loss of antagonism and increased BMP signalling in fibrodysplasia ossificans progressiva. This disorder is characterized by progressive heterotopic ossification of connective tissue<sup>86</sup>. Additionally, increased phosphorylation of SMAD2 and SMAD3 in HLA-B27-expressing T cells might promote T<sub>H</sub>17 cell formation in SpA<sup>87</sup>. These important observations will need to



**Fig. 5 | Convergence of endoplasmic reticulum stress and Toll-like receptor signalling pathways.**

The tendency of the HLA-B27 heavy chain to misfold can cause endoplasmic reticulum (ER) stress, triggering activation of the unfolded protein response (UPR). A complete UPR is generated when binding immunoglobulin protein (BiP) binds to misfolded proteins, such as HLA-B27, and is released from IRE1 $\alpha$ , PERK and ATF6 (not shown), which enables their activation, leading to a transcriptional response and upregulation of UPR target genes. UPR activation can enhance cytokine production in cells stimulated with Toll-like receptor (TLR) ligands derived from microbial products. **a**, Activation of PERK leads to phosphorylation of eukaryotic translation initiation factor 2 $\alpha$  (eIF2 $\alpha$ ), increased expression of ATF4 and upregulation of C/EBP homologous protein (CHOP), which in turn promotes

IL-23, IL-6, and TNF production in myeloid cells stimulated with TLR3 and TLR4 (TLR3/4) ligands. The double-stranded RNA sensor kinase (PKR) can also play a part in the stress response through eIF2B. **b**, Activation of IRE1 $\alpha$  activates *XBP1* mRNA splicing, and synthesis of the active XBP1 protein. Downstream effects include promoting expression of IFN $\beta$ , IL-6, and TNF in cells exposed to TLR ligands. HLA-B27 expression has also been linked to XBP1-dependent upregulation of retinoic acid receptor- $\beta$  (RAR $\beta$ ) with downstream upregulation of tissue non-specific alkaline phosphatase (TNAP) (which is encoded by *ALPL*). TNAP expression promotes mineralization of HLA-B27-expressing mesenchymal stem-cell-derived osteoblasts. Thus, HLA-B27 might promote bone formation independent from effects on inflammation. NF- $\kappa$ B, nuclear factor- $\kappa$ B. Adapted from ref. 40, Springer Nature Limited.

be confirmed and extended to better understand the mechanism and consequences of the HLA-B27–ALK2 interaction.

## Implications for spondyloarthritis treatment

Landmark studies identifying TNF messenger RNA in sacroiliac joint tissue from patients with AS provided a strong rationale for the use of biologic DMARDs targeting this cytokine<sup>88</sup>, a strategy that substantially advanced the treatment of AS in the early 2000s. The studies reviewed here implicating HLA-B27 in the activation of the IL-23–IL-17 axis contributed to the development and validation of IL-17A inhibition in the treatment of AS<sup>89</sup>. It should be noted that type 17 cells produce both IL-17A and IL-17F, and IL-17F might have pathogenic effects independent of IL-17A. To date only IL-17A inhibitors are approved for the treatment of axSpA. A 2024 study demonstrated the efficacy of bimekuzimab, which blocks both IL-17A and IL-17F, in active axSpA<sup>90</sup>. Additional studies will be needed to define the importance of IL-17F blockade with or without concomitant IL-17A blockade in the treatment of SpA. Interestingly, biologic drugs targeting IL-23 (and both IL-12 and IL-23) failed to show efficacy in AS or axSpA<sup>91,92</sup> despite strong genetic and pathophysiological evidence that the IL-23 signalling pathway is important in this disease, and that blockade of this pathway has clear therapeutic efficacy in psoriasis<sup>93</sup> and psoriatic arthritis<sup>94</sup>. The reasons for the failure of IL-23 inhibition, particularly in the context of the success of IL-17A and IL-17F blockade, are not clear. Potential reasons for the failure could include the possibility of an earlier therapeutic window that has closed in the patients with AS or axSpA who were studied in trials, and/or the importance of various innate immune cells that produce IL-17A and IL-17F independently of IL-23 stimulation, perhaps autonomously or through direct interaction with cell-surface HLA-B27 dimers that trigger NK cells or CD4<sup>+</sup> T cells<sup>95</sup> (Fig. 4). The Janus kinase (JAK) inhibitors upadacitinib and tofacitinib have also proven efficacious and are now approved for the treatment of axSpA<sup>96</sup>, although the major cytokine pathways that are affected remain unclear. It will be interesting to learn whether TYK2 inhibitors (such as deucravacitinib) are efficacious in axSpA, as they have the potential to be better for blocking type 17 innate and adaptive immune cell dysfunction<sup>97</sup>.

The success of blocking TYK2 in a preclinical model of SpA supports this idea<sup>98</sup>, but awaits confirmation.

The discovery and further characterization of T cells expressing HLA-B27-restricted TRA21–TRBV9 and other TCR motifs in AS provide an opportunity to test new cell-based therapies in this disease. For example, chimeric antigen receptor (CAR) T cells directed at the conserved TCR chains might be used to selectively deplete T cells that are critical to disease initiation and/or progression. Conceptually, therapies based on displacing targeted self-peptides or even reducing HLA-B27 expression could be considered. Intriguingly, a 2023 study reported that treatment of a patient who had had AS for >30 years with an anti-TRBV9 monoclonal antibody effectively depleted TRBV9-positive CD8<sup>+</sup> T cells in the peripheral blood<sup>99</sup>; this depletion was associated with substantial clinical improvement, including measures of mobility and function that are associated with ankylosis and usually considered irreversible<sup>100</sup>. The patient's previous treatment was also highly unusual, as they had received an autologous haematopoietic stem cell transplant for AS a decade earlier. This result is nevertheless very promising and justifies further study. Early results from a randomized, double-blind, placebo-controlled trial of BCD-180 (anti-TRBV9 antibody) were reported in 2024, demonstrating that up to 50% of patients who received BCD-180 achieved an ASAS40 response at week 24 compared with only 24% of those who received placebo<sup>101</sup>. The enthusiasm for anti-TRBV9 approaches must be tempered by the fact that there are TRBV5-4 and TRBV5-5 motifs that recognize the same antigen that could contribute to disease and yet not be targeted by anti-TRBV9 antibodies<sup>52</sup>.

## Future perspectives

Research into the mechanisms whereby HLA-B27 contributes to the pathogenesis of SpA continues to advance, although defining the most proximal and causal events in early disease remains a substantial challenge. Technological and conceptual advances in our understanding of innate and adaptive immunity and the effects of HLA-B27 expression have raised numerous questions that should be addressed by the research community (Table 1).

**Table 1 | Future perspectives for HLA-B27-related mechanisms**

Pathways related to HLA-B27	Important questions	Potential research strategies
Clonally expanded CD8 <sup>+</sup> T cells and recognition of arthritogenic peptides	What is the phenotype of TRBV9 <sup>+</sup> and TRAV21 <sup>+</sup> CD8 <sup>+</sup> T cells and are they expanded in entheses, synovial tissue and the gut? What is/are the autoantigenic (arthritogenic) peptide(s)? Are expanded CD8 <sup>+</sup> T cells with private TCRs HLA-B27 restricted? Do CD4 <sup>+</sup> T cells express TRBV9 or other expanded sequences?	Paired single-cell RNA and TCR sequencing and spatial analysis of affected tissues Screening of tissue expression libraries using TCRs constructed from expanded clones Further analysis of expanded CD8 <sup>+</sup> T cells In-depth analysis of CD4 <sup>+</sup> T cell TCRs in SpA
Regulatory CD8 <sup>+</sup> killer T cells	Are KIR <sup>+</sup> CD8 <sup>+</sup> T cells relevant to SpA? Are they regulated by aberrant HLA-B27?	Immunophenotyping and functional analysis of KIR <sup>+</sup> CD8 <sup>+</sup> T cells in SpA
Consequences of ER stress	What cells are affected by HLA-B27-mediated ER stress? Are gut epithelial cells affected? Is UPR activation incomplete, with enhanced <i>XBP1</i> splicing representing the key pathway?	Assess effects of HLA-B27 expression in gut epithelial and other cell types Does activation of <i>XBP1</i> splicing require additional stimuli in HLA-B27-expressing cells?
ALK2 inhibition and enhanced TGFβ and BMP signalling	How are immune cells altered by HLA-B27 effects on the ALK2 pathway? Does this mechanism alter bone formation?	Biochemical and molecular analysis of the ALK2–HLA-B27 interaction Analysis of immune cells, MSCs and osteoblasts

BMP, bone morphogenetic protein; ER, endoplasmic reticulum; KIR, killer-cell immunoglobulin-like receptors; MSCs, mesenchymal stem cells; SpA, spondyloarthritis; TCR, T cell receptor; TGFβ, transforming growth factor-β; UPR, unfolded protein response.

There is now direct evidence that CD8<sup>+</sup> T cells bearing  $\alpha\beta$ TCRs that are expanded in AS and AAU can recognize HLA-B27-bound peptides<sup>51</sup>, and some cells might develop after encountering bacterial antigens in the gastrointestinal tract, providing a clue to their origin<sup>52</sup>. This work has galvanized the field, but there is more to learn about the phenotype, function, and cytokine expression pattern of these T cells, as well as the identification of self-peptides that these CD8<sup>+</sup> T cells are targeting and if they are indeed autoreactive as is anticipated. CD8<sup>+</sup> T cells bearing these public TCRs (that is, a TCR that is shared between multiple individuals) have been found in the joints and vitreous fluid of patients with axSpA and AAU (with or without axSpA) and would be expected at other sites of inflammation such as entheses. In addition to public clones, private clones that are not broadly shared by patients have been discovered<sup>48</sup>; whether or not these clones are also HLA-B27 restricted or if other HLA class I alleles present in individuals who are HLA-B27 positive are the target of additional autoreactive CD8<sup>+</sup> T cells will be of interest. Such expansions could differ between individuals owing to HLA heterogeneity and would have escaped detection to date. Broader autoimmunity could be the case if aberrant properties of HLA-B27 work in concert as an innate immune stimulus to promote CD4<sup>+</sup> T<sub>H</sub>17 cells and/or CD8<sup>+</sup> T cell autoreactivity. Notably, *HLA-B\*27* positivity is associated with better outcomes following infection with viruses such as hepatitis C and HIV<sup>102,103</sup>. The basis for these associations is not known but could be related to oligomeric intracellular or extracellular forms of HLA-B27 serving as innate immune stimuli. If there is a more general phenomenon of autoreactivity, the question of whether other genetic factors contribute to a loss of immune tolerance should be considered. These questions cannot be addressed using existing animal models in which disease develops independently of CD8<sup>+</sup> T cells, and thus studies of human cells and tissues or new models will be required.

Although the arthritogenic peptide hypothesis in its original form proposes that autoreactive CD8<sup>+</sup> T cells are cytotoxic (that is, they kill target cells) it is plausible that they might function primarily via production of pro-inflammatory cytokines. Traditionally, these cytokines would include TNF and IFN $\gamma$ , although production of IL-17A and IL-17F by CD8<sup>+</sup> T cells can occur and might be expected in SpA. It will be important to perform detailed phenotyping and functional analysis of TRBV9-motif-bearing CD8<sup>+</sup> T cells in patients with SpA, ensuring that appropriate control samples are also analysed. Given the established importance of type 17 innate and adaptive lymphocytes and their cytokines in SpA pathogenesis, establishing a link between expanded CD8<sup>+</sup> T cells and T<sub>H</sub>17 cytokines is a high priority, as well as determining whether CD4<sup>+</sup> T cells express expanded  $\alpha\beta$ TCR motifs.

It would be surprising if the widespread tissue inflammation seen in axSpA (at sites such as entheses, axial and peripheral joints, gastrointestinal tract and eyes) will be explained by a single peptide targeted by autoreactive T cells, and thus looking for additional expanded TCR motifs and cross-reactive peptides is relevant. Characterization of expanded TCR motifs should extend to early disease, including juvenile-onset SpA, to establish the primacy of these cells and further specificity for HLA-B27.

The new developments discussed in this Review suggest the promise of novel biologic drugs and cell-based therapies aimed at eliminating the expanded TRBV9 and TRBV5-5-motif-bearing CD8<sup>+</sup> T cells. Early results from clinical trials are intriguing, but larger studies must be carried out to establish therapeutic efficacy and provide correlates with changes in imaging and tissue inflammation.

CD8<sup>+</sup> T cells expressing inhibitory KIRs have been shown to efficiently eliminate pathogenic gliadin-specific CD4<sup>+</sup> T cells from

leukocytes isolated from patients with coeliac disease in vitro<sup>104</sup>. Considering the previous evidence supporting the regulation of CD4<sup>+</sup> T cells and NK cells by HLA-B27 via its binding to KIR3DL2 (ref. 105), it is worth considering if KIR<sup>+</sup> CD8<sup>+</sup> T cells are relevant to SpA pathogenesis. More specially, KIR<sup>+</sup> CD8<sup>+</sup> T cells could regulate the expansion of T<sub>H</sub>17 cells. If this is the case, assessing whether the KIR<sup>+</sup> CD8<sup>+</sup> T cells in SpA are clonally expanded and restricted to HLA-B27 would be of interest. Future work performing immunophenotyping and functional studies of KIR<sup>+</sup> CD8<sup>+</sup> T cells from blood or joint tissue will be important.

Although ER stress and enhanced IL-23 production caused by HLA-B27 expression in myeloid cells have been established in rats<sup>42</sup>, similar consequences have not been observed in humans. Moreover, a mechanistic link between UPR-induced CHOP and subsequent IL-23 production has been ruled out as a potential mechanism for gut inflammation in rats<sup>61</sup>. A 2023 study demonstrated that T<sub>H</sub>17 cell-inducing gut bacteria can also induce ER stress in intestinal epithelial cells (IECs)<sup>106</sup>, which could potentially be exacerbated by HLA-B27 expression. Notably, activation of these IECs enhanced their production of both reactive oxygen species and purine metabolites to promote the accumulation of T<sub>H</sub>17 cells, which are key drivers of inflammation in SpA. Thus, it would be of interest to determine whether HLA-B27 enhances ER stress in IECs, which could alter T<sub>H</sub>17 cell generation and the gut microbiota independent of the effects of CHOP on IL-23.

The discovery that HLA-B27 can inhibit ALK2 function<sup>85</sup> has implications for altering signalling responses to TGF $\beta$  as well as BMPs, with potential downstream effects on immune homeostasis through regulation of T cell subsets (examples of cells regulated by TGF $\beta$  include T<sub>H</sub>17 cells, regulatory T cells and tissue-resident memory T cells)<sup>107</sup> as well as bone formation. The molecular basis and consequences of this effect of HLA-B27 require further study.

In addition to *HLA-B\*27*, other HLA class I alleles (such as *HLA-A\*02:01*, *HLA-B\*40:01* and *HLA-C\*12:02*) and some HLA class II alleles have been associated with an increased risk of developing AS (compared with the absence of the aforementioned other HLA class I alleles in *HLA-B\*27*-negative individuals<sup>34</sup>), although the effect size of each allele is much smaller than that of *HLA-B\*27*. Notably, although there is epitasis between *ERAPI* and *HLA-B\*40:01* in conferring risk for AS<sup>33</sup>, this phenomenon has not been reported for other HLA class I alleles. The association of multiple HLA class I and II alleles with AS makes it unlikely that a single arthritogenic peptide exhibiting promiscuous binding to all these alleles is causative. Although searching for expanded TCR clones in individuals with other risk alleles and/or assessing the biology of these risk alleles is attractive, it could be technically difficult because so few cases exist relative to *HLA-B\*27*-positive patients.

## Conclusions

Evidence supports distinct roles for peptide presentation, cell-surface dimerization and misfolding of HLA-B27 in the pathogenesis of SpA. CD4<sup>+</sup> T cell recognition of cell-surface dimers by innate immune receptors and the development of ER stress secondary to HLA-B27 misfolding have been linked to increased production of IL-17A, a major target in the treatment of SpA. In addition, HLA-B27 can promote aberrant bone formation through misfolding and XBPI activation. New evidence has revealed that TCRs on CD8<sup>+</sup> T cells that are expanded in SpA recognize peptides bound to HLA-B27, and on the basis of antibody-mediated depletion studies, these T cells could be pathogenic, supporting the concept of arthritogenic peptides. Continued research in each of these areas of investigation is needed to better understand the contribution

of HLA-B27 to disease pathogenesis. Occam's (or Ockham's) razor implies that the simplest explanation is usually the best one; unfortunately, the conundrum of HLA-B27 and SpA has so far defied simple explanations.

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# Antigen-specific immunotherapies for autoimmune disease

Jane H. Buckner  

## Abstract

Antigen-specific therapies have a long history in the treatment of allergy but have not been successful in autoimmunity. However, in the past 20 years, advances in the definition of the self-antigens that promote autoimmunity and the growing understanding of the mechanisms that maintain tolerance in health but fail in autoimmunity have led to antigen-specific approaches being considered for the treatment of autoimmune diseases. The core goal of each antigen-specific treatment approach is to remove the immune response that promotes autoimmunity whilst sparing protective responses. Approaches to antigen-specific therapy range from targeted deletion of autoreactive lymphocytes to tolerization of autoreactive T cells and active inhibition of autoimmune responses. Technologies such as vaccines, nanoparticles, cell-based therapies and gene editing are being harnessed to achieve these goals. Remaining challenges include the selection of the best antigen to target, modality and timing of administration of these therapies and the disease in which the therapies are used; overcoming these challenges will be vital to move antigen-specific therapies forward. Once established, antigen-specific therapy has the potential to be applied broadly in the area of autoimmunity.

## Sections

[Introduction](#)[Current challenges for developing antigen-specific therapies](#)[Types of antigen-specific therapies](#)[Selecting the antigen to target](#)[Setting the stage for antigen-specific therapies](#)[Conclusions](#)

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## Key points

- Current therapies for autoimmune diseases target inflammation but are immunosuppressive and do not directly address the underlying cause of disease, which is a failure of immune tolerance to self.
- Antigen-specific therapies have the potential to target pathogenic autoreactive lymphocytes whilst preserving protective immune responses.
- Approaches to antigen-specific therapy include deletion, tolerization and/or active suppression of autoreactive lymphocytes.
- Therapeutic challenges include the selection of the appropriate antigen to target, avoidance of off-target effects and identification of the appropriate stage of disease for therapy.

## Introduction

Autoimmune diseases are driven by a failure of immune tolerance whereby immune responses targeting self-antigens become pathogenic, which leads to localized or systemic inflammation (Fig. 1). Autoimmunity can manifest as inflammation in a single organ that is driven by a loss of tolerance to antigens specific to that tissue and mediated by autoantibodies or by autoreactive T cells. Examples of tissue-specific autoimmunity include Graves disease, in which anti-thyroid-stimulating hormone receptor antibodies result in hyperthyroidism, and type 1 diabetes mellitus (T1DM), in which autoreactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells target the insulin-producing  $\beta$ -cells in the pancreas<sup>1,2</sup>. Autoimmunity can also manifest as a systemic illness, driven by immune responses to autoantigens that are ubiquitous. Autoimmune diseases that are defined by systemic autoimmunity include systemic lupus erythematosus (SLE), in which immune responses target ubiquitous nuclear self-antigens, and seropositive rheumatoid arthritis (RA), in which immune responses target citrullinated self-antigens<sup>3,4</sup>.

The immune system maintains tolerance to self through multiple mechanisms. Autoreactive T cells with the potential to be pathogenic are deleted in the thymus and those that escape into the periphery are tolerized through anergy or suppressed by regulatory cells<sup>5</sup>. Thymically-derived CD4<sup>+</sup>FOXP3<sup>+</sup> regulatory T (T<sub>reg</sub>) cells are one form of regulatory cell that are central to maintaining self-tolerance. T<sub>reg</sub> cells are activated by self-antigens and upon activation suppress proliferation and cytokine production of effector T (T<sub>eff</sub>) cells through contact and non-contact mechanisms<sup>6</sup>. T<sub>reg</sub> cells mediate suppression by direct contact with T<sub>eff</sub> cells, but also through secretion of TGF $\beta$ , sequestration of IL-2 and alterations in antigen presenting cells (APCs) that limit their ability to activate T<sub>eff</sub> cells<sup>6</sup>. Notably, T<sub>reg</sub> cells not only suppress T<sub>eff</sub> cells that have the same specificity (that is, the MHC-peptide complex recognized by the T cell receptor (TCR)) but once activated also suppress bystander T cells, which includes T cells reactive to other self-antigens, which broadens the ability of T<sub>reg</sub> cells to control autoreactivity within a tissue<sup>6</sup>. B cells also undergo a process of selection, which limits the development of high affinity autoantibodies<sup>7</sup>. Regulation of autoreactivity is also mediated by the production of regulatory cytokines (for example, CD4<sup>+</sup> type 1 regulatory T (Tr1) cells produce IL-10 and TGF $\beta$ <sup>8</sup>). T cells and B cells influence each other: B cells function as APCs, which can promote autoreactivity, and T cells provide help to B cells which enables them to mature into antibody-producing cells<sup>9</sup>.

In the case of autoreactivity, a loss of tolerance in one compartment can promote the loss of tolerance in the other, resulting in autoimmunity and inflammation.

Current therapies for autoimmune diseases target the inflammatory processes. These approaches have led to marked improvement in controlling the symptoms and disability caused by many autoimmune diseases but result in immunosuppression, and the underlying cause of disease, failure of immune self-tolerance, is not directly addressed. The goal of antigen-specific therapies is not only to target pathogenic autoreactive lymphocytes but also to preserve protective immune responses. In this Review, I describe three different approaches to achieve this goal: deletion of pathogenic autoreactive lymphocytes, tolerization of pathogenic T<sub>eff</sub> cells and active regulation of autoreactive lymphocytes, with a focus on CD4<sup>+</sup> T cells. I address the evidence that indicates that these approaches can be successful, the challenges that must be overcome and the prospects for success in using these approaches to treat autoimmune and rheumatic diseases.

## Current challenges for developing antigen-specific therapies

A major challenge in the development of antigen-specific therapies for autoimmune diseases is that not all autoreactive lymphocytes promote disease. Indeed, autoreactive lymphocytes are also present in healthy individuals and promote tolerance, as is the case for T<sub>reg</sub> cells. Therefore, antigen-specific therapies must be designed to target autoreactive lymphocytes that promote disease whilst sparing the cells that contribute to health; to develop such antigen-specific therapies requires an in-depth knowledge of the specificity and pathogenic features of autoreactive lymphocytes that distinguish them from those that are important for maintaining health.

Antigen-specific therapy has been used for more than 100 years in the setting of allergy<sup>10</sup>, which is characterized by an inappropriate immune response to a benign foreign antigen. Allergen immunotherapy, which involves exposure to the allergen through mucosal or subcutaneous delivery can decrease symptoms and even prevent disease<sup>11</sup>. Despite the success of antigen-specific therapy in allergy, the translation of these approaches to autoimmunity has been challenging. Unlike in allergy, in which the target antigen is foreign, the target antigens for autoimmunity are self-antigens, which are often ubiquitous and must be targeted without impairing beneficial immune responses.

Other challenges include determining the best cell type to target and the specific antigen that promotes disease. The cell type could be selected on the basis of the understanding of the mechanism of disease pathogenesis. Thus, B cells should be targeted in settings in which autoantibodies are the prime mediator of disease, and T cells should be targeted when they are the key drivers of autoimmunity. However, selecting an approach based solely on the prime mediator of disease does not take into account the roles that B cells have as APCs, nor that T cell help is vital to promote B cell development. The choice and type of target antigen is also important and requires an in-depth knowledge of the antigen or antigens driving pathogenic responses. Additionally, B cells recognize whole antigens whereas T cells recognize peptides that are derived from whole antigens and presented via MHC on APCs. Despite these hurdles, multiple approaches currently show promise, including the deletion of autoreactive lymphocytes in vivo, the tolerization of autoreactive lymphocytes through anergy, alteration of their function or restoration of the ability of defective regulatory cells to control them, and the active regulation of pathogenic autoreactive T cell function via the delivery of regulatory cells (Fig. 2). The aim of

each of these approaches is to restore and maintain self-tolerance in patients with established autoimmune disease and to prevent the loss of self-tolerance in individuals who are at risk of developing autoimmune disease.

## Types of antigen-specific therapies

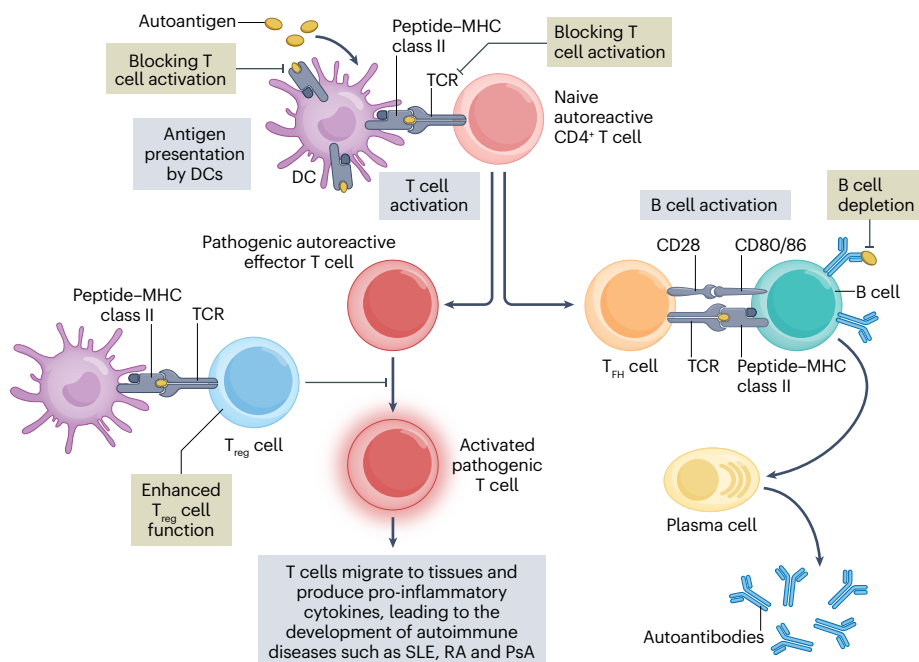
Despite the aforementioned hurdles, multiple approaches currently show promise for antigen-specific therapies, including the deletion of autoreactive lymphocytes *in vivo*, the tolerization of autoreactive lymphocytes through anergy, alteration of their function, or restoration of the ability of defective regulatory cells to control them, and the active regulation of pathogenic autoreactive T cell function via the delivery of regulatory cells (Fig. 2). The aim of each of these approaches is to restore and maintain self-tolerance in patients with established autoimmune disease and to prevent the loss of self-tolerance in individuals who are at-risk of developing autoimmune disease.

## Deletion of pathogenic autoreactive cells

Therapies that deplete total B cell populations have proven to be effective for the treatment or prevention of autoimmune diseases. Examples of these therapies include depletion of B cells via anti-CD20 monoclonal antibodies in RA, in vasculitis and in relapsing–remitting multiple sclerosis<sup>12–16</sup>, and anti-CD19 chimeric antigen receptor (CAR) T cell therapy in SLE, in idiopathic inflammatory myositis and in systemic sclerosis<sup>17–19</sup>. Although a decrease in autoantibodies has been seen in

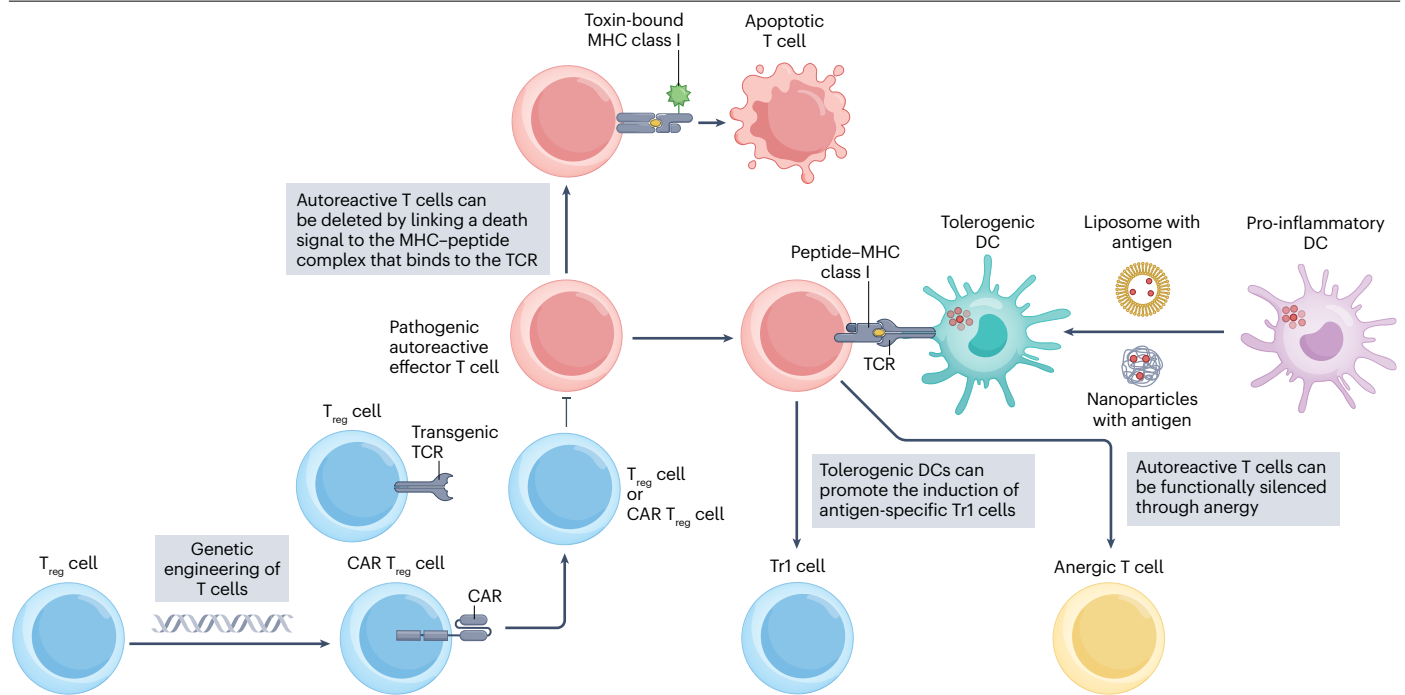
some settings with the use of B cell depletion therapies, it is not yet proven that the loss of autoantibodies is the basis for the therapeutic response. Indeed, in the case of anti-CD20 monoclonal antibodies, long-lived plasma cells and autoantibodies persist, raising the possibility that the production of antibodies by B cells could be less important than the additional roles that these cells have in the immune response, such as antigen presentation and cytokine production. Anti-CD19 CAR T cell therapy, however, does result in a deep depletion of B cells, and in SLE is accompanied by a drop in anti-DNA antibodies which suggests that loss of anti-DNA antibodies might be important to achieve a therapeutic response in SLE<sup>17,18,20</sup>. Less is known about the therapeutic efficacy of total T cell depletion in humans owing to concerns about profound immunodeficiency.

Despite the promise of lymphocyte depletion, these therapies are not antigen-specific, and result in substantial immunosuppression, including impaired responses to vaccines<sup>21,22</sup>. Thus, strategies for selective depletion of autoreactive lymphocytes are being actively pursued. Targeting of autoreactive B cells via the delivery of deletional signals through autoantigen binding to its receptor is being developed using particle-based therapies, soluble antigen multimers, cell-based therapies and fusion proteins, and is reviewed elsewhere<sup>23</sup>. As an example, liposomes coated with both factor VIII and CD22 (an inhibitory co-receptor) prevented bleeding in a mouse model of haemophilia, in which autoantibodies that target factor VIII were pathogenic<sup>24</sup>, and this type of approach could also be useful in autoimmune rheumatic diseases.



**Fig. 1 | Immune processes driven by antigen recognition in autoimmunity and points of antigen-specific intervention.** This figure demonstrates the key processes in the development of autoimmunity and how these processes can be targeted by antigen-specific therapies. Dendritic cells (DCs) presenting antigen on MHC class II activate naive autoreactive CD4<sup>+</sup> T cells and induce their differentiation into pathogenic T cells (such as T helper 1 (T<sub>H1</sub>), T<sub>H2</sub> or T<sub>H17</sub> cells) or T follicular helper (T<sub>H</sub>) cells. Autoreactive B cells are activated by T<sub>H</sub> cells and secrete autoantibodies. Regulatory T (T<sub>reg</sub>) cells can fail to suppress activated autoreactive T cells. Autoreactive T cells migrate to tissues

where they are activated and secrete pro-inflammatory cytokines resulting in disease pathology. Antigen-specific approaches (shown in brown boxes) include blocking T cell activation (either by stopping MHC–peptide binding to the T cell receptor (TCR) or preventing antigen presentation by DCs), depleting B cells (either by stopping antigen binding or preventing autoantibody production) and enhancing T<sub>reg</sub> cell function and numbers. Colours indicate T cell state: naive autoreactive CD4<sup>+</sup> T cell (pink), pathogenic T effector cell (red), T<sub>reg</sub> cell (blue), T<sub>H</sub> cell (orange). PsA, psoriatic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.



**Fig. 2 | Pathways to antigen-specific tolerance.** There are numerous processes that lead to the development of antigen-specific tolerance. Autoreactive T cells can be deleted by linking a death signal to the MHC-peptide complex that binds to the T cell receptor (TCR). Autoreactive T cells can be functionally silenced either by anergy or by tolerizing them to a type 1 regulatory T (Tr1) cell phenotype; both of these approaches rely on creating tolerogenic dendritic cells (DCs) leading to

antigen presentation in the context of tolerogenic signals via DCs, nanoparticles or DNA vaccines. Regulatory T (T<sub>reg</sub>) cells can be engineered to actively suppress autoreactive T cells either by introducing a transgenic TCR or genetically engineering chimeric antigen receptor (CAR) T<sub>reg</sub> cells. Colours indicate T cell state: pathogenic T effector cell (red), T<sub>reg</sub> cell (blue), anergic T cell (yellow), Tr1 cell (blue).

Notably, however, plasma cells, which produce antibodies, cannot be deleted by approaches that utilize antigen binding to the B cell receptor (BCR) as these cells do not express the BCR on their cell surface. Selective deletion of autoreactive CD4<sup>+</sup> T cells has gained interest, especially in the context of autoimmune diseases that are strongly associated with HLA class II alleles, such as RA and T1DM, in which, the death signal is linked to the MHC-peptide complex that binds to the TCR. However, there are several challenges with this approach; for instance, the autoantigen that drives pathogenic autoreactive T cells must be known, the therapy must be individualized on the basis of HLA type and the therapy needs to be specific to avoid off-target killing of T cells. In disease settings where germline encoded regions of the TCR of the autoreactive T cell are shared, deletion of this subset of T cells provides a way to overcome these challenges. For example, in a 2023 study, a cytotoxic antibody against a TRBV9-derived TCR motif on CD8<sup>+</sup> T cells was used to treat ankylosing spondylitis, a disease in which the TRBV9 TCR motif is enriched<sup>25</sup>. In a single patient, this approach led to the targeted depletion of TRBV9<sup>+</sup> T cells and clinical remission, and has led to larger clinical trials with this agent (NCT05445076, NCT06333210 and NCT05407779), thus underscoring the therapeutic potential of the depletion of autoreactive T cells<sup>25</sup>. However, the greatest limitation of lymphocyte depletion approaches is that, in many autoimmune diseases, tolerance is lost to multiple autoantigens or epitopes, and many of these TCR and BCR sequences are private; thus, targeting only one epitope, antigen or germline sequence is probably inadequate to control disease.

**Promoting tolerance by altering the function of pathogenic T cells**

An alternative to deletion is to make autoreactive T cells non-pathogenic by either deviating their lineage or functionally silencing them through anergy. These approaches rely on the context of T cell recognition of a self-antigen; each approach is designed to promote the recognition of peptides by autoreactive T cells in the absence of inflammation or co-stimulation signals or in the presence of tolerogenic signals. Multiple approaches have been developed to accomplish antigen delivery in the context of tolerogenic signals, but four delivery types predominate: tolerogenic dendritic cells (DCs); nanoparticles; DNA and messenger RNA vaccines; and peptides or whole-protein antigens. Antigen types include whole antigen, peptides or altered peptides, and the routes of administration can be oral, mucosal, subcutaneous, intra-nodal injection or intravenous.

**Tolerogenic dendritic cells.** DCs are professional APCs that are central to determining T cell lineage and fate in vivo through their ability to process self-antigens and present peptide in the context of MHC to T cells; DCs also provide co-stimulatory and cytokine signals that determine T cell fate. Thus, an early approach to antigen-specific therapy was the use of autologous tolerogenic DCs that were pre-loaded with autoantigens. Two phase I clinical trials demonstrated the safety of this approach in patients with RA or inflammatory arthritis<sup>26,27</sup>. Each trial used a different strategy to pre-load the DCs with autoantigen: in the first trial DCs were pre-loaded with four citrullinated peptides

derived from autoantigens that had previously been implicated in RA pathogenesis<sup>26</sup>, whereas in the second trial, DCs were exposed to autologous synovial fluid from the patient being treated<sup>27</sup>. DC-derived extracellular vesicles are also being developed as a cell-free therapy that can be stored for long periods of time and has a high biostability in circulation<sup>28</sup>; these vesicles have demonstrated efficacy in mouse models of autoimmunity, such as the collagen-induced arthritis (CIA) model of RA<sup>29,30</sup>. These approaches have led to the development of carriers such as nanoparticles, liposomes and red blood cells used to deliver antigens to DCs and promote tolerogenic features in these cells<sup>31</sup>.

**Liposomes and nanoparticles.** Liposomes have been used to deliver antigen as well as other molecules or substances to DCs that modulate their function; for example, the addition of an nuclear factor- $\kappa$ B (NF- $\kappa$ B) inhibitor to liposomes results in DCs that promote the development of T<sub>reg</sub> cells<sup>32</sup>. On the basis of this finding, a clinical trial in RA using liposomes loaded with the type II collagen<sub>259–273</sub> peptide and the NF- $\kappa$ B inhibitor calcitriol was carried out<sup>33</sup>. This trial demonstrated safety and a modest improvement in disease activity and provided insights into underlying immune mechanisms of RA. An increase in PD-1 expression on type II collagen-specific T cells and exhaustion signatures suggested an increase in tolerance<sup>33</sup>. Furthermore, there was evidence of changes in cit-vimentin-specific T cells, suggesting bystander effects on other autoreactive T cell specificities that target other RA antigens<sup>33</sup>. The next steps for this approach include identification of the ideal antigen and/or peptides and other additional factors that could further enhance the tolerogenic effect of liposomes. An exciting extension of this work is the use of liposomes for the delivery of nucleoside-modified messenger RNA that encodes autoantigens to CD11c<sup>+</sup> APCs. Studies in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, have shown that the aforementioned liposome treatment approach increases T<sub>reg</sub> cells and ameliorates disease<sup>34</sup>.

Similarly to liposomes, polymer-based nanoparticles are also being used to deliver both antigens and tolerogenic signals. Nanoparticles bound to antigens can be directed to sites in the body that promote tolerance, such as the liver and spleen, or loaded with immunomodulatory chemicals that provide a source of antigen and chemical signals that alter APCs, which can also promote tolerance<sup>35</sup>. For example, microparticles that are made of all-*trans*-retinoic acid and encapsulated in hydrolytically degradable poly-(lactic-co-glycolic) acid can be delivered to the joint in the SKG mouse model of RA; this method was efficacious and conferred protection without global immunosuppression<sup>36</sup>. In addition, nanoparticles can be targeted to organs or cells via their physicochemical properties; for example, targeting plasmacytoid DCs in the spleen or liver can promote tolerogenic phenotypes<sup>37,38</sup>.

Polymer-based nanoparticles coated with MHC class I or II-peptide complexes can directly interact with autoreactive T cells; evidence that supports this approach has been demonstrated in rodent models of autoimmune disease<sup>37</sup>. Nanoparticles coated with MHC class I-peptide complexes (IGRP peptides) or MHC class II-peptide complexes (chromogranin A peptides) induced expansion of antigen-specific T<sub>reg</sub> cells and halted disease progression in the non-obese diabetic (NOD) mouse model of T1DM<sup>39</sup>. Nanoparticles coated with MHC class II-peptide complexes have also been shown to effectively reduce disease severity in mouse models of RA. In the CIA model of RA, nanoparticles coated with MHC class II complex A<sup>q</sup>-galCOL2 ameliorated autoimmune arthritis; in the HLA DR4-transgenic model of RA a nanoparticle coated with HLA class II DR4-mouse type II collagen<sub>259–273</sub> complex successfully controlled arthritis through the promotion of Tr1 cell development;

Tr1 cells produce the regulatory cytokines IL-10 and TGF $\beta$ <sup>40</sup>. This approach has also been extended to EAE mouse models of multiple sclerosis<sup>40</sup>. Clinical trials of antigen-carrying nanoparticles are underway<sup>31</sup>. A phase I trial in multiple sclerosis (EudraCT 2008-004408-29) used myelin peptides bound to apoptotic red blood cells that trafficked to the spleen and liver to deliver antigen; the outcome of this trial suggests that there is a decrease in T cell responses to the myelin peptides *in vitro* after therapy<sup>41</sup>. Liposomes that carry myelin-derived peptides and target CD206 have been well tolerated and decrease inflammatory mediators in the serum of individuals with multiple sclerosis (Russian Public Health Ministry #930 (FASEMS-01/01))<sup>42,43</sup>. In a phase IIa trial, treatment of coeliac disease with poly-(lactic-co-glycolic) acid nanoparticles that contained gluten protein led to a decrease in circulating CD4<sup>+</sup>CD38<sup>+</sup> $\alpha$ 4 $\beta$ 7<sup>+</sup> T cells, a cell type thought to contribute to the pathology of coeliac disease, but did not change clinical outcomes (NCT03486990 and NCT03738475)<sup>44</sup>.

**Vaccines: whole-antigen, peptide, DNA or mRNA.** The use of antigens (either individual peptides or whole antigens) is an approach that relies on the principle that either the peptide itself has tolerogenic features or the mode of delivery of the antigen will enable development of tolerance. One approach to antigen delivery is the use of vaccination, whereby an autoantigen together with an adjuvant that is designed to divert pathogenic T cells (such as T helper 1 (T<sub>H</sub>1) cells or T<sub>H</sub>17 cells) towards a T<sub>H</sub>2 cell-like phenotype is administered. This approach has been carried out with an adjuvant mixed with peptide or whole antigen; for example, a glutamic acid decarboxylase (GAD)-alum vaccine is being tested in T1DM<sup>45–47</sup>. Alternatively, a peptide can be engineered so that it is linked to cell-binding proteins that promote T<sub>H</sub>2 cell cytokine secretion, as is the case for the DerG-LEAPS conjugate, which incorporates proteoglycan and aggrecan epitopes and has demonstrated efficacy in the recombinant human proteoglycan aggrecan-G1 domain-induced arthritis (GIA) mouse model of RA<sup>48</sup>. Similarly, vaccination with a fructosylated peptide derived from the immunodominant T cell epitope of bovine type II collagen is effective in the fibrinogen-induced arthritis (FIA)-CIA mouse model<sup>49</sup>.

Delivery of a protein via DNA or RNA is an approach that is gaining momentum and enables the *in vivo* production of antigen that is linked with co-expression of regulatory molecules, such as IL-4 and IL-10. DNA-mediated protein delivery has been achieved in rat models of RA, in which a DNA vaccine encoding chicken type II collagen has shown therapeutic efficacy<sup>50–52</sup>. The safety of this approach in humans is now being tested in the TOPPLE T1DM trial (NCT04279613) in which a plasmid encoding pre-proinsulin, along with TGF $\beta$ 1, IL-10 and IL-2, is delivered subcutaneously<sup>53,54</sup>.

**Apitopes and altered peptide ligands.** Other approaches utilize selected peptides alone to confer tolerance, such as antigen processing independent T cell epitopes (known as apitopes), which are administered subcutaneously and promote IL-10 production and induction of Tr1 cells<sup>55</sup>. Apitopes are water soluble, can bind to MHC with high affinity and mimic the native peptide. Apitopes are thought to be bound preferentially by CD11c<sup>+</sup> DCs in lymph nodes and in the spleen, and have a short half-life. Clinically, apitopes are being applied in Graves disease and multiple sclerosis<sup>55</sup>. Other studies have identified tolerogenic peptides that occur when the sequence of a self-epitope is slightly altered from the native peptide; these are referred to as altered peptide ligands (APLs)<sup>56</sup>. In both the EAE model of multiple sclerosis and the NOD model of T1DM, APLs have been shown to effectively

block or alter autoreactive T cell responses owing to alterations in their binding to MHC or contact with TCRs<sup>57,58</sup>. These approaches have been extended to human trials and have been shown to be safe in new-onset T1DM (NCT03272269)<sup>59,60</sup>; however, conflicting outcomes have been reported in multiple sclerosis. In one study ( $n = 142$ ), administration of myelin basic protein (MBP) APL reduced the number of lesions appearing on MRI and skewed T cells towards the less pathogenic T<sub>H</sub>2 cell lineage (NCT00079495)<sup>61</sup>, whereas in another study of eight individuals, administration of MBP APL (NCT00001781)<sup>62</sup> led to exacerbations of multiple sclerosis and an increase in pathogenic T<sub>H</sub>1 MBP-specific T cells<sup>62</sup>. Both studies were suspended because of safety concerns, highlighting the risk of using an antigen or peptide to vaccinate patients and the need to consider mode of delivery to ensure that the antigen is delivered into a tolerogenic space, such as the spleen or liver. Importantly, these tolerogenic spaces might be influenced by factors beyond the product itself (that is the peptide or antigen) and could also be influenced by ongoing inflammation at the time of treatment, as a result of the disease itself or intercurrent infections. Furthermore, in the case of APLs, it remains unclear if a tolerogenic peptide in some patients could be pathogenic in others. Ultimately, important considerations for this approach are what antigen to use and whether a single antigen or peptide works for everyone. These are issues that ongoing research, clinical trials and associated mechanistic studies will help to address.

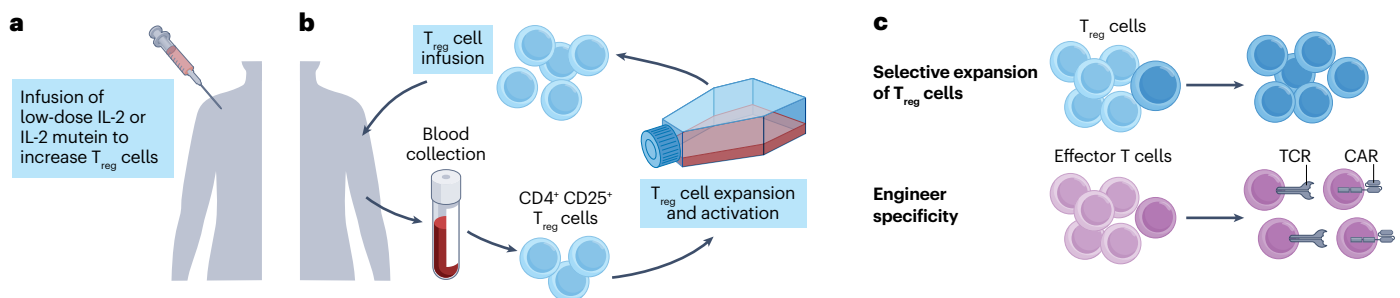
## Promoting tolerance through active regulation

A third approach to antigen-specific therapy is active regulation, which is inspired by the demonstration that peripheral regulation of auto-reactivity is central to immune regulation. This peripheral regulation is achieved through immune cells, which have the capacity to regulate the immune response. Although multiple regulatory cells have been described, CD4<sup>+</sup> T<sub>reg</sub> cells are the most well-characterized, and these cells express the transcription factor FOXP3, which has been described as the master regulator of T<sub>reg</sub> cells<sup>63</sup>. In humans, the importance of T<sub>reg</sub> cells is demonstrated by IPEX (immune dysregulation, poly-endocrinopathy, enteropathy, X-linked) syndrome whereby mutations in *FOXP3* result in the absence of T<sub>reg</sub> cells and the subsequent development of profound autoimmunity from birth including early-onset T1DM<sup>64</sup>. Similarly to other CD4<sup>+</sup> T cells, T<sub>reg</sub> cells are activated through their TCR upon recognition of an MHC-peptide complex on an APC. T<sub>reg</sub> cells recognize self-peptides and upon activation block the proliferation and function of T<sub>eff</sub> cells, through contact and non-contact mechanisms<sup>65,66</sup>. Importantly, T<sub>reg</sub> cells suppress bystander T cells as well as those specific for the same target antigen<sup>67</sup>. Thus, these cells are

only active in the presence of a specific self-antigen but once activated have the ability to suppress local T cells irrespective of specificity, thus promoting tissue tolerance. Therefore, it might not be essential to identify all antigens involved in pathogenesis if the immunotherapy induces bystander suppression.

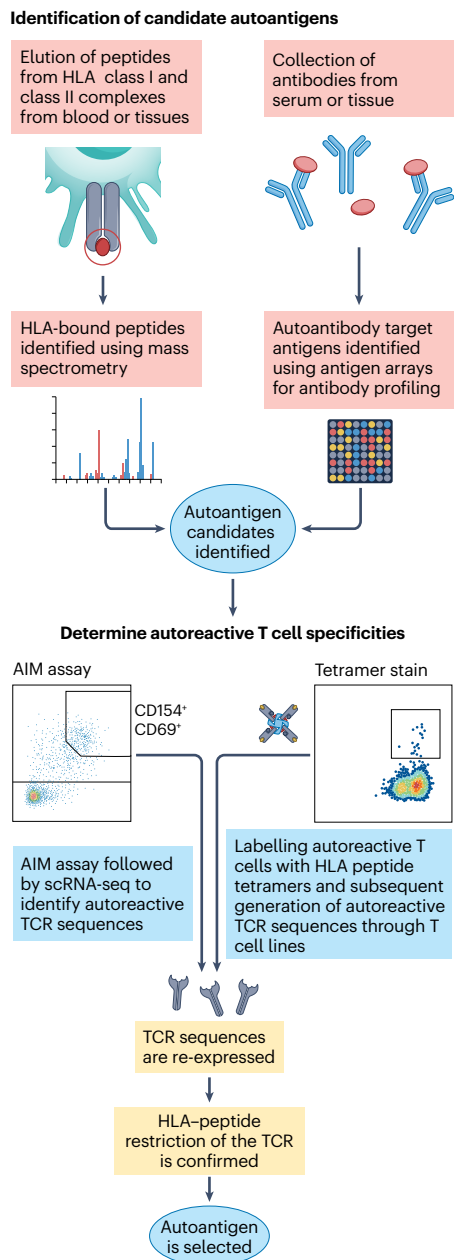
Increasing the number of T<sub>reg</sub> cells in an individual as a treatment approach for autoimmunity has been attempted in multiple settings (Fig. 3). Providing IL-2 as a growth factor has been shown to promote the expansion of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells in vivo; for example, treatment with IL-2 led to a partial clinical response in patients with graft-versus-host disease<sup>68</sup> and some early success was reported in vasculitis<sup>69</sup>. Yet, IL-2 has additional immune effects that can promote inflammation or expand unwanted T<sub>eff</sub> cell populations in the setting of autoimmunity. To address these concerns, new therapeutics that mimic IL-2 and specifically target T<sub>reg</sub> cells are being developed, therefore avoiding activation of other cell populations; many of these IL-2 mimics are currently being assessed for efficacy in both cancer and autoimmunity<sup>70</sup>. Notably, the pegylated IL-2 biologic repegaldesleukin, which targets the IL-2 receptor on T<sub>reg</sub> cells, did not meet its primary end point in a phase II study in 291 adults with moderate-to-severe SLE<sup>71</sup>, and a phase IIb trial of an IL-2 mutein Fc fusion protein, efavaleukin alfa (NCT04680637), was stopped because of futility in SLE<sup>72</sup>. An alternative approach is the expansion of autologous polyclonal T<sub>reg</sub> cells ex vivo followed by infusion of the expanded cells; this approach has been shown to be safe<sup>73</sup> but clinical efficacy has not been shown in autoimmunity<sup>74</sup> (Fig. 3). However, none of these approaches provides an antigen-specific form of therapy.

Active antigen-specific regulation requires the promotion or delivery of a regulatory cell that is specific to the target autoantigen. To achieve antigen-specific regulation, autoreactive T cells can be redirected in vivo towards a regulatory phenotype or modified ex vivo to promote antigen-specific tolerance<sup>66,75</sup>. Peptide immunization alone or nanoparticles that deliver antigen to APCs can promote the induction of antigen-specific Tr1 cells in vivo<sup>76</sup>. Similar approaches can also promote adaptive T<sub>reg</sub> cells<sup>77</sup>. A second approach is adoptive transfer of antigen-specific T<sub>reg</sub> cells; this approach has been pioneered in the transplantation field. The use of donor-alloantigen-reactive T<sub>reg</sub> cells is an approach that increases the specificity of an autologous T<sub>reg</sub> cell product without genetic modification<sup>78</sup> through ex vivo expansion of recipient T<sub>reg</sub> cells. This approach was tested in the ARTEMIS trial in liver transplantation (NCT02474199)<sup>79</sup> but the study was limited owing to the dysfunction of T<sub>reg</sub> cells in liver transplant recipients, which in some patients prevented the generation of a sufficient number



**Fig. 3 | Increasing the frequency of T<sub>reg</sub> cells as a therapeutic approach. a.** One method to increase the number of regulatory T (T<sub>reg</sub>) cells in vivo is the injection of low-dose IL-2 or IL-2 muteins. **b.** Another method is adoptive transfer of T<sub>reg</sub> cells, whereby blood is collected, T<sub>reg</sub> cells are purified and expanded ex vivo

and then the T<sub>reg</sub> cells are infused back into the same patient. **c.** Approaches to increase antigen-specific T<sub>reg</sub> cells include selective expansion of T<sub>reg</sub> cells with the right antigen specificity or engineering T cell receptors (TCR) or chimeric antigen receptors (CAR) that are specific for the target antigen.



**Fig. 4 | Pipeline for selecting target autoantigens for antigen-specific therapy.** The candidate T cell autoantigen can be identified by the elution of peptides from HLA class I and HLA class II complexes that are isolated from the tissue of interest followed by mass spectrometry peptide identification. Alternatively, antibodies can be collected from serum or tissue and the autoantibody antigen targets identified using approaches such as antigen arrays for antibody profiling. The next step is determining the specificity of the autoreactive T cell. Autoreactive T cell specificities can be determined using an activation-induced marker (AIM) assay where T cells responding to antigen are identified by activation markers (such as CD154 and CD69) or through the use of HLA peptide tetramers. Autoreactive T cell receptors (TCRs) are sequenced and re-expressed to determine the specificity of autoreactive T cells. The restriction of the TCR is then confirmed and the target autoantigen selected. scRNA-seq, single-cell RNA sequencing.

of donor-alloantigen-reactive  $T_{reg}$  cells for adoptive transfer. Alternatively, the expression of A2-specific CAR T cells exemplifies the approach of expressing an antigen receptor that confers specificity to a  $T_{reg}$  cell product. There is an expanding body of literature that supports the development of this approach to provide specific tolerance to the allograft; in 2024 a preprint provided evidence that this approach might promote long-term tissue-specific tolerance through infectious tolerance, a process whereby tolerance is naturally passed between lymphocyte populations<sup>80</sup>.

In transplantation the target antigen is foreign (known as an alloantigen), but insights from transplantation studies suggests that targeting  $T_{reg}$  cells to autoantigens might be useful in the treatment of autoimmunity. Animal models have clearly demonstrated that  $T_{reg}$  cells that target a tissue-specific antigen are able to protect and reverse autoimmunity<sup>81–83</sup>;  $T_{reg}$  cells that are specific to heat shock protein 70 suppress established proteoglycan-induced arthritis<sup>84</sup>. To that end, current approaches use autologous cells, either natural  $T_{reg}$  cells or conventional T cells, which are modified to have regulatory function and express a CAR that is targeted to an antigen uniquely seen in the target tissue<sup>85</sup>, a TCR with specificity for a relevant autoantigen<sup>86</sup> or, alternatively, a CAR recognizing a specific MHC–peptide complex, similar to a TCR<sup>87</sup>. These approaches have followed the success of CAR T cell therapy in cancer<sup>88</sup> and have become more feasible with the advent of genome editing, which enables the modification of TCRs and genes involved in  $T_{reg}$  cell function, commitment and stability<sup>86,89–91</sup>.

## Selecting the antigen to target

An important consideration in the development of antigen-specific therapies for autoimmunity is the antigenic target. In a small number of autoimmune diseases, the autoantigens that are targeted in the disease are clear. Typically, these diseases have autoantibodies directed at a tissue antigen, such as transglutaminase in coeliac disease, thyroid-stimulating hormone receptor in Graves disease and thyroid peroxidase in Hashimoto disease. In other autoimmune diseases, the presence of autoantibodies against antigens found in the target tissue suggest potential therapeutic targets, as is the case in T1DM whereby the presence of numerous islet autoantibodies (such as anti-insulin, anti-GAD, anti-protein phosphatase-like IA-2 and anti-zinc transporter 8 antibodies) indicates a broad reactivity to antigens found in  $\beta$ -cells. Autoantigens can also be more ubiquitous, as is the case for autoantibodies directed to nuclear antigens in SLE, systemic sclerosis and dermatomyositis. There is growing recognition that post-translationally modified antigens are targets in autoimmunity, as best exemplified by anti-citrullinated protein autoantibodies in RA. However, there are some autoimmune diseases in which autoantibodies are not well described. Additionally, there might be antigens and/or epitope specificities that promote improved induction of or protection by  $T_{reg}$  cells. As methods of  $T_{reg}$  cell isolation at the single-cell level and the investigation of  $T_{reg}$  cell specificities improve, the ideal peptide and antigens for protection will become clearer.

The specificity of autoantibodies has been a helpful guide in identifying T cell epitopes in autoimmune diseases as there are clear examples of shared autoantigens and even epitopes between B cells and T cells<sup>92</sup>. However, not all T cell autoantigens and epitopes are shared with autoantibodies, making the identification of T cell autoepitopes an even greater challenge. In the case of T cell antigens, where the goal is to delete, tolerize or use  $T_{reg}$  cells to actively suppress the autoreactive response, the epitope and MHC allele to which that epitope is restricted must be elucidated. Although identification of T cell

epitopes has been underway for years, and T cell epitopes are known in some autoimmune diseases, there remains a substantial gap in the knowledge surrounding T cell epitopes in many diseases. As a result, there is considerable research effort focused on addressing this knowledge gap with studies of disease-affected tissue to explore the specificities of T cells infiltrating the affected tissues and the application of single-cell technologies, including tetramers<sup>93,94</sup>, activation-induced marker assays<sup>95</sup> and single-cell RNA sequencing<sup>96,97</sup>. These new technologies enable investigators to identify potentially pathogenic T cells and confirm their specificity (Fig. 4). Moreover, studies identifying the antigens presented on MHC molecules in the affected tissue through mass spectrometry-based identification of MHC-associated peptides<sup>98</sup> are also expanding the understanding of the antigens that might drive disease. The field is well on its way to identifying the antigenic targets in many diseases. Another factor to consider is when in the course of disease an autoantigen will be a relevant target. At initiation of a disease, the autoimmune response could be targeted to a single antigen but with time multiple antigens could become targets; this phenomenon, referred to as epitope spreading, has been well described in mouse models of autoimmunity, including arthritis models<sup>99</sup>. Thus, there could be certain antigens that are dominant prior to disease onset when prevention strategies are most fruitful, whereas other specificities might be involved later in the disease course. Understanding which antigen specificities to target on the basis of disease stage will be vital as antigen-specific therapies develop.

## Setting the stage for antigen-specific therapies

The context in which any therapy is given influences its efficacy; in antigen-specific therapy context is particularly important, as the immunological milieu into which the antigen-specific therapy is given could have a profound influence on therapeutic efficacy. For example, the induction of anergy relies on a lack of co-stimulation which can only be achieved in an immunologically quiescent state; approaches that promote alterations in T cell lineage rely on signals from tolerogenic cytokines that could be negated by those from pro-inflammatory cytokines that are present in the local environment of patients with active disease. Antigen-specific therapy might therefore require control of inflammation prior to or congruent with administration of the antigen-specific therapy. In addition, T cell responses in individuals with autoimmunity might be resistant to tolerogenic therapies; for example, resistance to T<sub>reg</sub> cell-mediated suppression is well described in human autoimmune diseases (such as T1DM<sup>100–102</sup>, SLE<sup>103</sup>, multiple sclerosis<sup>104</sup> and active RA<sup>105</sup>), and autoantibody-producing plasma cells persist despite current therapies that deplete B cells<sup>106</sup>. Once established, tolerance might need to be boosted in order to be maintained because of intrinsic defects in the immune response in individuals with autoimmunity owing to underlying genetic factors linked to development of autoimmunity. These genetic risk variants can lead to altered responses to TCR activation, expression of co-stimulatory signals or cytokine responses<sup>107–110</sup>. Timing of the administration of antigen-specific therapies in the context of disease course is also important. Antigen-specific therapies could be more appropriate prior to the development of clinical disease, when there is limited inflammation and at a time prior to epitope spreading when there might be fewer autoantigens. The timing of therapy could also depend on the type of treatment, the efficacy under different conditions and the associated risks and costs of the therapy. Understanding the context in which an antigen-specific therapy is given will be vital for its success; knowing the immune environment of the target tissue during disease will be key

and could require the introduction of therapies that can promote an ideal milieu into which to apply an antigen-specific therapy to achieve and maintain tolerance.

## Conclusions

Antigen-specific approaches have shown success in animal models of autoimmunity and now have shown promise in phase I and II clinical trials of autoimmune diseases with larger trials underway using a range of approaches to achieve the goal of antigen-specific tolerance. Yet, challenges remain: the antigen or antigens targeted by the autoimmune response need to be identified; treatment approaches need to be tailored to the specific immune pathology of the autoimmune disease being treated; and off-target effects or promotion of autoimmunity need to be avoided to ensure safety. Finally, antigen-specific therapies need to be accessible and long-lasting. To meet these challenges, research to determine the underlying mechanisms that promote autoimmune pathology must continue; investigators must harness as much information as possible from clinical trials that are using antigen-specific therapies to determine the modalities that are successful and when in the course of disease to deliver these therapies. Combined, these strategies will improve antigen-specific therapies and accelerate progress with the goal of getting antigen-specific therapies into the clinic and re-establishing tolerance in patients with autoimmune diseases.

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## Competing interests

J.H.B. is a scientific co-founder and scientific advisory board member of GentiBio, consultant for Bristol Myers Squibb and Hotspot Therapeutics, and has past and current research projects sponsored by GentiBio, Amgen, Bristol Myers Squibb, Janssen, Novo Nordisk and Pfizer. She is a member of the Type 1 Diabetes TrialNet study group, a partner of the Allen Institute for Immunology, and a member of the scientific advisory boards for the La Jolla Institute for Allergy and Immunology and BMS Immunology.

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# Breaking research silos to achieve equitable precision medicine in rheumatology

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## Abstract

Health disparities in rheumatic disease are well established and urgently need addressing. Obstacles to precision medicine equity span both the clinical and the research domains, with a focus placed on structural barriers limiting equitable health care access and inclusivity in research. Less articulated factors include the use of inaccurate population descriptors and the existence of research silos in rheumatology research, which creates a knowledge gap that precludes addressing the health disparities and fulfilling the goals of precision medicine to understand the ‘full patient’. The biopsychosocial model is a research framework that intertwines layers of biological and environmental effects to understand disease. However, very limited rheumatology research bridges across molecular and epidemiological studies of environmental exposures, such as physical and social determinants of health. In this Review, we discuss clinical obstacles to health care equity, including access to health care and the use of inaccurate language when labelling population groups. We explore the goals and data needed for research under the biopsychosocial model. We describe results from a rheumatic disease literature search that highlights the paucity of studies investigating the molecular influences of systemic exposures. We conclude with a list of considerations and recommendations to help achieve equitable precision medicine.

## Sections

Introduction

Disparities in health care access

Describing population groups

The biopsychosocial model

Epidemiological research silo

Biological research silo

The knowledge gap from research silos

Breaking research silos

Conclusions

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## Key points

- Health disparities in rheumatic disease are well established and need addressing urgently.
- Currently, epidemiological and biological research exists in silos, which hampers our ability to elucidate the interplay and underlying molecular mechanisms by which environmental exposures yield biological changes.
- Poor diversity and inclusivity in research datasets, individual and structural barriers in access to research and care, inaccurate use of population descriptors, and research siloing limit equal and equitable precision medicine and health.
- Embracing ecological frameworks such as the biopsychosocial model will enable research that better accounts for the multi-faceted factors contributing to rheumatic disease prevalence and progression.
- The continued development of omics technologies and emerging large population-based registries offer great opportunities for identifying and alleviating health disparities.

## Introduction

The goal of precision medicine is to provide a more precise approach to the prevention, diagnosis and treatment of disease<sup>1</sup>. To achieve this goal, precision medicine must be equitable; that is, it must be fairly applicable, available and accessible to all<sup>2</sup>. Unbiased and accessible precision medicine can contribute to reducing avoidable and systemic inequalities in health status, that is, it can contribute to health equity, the state in which everyone has a fair and just opportunity to attain their highest level of health<sup>3</sup>.

Disparities in prevalence, incidence, clinical and serological manifestations, and outcomes of rheumatic conditions and rheumatological manifestations are well established, with some examples highlighted in Box 1. These disparities are pronounced in under-represented populations, namely racial minority and ethnic groups, medically underserved urban and rural communities, the uninsured and underinsured, older adults, and those with lower education and income<sup>4</sup>. Despite the disproportionate impact of many rheumatic diseases on these communities, the causes of these disparities remain elusive. Identifying the factors that promote disparities is a critical step towards equitable care and precision medicine<sup>5–7</sup>.

Developing successful precision medicine initiatives necessitates addressing health disparities. Although precision medicine is meant to focus on individual variability (such as genes, environment and lifestyle), these initiatives are predominantly informed by population-level research<sup>8</sup>. The consequence is that if health disparities are not properly identified and addressed in research, precision medicine is poised to worsen health disparities for groups already disadvantaged by current practices and interventions<sup>8–10</sup>. Frameworks for studying health disparities emphasize the need for ecological models that consider biology and environment, including social factors. In theory, this sounds akin to the needs of precision medicine research but in reality there is substantial isolation of biological research from epidemiological studies on environmental determinants of health.

Despite the recognized influence that social and physical environmental experiences have on rheumatic disease, it is not known how these experiences and exposures interact with biology to influence disease outcomes. To date, rheumatic disease research has largely focused on biological mechanisms without inclusion of effects by environmental exposures. Furthermore, genetic studies frequently fail to acknowledge the fact that non-genetic factors are substantial contributors to the disparities. In addition, the persistent use of vague and arbitrary categories of population differences continues to hamper rigorous and reproducible research<sup>11</sup>. These issues have resulted in a knowledge gap regarding the interactions between genetic and environmental factors that contribute to disparities in disease outcomes. We explored the extent of this knowledge gap in rheumatic diseases by conducting a literature review of studies that intersected both molecular biology and social determinants of health (SDOH), including racism and discrimination. We identified a striking shortage of literature spanning both molecular and epidemiological disciplines. Further, we identified a strong bias in the amount of research focusing on molecular biology in comparison with epidemiological SDOH. This finding highlights the ‘siloing’ of research entities and the general challenges (such as data availability and research infrastructure) in implementing a true precision medicine paradigm that considers the full biological and environmental experiences of a patient; this phenomenon has been identified in other disease categories and has been presented as a cause of hampered progress in alleviating health disparities<sup>12,13</sup>.

In this Review, we start by discussing clinical obstacles to health care equity, including access to health care and the use of inaccurate language when labelling population groups. We describe the biopsychosocial model and necessary data towards studying health care disparities, followed by a description of existing barriers in research, focusing on limitations in available data, research silos and the subsequent knowledge gap created in rheumatic disease studies. We then propose solutions and considerations, working towards a better understanding of the biology of rheumatic disease in the context of health disparities. Addressing this knowledge gap is expected to improve precision medicine diagnostics and therapeutics for underserved patients, thus working towards a more equitable paradigm in research and clinical practice.

## Disparities in health care access

In addition to disparities in disease susceptibility, prevalence and incidence, another source of health disparities occurs in the health care setting. Health care disparities can manifest in different capacities, leading to delayed or deficient patient care and education and poor patient outcomes. Travel distance to receive specialized care is often a deterrent and a major source of delayed patient care. When examining patients in the Medicaid beneficiary cohort (a government health insurance program that provides health care to some of the most vulnerable populations in the USA), poor access to specialized care for patients with systemic lupus erythematosus (SLE) occurred more often in those enrolled in Medicaid than in those with other health insurance<sup>14</sup>. Patients only enrolled in Medicaid travelled 11.5 more miles than those with additional or other health insurance and on average more than 19.8 miles to see a rheumatologist<sup>15</sup>. Consequently, patients need to use the emergency department not only for acute care but also as their primary source of medication prescriptions<sup>14</sup>. In patients with rheumatoid arthritis (RA) in the Canadian universal public health care system, those who lived >100 km from a rheumatologist were 50% less

likely to be seen by a rheumatologist within 3 months of a suspected RA diagnosis by a primary care physician and had decreased continuity of care within the first year of diagnosis<sup>16</sup>, both of which are important for early initiation of therapy to prevent disability and damage. As a result of these delays, patients have an accumulation of joint damage requiring surgeries<sup>17</sup>. Not only is access to specialized clinical care and medications a source of unequal outcomes but it also contributes to a deficiency in patient education, specifically in addressing disease comorbidities, which further promotes health disparities.

These structural barriers to health care access are also deterrents to the achievement of the goals of precision medicine. Access to health care is not guaranteed in many countries. A comparison of health care models in the USA, Austria and Denmark showed that the effects of precision medicine depend on the structure of the local health care system; in contrast to the Austrian and Danish health care systems, the USA for-profit and insurance-based structure increases the risk that precision medicine will exacerbate existing health disparities and structural injustice<sup>18–20</sup>. In addition to health care coverage, the current high costs of precision medicine therapies also preclude equity by excluding those who cannot access them. Changes are needed to prevent health care structures and pharmaceutical business models from supporting existing structural injustices and destabilizing egalitarian health policies<sup>18</sup>. Such changes include revisiting, monitoring, and regulating drug pricing models, to encompass the values of social equity and solidarity<sup>18</sup>.

## Describing population groups

Low representation in clinical trials and genomic studies is known to be perpetuated by multiple factors, including geographical distance, mistrust of the health care system, language barriers and fear of encountering implicit bias and stereotyping during care. Under-represented communities are often at a greater risk of poor health outcomes<sup>21</sup>. The confusion about population descriptors and lack of rigorous language when describing population categories continue to stigmatize many communities. Addressing individual biases and using clear and respectful population labels are paramount to realizing the promise of precision medicine<sup>22</sup>.

Many studies, especially in the USA, describe the epidemiology of rheumatic disease in terms of socially defined, discrete racial

categories. Similarly, health disparities research traditionally focuses on self-reported race. However, this methodology poses challenges and limitations. First, race and ethnicity are imperfect proxies for important epidemiological information, including SDOH, such as racism and discrimination, economic stability, health care access and quality, education access, and physical and/or built environmental exposures<sup>23</sup>. These social and physical environmental determinants are differentially experienced across racial and ethnic groups owing to historical and contemporary discriminatory policies and practices, resulting in health disparities across groups and geography. Hence, it is not race and ethnicity, but the lifetime of exposures, that contributes to health disparities. Consequently, discrete racial categories are unable to appropriately capture the variability of risks one person might be exposed to in their lifetime. As such, limiting health disparities research to racial categories can impose harm through ascribing risk to arbitrary groupings of heterogeneous individuals while also obscuring the causal exposures facilitating risk<sup>24</sup>. Another weakness of limiting research to racial categories is that many identities intersect, and thus, risk factors might impose differential or compounded effects. For instance, although increased socioeconomic status (SES) is protective, increased exposure to discrimination has the potential to offset these benefits<sup>25–27</sup>. In addition, the concept of race is dynamic and changes over time in the wake of administrative decisions and cultural shifts; for example, the list of racial groups on the US Census has changed nearly every decade since the first enumeration in 1790 (ref. 28). Race is also a complex population descriptor because it can be interpreted and measured in many ways (for example, racial identity, racial self-classification, observed race, reflected race)<sup>29</sup>. Although it may be a useful population descriptor for researchers who wish to measure a consequential form of social status and affiliation, the concept of discrete populations that are static in place and time does not apply to humans.

Another issue is the frequent conflation between race and genetic similarity groups<sup>22</sup>, often referred to as genetic ancestry groups. Genetic ancestry is a statistical estimate inferred from measures of genetic similarity across individuals using available data, thus without any relationship to the concept of race. The epidemiological literature reveals rheumatic disease disparities based on ascribed race, and as discussed below, the genetics literature has found rheumatic disease disparities based on genetically inferred groups. However, equating these population descriptors is a fallacy, as race is an imperfect proxy for SDOH, and the other captures genetic risk factors. Altogether, this issue highlights the necessity of a multi-faceted approach to studying health disparities, one that includes both genetic and non-genetic, individual-level and population-level stressors and exposures<sup>30</sup>. Moving forward, it is critical for researchers to utilize accurate and appropriate population descriptions, such as those described by the National Academies of Science, Engineering, and Medicine<sup>28</sup> and by Feero et al.<sup>31</sup>

## The biopsychosocial model

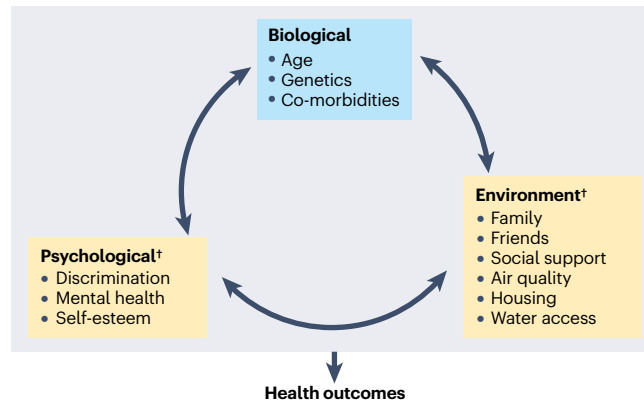
The biopsychosocial model was proposed in response to the reductionist paradigm of viewing diseases through a strict 'biomedicine' lens. Instead of viewing the full scope of phenotype and symptoms as explainable by molecular processes (biomedicine model), the biopsychosocial model asserts that health and disease result from an interplay between biological, psychological and social factors<sup>32</sup> (Fig. 1a). The biopsychosocial model emphasizes the importance of integrating different domains (such as biological, behavioural, physical

### Box 1 | Examples of known health disparities in rheumatic diseases

Overview of some documented differences in prevalence, incidence and severity across ten rheumatic diseases emphasizes the necessity of addressing these issues in research.

- Higher prevalence in African Americans: gout<sup>51</sup>, knee osteoarthritis<sup>50</sup>, rheumatoid arthritis<sup>140</sup>, sarcoidosis<sup>53,141</sup>, systemic lupus erythematosus<sup>142</sup> and systemic sclerosis<sup>52</sup>.
- Higher prevalence in Mediterranean countries, the Middle East, and Southeast Asia: Behçet's disease<sup>143</sup>.
- Higher prevalence among European Americans: psoriatic arthritis<sup>144</sup>.
- Higher prevalence among non-European populations: Sjögren's syndrome<sup>145,146</sup>.
- Worse severity of disease for patients with lower socioeconomic status and/or countries with lower GDP: ankylosing spondylitis<sup>147,148</sup>, psoriatic arthritis<sup>57,149</sup>.

## a Biopsychosocial model



## b Environmental determinants of health

Domain	Physical environment	Health care access	Biological	Chemical	Economic security and equality	Health behaviours	Social and community support
Subdomains	Air and water quality	Access	Animal dander	Air pollutants	Education access	Alcohol use	Conflict, crime and violence
	Climate and disasters	Affordability	Bacteria	Heavy metals	Education quality	Nutrition	Discrimination
	Energy/utilities access	Health system (for example, health workers per 1,000 people)	Cockroaches	Pesticides	Employment	Physical activity	Forced displacement/migration
	Healthy workplace		Mildew/mould	Food insecurity	Tobacco use	Gender equality	
	Housing and safe house		Mites		Income inequality		Healthy ageing
	Road safety, traffic congestion and transportation systems		Pollen		Poverty		Incarceration
	Sanitation and hygiene		Viruses				Social capital, cohesion, connections and participation
	Urbanization						

**Fig. 1 | The biopsychosocial model and domains of environmental determinants of health.** **a**, The biopsychosocial model comprises biological, psychological and social factors; extensions of this model into an ecological

model include other environmental factors. †Sources of social determinants of health. **b**, Primary domains and subdomains of environmental determinants of health.

and/or built, sociocultural, health care system) and levels (for example, individual, family, community, society). In the context of health disparities, this conceptual framework posits that individuals who perceive certain circumstances as stressful experience a physiological stress response that can be modulated by adverse or protective sociodemographic factors (such as SES), psychological characteristics (for example, depression), behavioural factors (smoking, alcohol use), and coping responses (for example, ability to mobilize social support) to such experiences<sup>30,33</sup>. Expansions of this model also include physical environmental exposures (for example, air and water quality)<sup>34</sup>. Such environmental exposures can enact effects independent of individual perception and/or awareness and can be disproportionately distributed among neighbourhoods and by geographic region<sup>35,36</sup>.

Successful implementation of the biopsychosocial model requires availability of appropriate data, measurements, and instruments for the multiple domains contributing towards health disparities (Fig. 1b). Physical environmental exposures are increasingly available at varying geographic granularity. For example, studies of air quality, ranging from local or regional measures<sup>37</sup> to national measures<sup>38</sup>, highlight the increased prevalence of detrimental environmental exposures among minority and low-income populations. Broadly, environmental factors or determinants to health are usually

classified into biological, chemical, physical and social environments. As defined by the WHO, other SDOH include economic security and equality, health behaviours and health care resources<sup>39,40</sup>. There are many indicators for capturing environmental determinants of health; methodologically, these can be measured via self-reporting (for example, surveys) and/or aggregated data from population statistics with varying granularity<sup>41,42</sup>. Given the broad domains of environmental determinants of health, it is critical to recognize that any single indicator will be unable to capture the full complexity of an individual's environmental determinants of health, including SDOH<sup>40</sup>. For instance, subjective social status instruments in which participants rank their circumstances (such as, income) relative to others in their community (for example, social ladders), have been found to capture perceived well-being<sup>43</sup>, and thus might capture aspects such as perceived discrimination and social stress, whereas multiple deprivation measures might be better suited to capturing material and/or resource-based socioeconomic factors. Given the intersectionality of identities, and thus experiences and exposures, SDOH measures that are multi-faceted (including more than one indicator) and multi-level (that is, individual, community) are more likely to appropriately capture the complexity of these effects<sup>40,44,45</sup>. In rheumatic diseases, data from each of these broad categories

(biological, SDOH) are abundantly available. However, these fields are rarely intertwined to investigate disease outcomes under an ecological, combined molecular and SDOH model, severely hindering progress in health disparities research.

## Epidemiological research silo

Decades of epidemiological research of health disparities in rheumatic diseases have highlighted the existence of systemic issues that drive poor outcomes for patients from under-represented and underserved communities (Table 1). For example, multiple social stressors, such as low household income, poverty, unemployment and racial discrimination, are associated with increased risk and severity of SLE<sup>46,47</sup> and RA<sup>48,49</sup>. A marked socioeconomic gradient was reported for these diseases, with individuals in the most deprived group being up to 70% more likely to develop the disease than the least deprived group<sup>49</sup>. Low income was associated with greater odds of osteoarthritis<sup>50</sup> and gout<sup>51</sup>, and worse outcomes for systemic sclerosis (SSc)<sup>52</sup> and sarcoidosis<sup>53,54</sup>. Social deprivation and social vulnerability were associated with osteoarthritis, RA, ankylosing spondylitis, sarcoidosis, and SSc<sup>50,55,56</sup>. In countries with lower GDP, patients with psoriatic arthritis had higher levels of disease activity and more patient-reported impact of disease<sup>57</sup>. Also, women, in particular women of understudied and under-represented populations, are disproportionately affected by rheumatic disease;

and women, especially African American women, disproportionately experience psychosocial stressors<sup>55</sup>.

Despite stressors co-existing in areas of concentrated poverty and with other environmental factors, protective factors might buffer the negative impacts of stressors<sup>58</sup>. For example, social support decreased depression and anxiety in African American women with SLE<sup>59–62</sup>, perceived social support improved depression, anxiety and quality of life in patients with RA<sup>63–65</sup>, and social support and spiritual well-being were associated with life satisfaction in patients with SSc<sup>66</sup>. Additionally, alleviation of stressors can influence disease. For instance, for patients with SLE, exiting poverty can mitigate the strong effect of living in concentrated poverty on disease damage<sup>67</sup>. Most epidemiological research has focused on the identification of risk factors, but understanding the mechanisms and extent to which protective factors can offset risk factors is essential to address health inequities (Table 1).

Although critical, the historical focus on epidemiological issues in rheumatic disparities research has sustained an epidemiological research silo (Fig. 2 and Supplementary Table 1, Supplementary Fig. 1) through its failure to intertwine molecular and biological effects. That is, the molecular and physiological mechanisms through which social and physical and/or built environment exposures affect disparities are unknown. For example, air quality and particulate exposures have been associated with disease activity and drug response in rheumatic diseases<sup>68–70</sup>, but the biological mechanisms require more exploration. Similarly, epigenetic marks affect gene expression and can govern cell function and physiological response to environmental factors, including SDOH such as SES<sup>71,72</sup> and general perceived stress<sup>73</sup>. Yet, despite the growing awareness of the effect of social stressors on the epigenome, research aimed at identifying and quantifying the regulatory mechanisms through which social exposures influence rheumatic outcomes is only just emerging<sup>74</sup>. Understanding the interplay and underlying mechanisms by which various positive and negative determinants of health influence epigenomic variation, and how the resulting biological changes might contribute to rheumatic health disparities, will enable biomedical research to directly address outcome disparities.

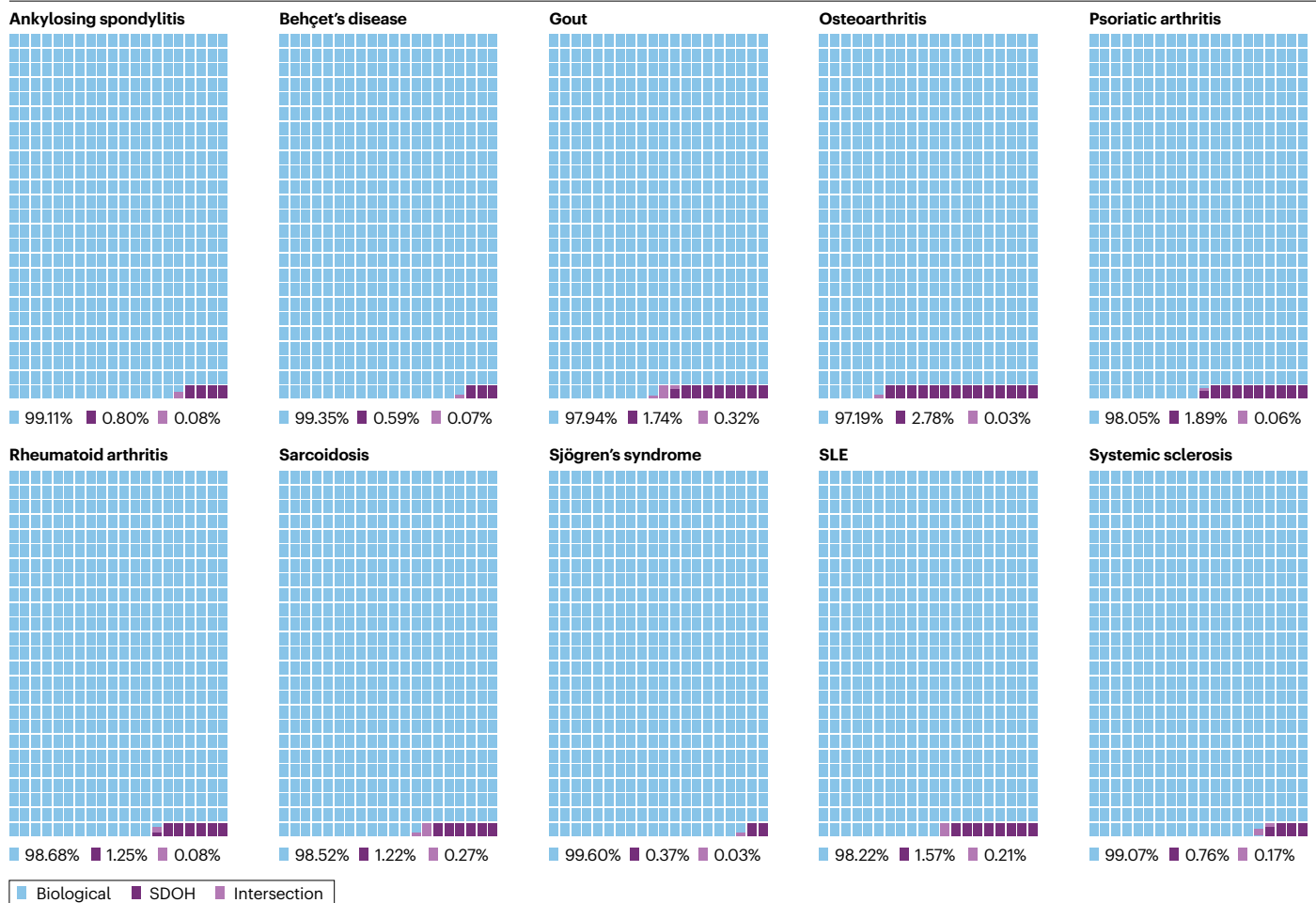
## Biological research silo

Large-scale studies into the biological aetiology of rheumatic diseases most commonly focus on genetic variation associated with disease. This line of research has been productive and important. Genome-wide association studies (GWAS) have identified hundreds of genomic loci associated with rheumatic diseases, providing important insights into the biological pathways contributing towards disease. Given the potential to facilitate disease diagnosis, support risk prediction, improve cost and efficiency of trials, and accelerate personalized care, results from these GWAS have been used to construct genetic risk scores (GRS)<sup>75,76</sup>. Given that many of these risk factors have been reported to vary among populations (for example, along geographic gradients), genetic variation has been proposed as a partial contributor towards disparities<sup>77,78</sup>. In fact, the 2006–2009 strategic plan of the US National Institute of Arthritis and Musculoskeletal and Skin Diseases prioritized the identification of genetic contributions to health disparities, citing as a rationale that rheumatological conditions, including SSc, SLE and osteoarthritis, cluster in populations targeted by health disparities initiatives<sup>79</sup>. However, to date, few genetic risk factors for rheumatic diseases have been linked to health disparities, which is due, in part, to limitations of genetic data, current data availability and the general siloing of genetic research from pertinent environmental and exposure data.

**Table 1 | Effect of environmental factors on rheumatic diseases**

Environmental factors	Rheumatic disease	Epidemiological conclusions
<b>Negative outcomes</b>		
Low household income, poverty, unemployment	SLE and RA	Increased disease risk and severity <sup>46–49</sup>
	Gout and OA	Greater odds of developing disease <sup>50,51</sup>
	SSc and sarcoidosis	Worse outcomes <sup>52–54</sup>
Diet (purine rich)	Gout	Increased chance of experiencing flares <sup>127</sup>
Social deprivation and social vulnerability	OA, Ra, ASp, sarcoidosis and SSc	Associated with rheumatic disease development <sup>50,55,56</sup>
Air quality and particulate exposures	RA	Worse disease activity and drug response <sup>68–70</sup>
Cigarette smoking	SLE	Worse disease activity <sup>128–131</sup>
	RA	Increased risk of developing disease <sup>132</sup>
<b>Positive outcomes</b>		
Social support, improved life satisfaction	SLE	Decreased depression and anxiety <sup>59–62</sup>
	RA	Improved depression, anxiety and quality of life <sup>63–65</sup>
Exiting poverty	SLE	Decreased disease damage <sup>67</sup>
Social support and spiritual well being	SSc	Improved life satisfaction <sup>66</sup>
Diet (Mediterranean)	SLE, RA	Improved disease activity <sup>133,134</sup>
Physical activity	SLE, gout, RA	Improved disease activity <sup>135–139</sup>

ASp, ankylosing spondylitis; OA, osteoarthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.



**Fig. 2 | Siloing of research in rheumatic diseases.** Percentages of biological research, social determinants of health (SDOH) research and intersecting research were calculated based on a PubMed literature search. Percentages are visualized as rates of publishing per 500 papers. Icons are coloured according to research

category and are rounded to the nearest quarter. Manuscripts spanning biological and SDOH topics account for <1% of publications across each of the evaluated diseases (see Supplementary Table 1 and Supplementary Fig. 1 for PubMed search criteria). SLE, systemic lupus erythematosus.

First, it is crucial to understand the general limitations of genetic data, especially in the context of health disparities research. In genetic studies, individuals are categorized into groups on the basis of genetic similarity to genomic reference groups, such as the 1000 Genomes Project populations<sup>80</sup>. Importantly, however, these genetically assigned categories, often labelled continental ‘ancestry’ groups, are not equivalent to self-identified and socially defined categories of race and ethnicity<sup>81,82</sup>. Thus, risk allele differences among these genetically defined groups cannot be equated to explaining observed racial health disparities. Furthermore, mapping differences in genetic risk variants among populations requires careful interpretation. Genetic studies disproportionately comprise European-ancestry populations. Even studies including under-represented populations are often more limited in total sample size. Hence, comparison and portability of identified risk variants should always be considered in the context of potential differences in statistical power<sup>83,84</sup>. Another complicating factor is linkage disequilibrium, which refers to the tendency of linked alleles to be co-inherited. Most GWAS-reported associations are not causal variants, but instead, ‘tagging’ variants that are highly

correlated with the functional variant. Linkage disequilibrium varies by geographically or ancestrally distinct populations. Thus, a genetic association (tagging the causal variant) in one population might not reproduce in another. In studies comprising samples from multiple distinct study populations, linkage disequilibrium can be a powerful fine-mapping tool, as illustrated by SLE studies<sup>85–87</sup>. Unfortunately, the availability of large, multi-population cohorts remains limited for most rheumatic diseases. The consequence is that, to date, GRS are built upon associated, tagging variants, which frequently show poor predictability in diverse populations<sup>88</sup>. Thus, as they are currently built, these genetic prediction tools do not equally benefit all patients (particularly patients from populations not used to build these tools), precluding health equity in precision medicine<sup>88</sup>. A potential solution is to move towards GRS that are built upon causal versus tagging variants, which requires extensive functional studies to disentangle linkage disequilibrium in associated regions. As reviewed elsewhere<sup>89</sup>, ongoing research aimed at unveiling the aetiology of rheumatic diseases includes individual molecular profiling, incorporating genetic risk, cell-specific transcriptional signatures, epigenomics, mass spectrometry, tissue

and *in vitro* molecular signatures, and environmental factors. However, the majority of current molecular omics research still focuses on individuals with two copies of chromosome X and of genetic European ancestry<sup>89</sup>, and so the results from this progress are not necessarily translatable or applicable to diverse groups.

The second challenge of applying genetics data to health disparities research is that it must be studied within the context of complex disease aetiology, and not in isolation from influencing environmental and gene–environment factors. Importantly, inherited genetic variants, which are fixed within the genome, do not directly capture alterations imposed by environmental and gene–environment factors. Inclusion of additional omics such as transcriptomics (RNA level), proteomics (protein level) and/or epigenomics (for example, cytosine methylation) offers the potential to capture biological changes caused by stress attributable to systemic racism, diet or other lifestyle factors or exposures that more often affect underprivileged patients. Hence, applying genetics and/or omics knowledge also requires comprehensive and high-quality environmental data to control for environmental confounding, to identify gene–environment associations, and to identify the molecular mechanisms through which environmental exposures affect disease outcomes<sup>90,91</sup>. Genes and environmental exposures are inherited together; individuals with more genetic similarity, such as families or groups from the same region, are also exposed to more environmental similarity<sup>90,91</sup>. Yet, despite this shared environment, molecular research often negates these factors from studies, contributing to the siloing of biological research. For example, a review of sarcoidosis prevalence emphasized that most family studies focus solely on genetics, without leveraging the opportunity to explore shared environment effects<sup>92</sup>.

Epigenetic studies are well poised to elucidate the relationships between genetics, biology and environmental exposures. Epigenome-wide association studies (EWAS) have identified cell-type-specific DNA methylation changes associated with multiple rheumatic diseases<sup>78</sup>. As reviewed, although DNA methylation varies among population groups, and this variation is partially explained by their distinct genetic ancestry, environmental factors not captured by genetic ancestry are important contributors to variation in DNA methylation. This fact supports the notion that an interaction between environmental, genetic and epigenetic factors underlies health disparities in rheumatic conditions. Mounting evidence suggests that environmental exposures influence epigenetic modifications and trigger alterations in multiple regulatory mechanisms that interact to create a systemic response<sup>93,94</sup>. For example, air pollution exposure downregulates hsa-miR-137, which leads to a pro-inflammatory state that contributes to the pathogenesis of RA<sup>95</sup>. In rheumatic diseases, EWAS have largely focused on epigenetic associations with disease, without incorporation of environmental factors linked to determinants of health.

Our review of the literature shows a considerable bias in the number of studies on biological outcomes compared with epidemiological, SDOH research (Supplementary Table 1). On average, the amount of published biological research was 106:1 compared with SDOH research across ten rheumatic diseases (range: 35–271 times). As explored below (in The knowledge gap from research silos), overlap between biological and SDOH research is minimal (<1% for each of the rheumatic diseases explored) (Fig. 2). Despite the accepted influence of environmental factors on cell processes, including the role of stress in inflammation<sup>96,97</sup> and diseases such as SLE, RA and Sjögren's syndrome<sup>98</sup>, exploration of the biological mechanisms that these exposures enact within the context of health disparities research is limited.

## The knowledge gap from research silos

In our review of ten major rheumatic diseases, we identified 71 unique manuscripts that spanned both molecular biology and SDOH topics in the manuscript (Supplementary Table 2). The majority (60.5%) of the 71 manuscripts were classified as reviews, perspectives or protocol papers (Supplementary Fig. 2). Of manuscripts classified as original research, most were genetic studies stratified by race or that adjusted for SDOH in their analytic models. Notably, none reported on the mechanistic links between SDOH and molecular biology outcomes. We recognize that some biologically centred studies that included factors such as income or poverty, but not specific terms such as SDOH or SES, might have been excluded from our search. However, we note that including such factors in the absence of common SDOH or SES identifiers fails to acknowledge their context within a biopsychosocial model and their potential relevance to health disparities. Hence, we limited our intersection search to those that explicitly denoted health disparities, racism, SES and/or SDOH in the manuscript. Altogether, the scarcity of articles identified emphasizes the current knowledge gap of biological–SDOH mechanisms within rheumatic diseases.

After reviews and commentaries, the majority of identified rheumatological research that included biological and epidemiological factors could be classified as genetic risk studies that adjusted for SDOH, primarily using measures of SES. Of note, Cui et al. studied risk prediction for incident SLE among women in the Nurse's health study cohorts and found that prediction models that included genetics, family history and lifestyle (including income and US geographic region) yielded better performance than models built solely on genetics<sup>99</sup>. This finding highlights the value of incorporating variables beyond biology in prediction models. Other studies focused on assessing genetic risk while adjusting for an SES factor, including single variables (for example, income and education level), social ladders and indices of multiple deprivation. Although multiple deprivation indices and social ladder surveys are more multi-faceted than single variables, we note that these are not without limitations<sup>45,100</sup> and cannot be considered to fully capture the complexity of SDOH. That is, the absence of SDOH effects in a biological model should not be concluded when only a limited measure (such as income) was considered. In our search, we identified several examples in which potential influences of SDOH were downplayed because genetic associations remained after adjustment for single-faceted SDOH variables, further illustrating the siloing of SDOH and molecular research.

## Breaking research silos

The results of this literature search highlight the need for growth in the field. Here, we outline some key considerations and potential solutions towards enabling biopsychosocial research in rheumatic diseases and offering paths towards identifying and alleviating health disparities (Box 2).

### Structural barriers

So far, and across disciplines, addressing equity in precision medicine has focused on increasing inclusivity in research. Two leading precision medicine initiatives, the All of Us research program (USA) and Genomics England (UK), placed diversity and inclusion at the core of their efforts to promote equity. However, as argued by Galasso<sup>101</sup>, efforts at inclusion upstream (by removing barriers to participation in research) do not correspond with efforts for inclusion downstream (by facilitating access to the benefits deriving from precision medicine), and this imbalance jeopardizes the equitable goals of the programmes. For example, to

## Box 2 | Addressing obstacles to precision medicine equity

To achieve equity in precision medicine, the research silos, social and structural barriers in access to research, care, behavioural or lifestyle options, and biomedical benefits must be addressed. The priorities with which to address these barriers include the following:

### Structural issues

- Address delayed access to specialized clinical care
- Improve access to medical therapy and benefits from precision medicine (e.g. owing to health care coverage, or medication costs)
- Reform health care structures and pharmaceutical business models (e.g. drug-pricing models)
- Remove legal barriers (e.g. inadequate privacy and anti-discrimination protections for research participants, lack of health coverage and funding for follow-up care, failure to use law to ensure access to genomic medicine, and practices by research sponsors that tolerate and entrench disparities)
- Address the impacts of structural drivers of health, including racism
- Use knowledge to inform and incentivize sociopolitical reforms to reduce social inequalities and inequities
- Remove barriers to participation in research (e.g. geographical distance, mistrust of the health care system, language barriers) to increase inclusivity in research
- Consider precision medicine in a socioecological model, including environmental and structural factors that are beyond the individual's control

### Diversity in backgrounds and expertise

- Foster collaborations in team science, utilizing international collaboration
- Support collaborations between biological and epidemiological scientists, academics and clinicians, and partnerships with institutions, community groups and patients

- Encourage commitment from biotechnology and pharma industries, health care systems and governments
- Include policymakers and legal experts to address legal barriers
- Address the conditions that structure power within research teams

### Diversity in data resources

- Address the structural issues that foster the under-representation of some populations in basic and clinical research
- Improve diversity in population-based cohorts with validated diagnoses, clinical, exposure/environmental and omics data
- Support efforts to collect granular sociocultural and physical longitudinal exposures data and develop approaches to analysing their effects on molecular responses

### Infrastructure for research development

- Use multi-faceted frameworks for studying health disparities that include data for the multiple domains that contribute towards health disparities
- Adjust funding priorities

### Disseminating research responsibly

- Engage in training to become aware of the ethical, legal and social implications of research and health disparities
- Assess and address individual biases
- Incorporate equity-related concerns in research and care
- Use clear, rigorous and respectful population labels that move away from crude and oversimplistic, discrete racial categories to describe exposures such as environmental determinants of health
- Acknowledge limitations of findings, such as explicit definitions of what environmental and/or social factors might not have been captured

address the objective upstream barriers (such as time, technological literacy, language fluency) and subjective upstream barriers to participation (including mistrust, fear and stigma). All of Us implemented a comprehensive series of strategies to facilitate inclusive participation by addressing communities' mistrust, mobility, language and tech-literacy barriers<sup>102</sup>, such as including educational content in several languages, assistance via telephone, arranging home visits and providing dedicated informative material prepared in collaboration with community members. Legal barriers, such as inadequate privacy and anti-discrimination protections for research participants, and practices by research sponsors that tolerate and entrench disparities, also limit diversity and inclusion in genomic research<sup>103</sup>.

Despite efforts on inclusion upstream, downstream inclusion has remained challenging. Structural barriers preventing the affordability and availability of precision medicine outputs are the chief downstream obstacle, most notably poverty. Access to biomedical benefits are constrained by the costs of private structures and by the long waiting lists and under-resourcing of publicly funded health care<sup>101</sup>. For example, the costs of CAR-T therapies make them unavailable to individuals or countries who cannot afford them. Legal barriers

can also limit the implementation of equitable precision medicine, including lack of health coverage and funding for follow-up care, and failure to use law to ensure access to genomic medicine<sup>103</sup>. Structural factors can also prevent behavioural or lifestyle changes. Because the rheumatology community is acutely aware that health disparities and lifestyle choices negatively impact disease outcomes, our discipline is uniquely positioned to advocate for positive, broad change. However, owing to the mistrust that exists towards the health care and research communities, academic professionals need to form relationships with trusted community members who understand the needs of patients and can influence their lifestyle choices, thereby creating lasting change. Consequently, the formation of community-academic partnerships is a solution that helps engage the community in medical care, education and research, while highlighting the social resources needed for a holistic approach to patient care<sup>104</sup>. These partnerships are also positioned to examine the effect of specific SDOH in rheumatic diseases within a community<sup>105</sup>.

Another issue is the current focus on individualized risk scores and preventive strategies at the cost of structural drivers of health, including racism, and prevention of disease. This focus on the individual

might increase responsabilization and stigmatization of individuals, while neglecting how health outcomes are inseparable from environmental and structural factors beyond the individual's control<sup>18</sup>. This issue is particularly relevant in societies in which historical and contemporary discrimination not only cause health disparities but continuously prohibit opportunities for lifestyle changes for those at highest risk owing to a lack of basic resources and environmental opportunities for making healthier choices<sup>18</sup>. Recommendations can guide researchers on how to address the impacts of structural drivers of health, including racism, at all stages of the research process<sup>5</sup>. Precision medicine can catalyse knowledge and attention to inform and incentivize sociopolitical reforms to reduce social inequalities and inequities<sup>101</sup>. Specifically, All of Us and Genomics England can generate important information on social and environmental determinants of health, which might not be actionable for research participants, but would be actionable for institutions and policy makers<sup>101</sup>. This is the goal of precision public health.

## Multidisciplinary teams

Diversity in backgrounds and expertise is crucial to break the current research silos and address health disparities in precision medicine. Transdisciplinary collaboration is also needed between biological and epidemiological scientists, academics and clinicians, and partnerships with institutions, community groups and patients that represent diverse perspectives. Diverse and transdisciplinary teams are needed at all stages, from study design (what biological and SDOH measures to collect, what communities to include), to action (how to analyse and interpret results), writing and dissemination of results (how to communicate results to benefit instead of discriminate against vulnerable groups), and implementation of strategies to mitigate health disparities. A study focused on precision medicine research teams found the following points: one, existing hierarchies and power structures in the research ecosystem compound challenges for equitable diversification of teams; two, tokenism and instrumental diversity (to advance corporate interests) jeopardize goals to diversify research teams and risk merely transient and superficial diversification; and three, the siloing of the expertise of under-represented team members to frontline and diversity-only activities might perpetuate a turnstile effect, in which these team members move from study to study laterally, or altogether leave the field because of the lack of advancement opportunities<sup>106</sup>. Without considering the power structure within research teams, tokenism can be misrecognized as inclusion<sup>106</sup>.

Furthermore, to address health disparities in precision medicine, commitment from biotechnology and pharma industries, health care systems, and governments is also needed<sup>21</sup>. The inclusion of legal experts is needed to address legal barriers that limit broad inclusion in genomic research and the development of precision medicine to advance health equity<sup>103</sup>. Policymakers are essential to assess and address the effects of the sociopolitical, economic and structural contexts of health care implementation<sup>18</sup>. Without concerted public health action, health disparities could widen in the next decade<sup>4</sup>.

## Diversity in data resources

Identifying and addressing health disparities in rheumatic diseases requires greater sample diversity in datasets<sup>107</sup>. There has been a growing push to make GWAS analyses more equitable through sample diversity and using genotyping platforms better suited to capturing global variation. One such example is the MEGAchip, which is a consortium effort that developed a genotyping array to better capture genetic

diversity across global populations<sup>84,108</sup>. For sequencing studies, reference genomes have been developed that are more appropriate for capturing rare sequencing variants in diverse populations<sup>109,110</sup>. Importantly, diversity in biological data is also needed beyond studies strictly identifying genetic risk variants. As described above, it is essential to pinpoint the functional variants contributing to disease, requiring extensive functional annotations and tissue-specific data. Repositories used for these goals have not been representative of global diversity. Long et al. explored this issue among datasets used to link genetic variants to gene expression (expression quantitative trait loci) and found that three major resources overwhelmingly comprised European ancestry samples (82–95% of samples)<sup>111</sup>. The lack of diversity in large datasets is even more apparent for cohorts containing environmental and lifestyle data, particularly for rheumatic diseases. For example, the US National Institutes of Health-funded Polygenic Risk Methods in Diverse Populations Consortium pools genomic and phenotypic information from diverse populations<sup>112</sup>. However, at present, the phenotypic and environmental data are limited.

The paucity of large and diverse population-based cohorts with validated diagnoses has been a substantial barrier to better understanding the true clinical burden of disease, as well as the many unanswered questions related to treatment, health care access, and natural history<sup>113</sup>. The Environmental Influences in Child Health Outcomes, All of Us<sup>114</sup>, and Genomics England programmes were created to mitigate against these disparities<sup>115</sup>. However, given that these programmes reflect broad (non-disease-focused) recruitment efforts, sufficient numbers for conducting studies in rheumatic diseases remains challenging. Efforts to include communities with worse and better outcomes will be important, to fully understand the interplay among risk factors. Researchers collecting biological samples should make efforts to collect granular sociocultural and environmental data, moving beyond the scope of the limited racial and ethnic categories currently used by the US Office of Management and Budget.

As previously described, many instruments can be used to capture various aspects of SDOH. Although we advocate for using multi-dimensional tools that capture both materials and/or resources (for example, income) and exposures (such as stress, environment), the feasibility might vary by study. For any instrument used, it is critical to be cognizant of and report limitations of the study (for example, factors not captured by the data). Electronic health record (EHR) data might provide a promising venue to facilitate SDOH data collection; however, logistical challenges, such as infrastructure and consensus of standards, might affect the speed of implementation<sup>116</sup>. The EU-funded SPIDeRR consortium (Stratification of Patients using advanced Integrative modelling of Data Routinely acquired for diagnosing Rheumatic complaints) is aimed at leveraging EHR data from seven countries to streamline early diagnosis of rheumatic diseases. In addition to using GRS for disease differentiation, SPIDeRR is also aimed at investigating the interplay between genetics and the environment<sup>75</sup>.

## Infrastructure for research development

Patients with rheumatic diseases require a transformation in both patient care and research infrastructure to address current and future health disparities. As the benefits of collaborations in team science are increasingly recognized, utilizing international collaborations is an additional method to diversify patient recruitment to investigate and minimize disparities in rheumatic disease. For example, in China, a clinical trial focused on multidisciplinary care for patients with SLE showed a substantial decline in disease activity in the intervention group and an

improvement of health quality<sup>117</sup>. A subsequent study reviewed the pros and cons of this approach, specifically in the care of patients with lupus nephritis<sup>118</sup>. Another example is the Sjögren's International Collaborative Clinical Alliance, which was created to expand the infrastructure for engagement and to be used as a research tool to better understand the pathogenesis of Sjögren's disease. Several publications to date have used this collaboration as the basis for manuscripts, including investigating associations between clinical disease manifestations and autoantibody profiles<sup>119</sup>. This international collaborative approach broadens not only the number of potential patients but also invites the participation of diverse researchers, which will enlarge the range of research topics and ideas to address research disparities.

Building multidisciplinary teams and more robust data resources will require significant infrastructure. Previous inquiries on the slowed progress and siloing of health equity research identified funding as a meaningful influence on multidisciplinary work<sup>120,121</sup>. That is, funding mechanisms and priorities have the potential to facilitate the bridging or sub-division of disciplines. Although historic commitments have been made towards health disparities research, including the US Healthy People 2010 programme (funded in 2000) and the aforementioned 2006–2009 strategic plan of the US National Institute of Arthritis and Musculoskeletal and Skin Diseases, we posit that these were inadvertently, largely, siloing infrastructures – the former focusing on epidemiological initiatives and the latter prioritizing the identification of genetic contributions to health disparities. Opportunely, in 2023, the US National Institutes of Health began funding projects to design, develop and implement an Exposome in Autoimmune Diseases Collaborating Teams (EXACT) Network<sup>122</sup>. Its goal is to enable institutions to plan research strategies and develop partnerships, infrastructure and capabilities. The EXACT initiative is envisioned as a multisite, collaborative network that will adopt a team science approach to produce a systems-level understanding of the role of the exposome in autoimmune diseases. Focused investment is needed for similar projects and programmes that will combine clinical and analytical teams to collect and integrate clinical, omics and exposure data to enable analyses focused on elucidating how specific exposures govern biological responses in patients with rheumatic diseases. Longitudinal follow-up and collection of biospecimens is also challenging<sup>113</sup>; to address this limitation, the US Centers for Disease Control and Prevention has funded five population-based SLE registries<sup>107</sup>.

## Disseminating research responsibly

Finally, understanding and alleviating health disparities requires care in the reporting and dissemination of results. These studies have the potential to inform diagnoses, interventions and future policies, but they also have the potential to produce harm. As previously discussed, researchers should avoid conflating self-reported race with genetic ancestry. However, even with repeated emphasis in reporting guidelines<sup>11,31,123</sup> distinctions can still be blurred. For instance, owing to sampling strategies and analytical challenges, some studies still assign genetic ancestry categorically rather than as a continuum, which can reinforce the erroneous interpretation that discrete continental population labels reflect discrete genetic differences among samples<sup>124</sup>. Additionally, studies must thoroughly acknowledge limitations of findings, such as explicit definitions of what environmental and/or social factors might not have been captured or included in analyses and results. These issues are also pertinent in the age of large language models, which are increasingly available to and leveraged by the public and search engines. These models synthesize large quantities of data

and can be influenced by biases present in training data<sup>125</sup> with the potential to reiterate racist ideologies<sup>126</sup>. Thus, all roles contributing towards health disparities research, including researchers, journal editors and peer reviewers must remain diligent in assessing manuscripts for clarity and precision on these issues.

## Conclusions

Although precision medicine has been promoted for over a decade, a lack of understanding of the effects of determinants of health on health disparities remains a barrier to achieving equitable access to precision medicine<sup>21</sup>. To date, crossover between molecular and SDOH research groups remains limited; this challenge is pervasive across disciplines, but awareness of the problems it poses is growing<sup>12,13</sup>. We have illustrated the disciplinary siloing between molecular and epidemiological research by quantifying the current publication gap of integrative research across ten rheumatic diseases. Current work has already shown how inclusion of additional exposures and SDOH can improve biological risk models, but more data are needed to further this area of research. Until we diversify rheumatic disease research, it will remain impossible to implement precision medicine that is informed by both environmental and genetic factors for all patient demographics. Our presented solutions include breaking the epidemiological and biological silos, as well as commitments to equity and structural reform. These changes are necessary to elucidate how exposures affect the heterogeneous molecular and cellular pathways leading to pathology, and might highlight novel approaches to avoiding or neutralizing interactions that contribute to rheumatic disease. We note that these challenges are not unique to rheumatic diseases and thus there is benefit in uniting efforts across disease-focused fields (for example, towards standardized SDOH and environmental exposure data, and society-level changes towards better inclusion). The continued development of EHR data, high-throughput omics and molecular profiling offers great opportunities but only if the field works to ensure these data are truly representative of the complex biological and environmental relationships within patient populations.

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## Author contributions

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## Competing interests

The authors declare no competing interests.

## Additional information

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# Opportunities and limitations of B cell depletion approaches in SLE

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## Abstract

B cell depletion with rituximab, a chimeric monoclonal antibody that selectively targets B cells by binding CD20, has been used off label in severe and resistant systemic lupus erythematosus (SLE) for over two decades. Several biological mechanisms limit the efficacy of rituximab, including immunological reactions towards the chimeric molecule, increased numbers of residual B cells, including plasmablasts and plasma cells, and a post-treatment surge in B cell-activating factor (BAFF) levels. Consequently, rituximab induces remission in only a proportion of patients, and safety issues limit its use. However, the use of rituximab has established the value of B cell depletion strategies in SLE and has guided the development of several improved B cell depletion therapies for SLE. These include enhanced monoclonal antibodies, modalities that redirect the specificity of patient T cells using chimeric antigen receptor T cells or bispecific T cell engagers, and combination treatment that simultaneously inhibits the BAFF pathway. In this Review, we consider evidence gathered from over two decades of using rituximab in SLE and examine how B cell depletion therapies could be further optimized to achieve immunological and clinical efficacy. In addition, we discuss the prospects of B cell depletion strategies for personalized treatment in SLE based on genetic research and studies in pre-symptomatic individuals.

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Introduction

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## Key points

- Although the B cell depletion agent rituximab failed to reach its primary end points in randomized controlled trials in systemic lupus erythematosus (SLE), favourable clinical experience has led to its frequent off-label use in patients with SLE.
- Deep B cell depletion of prolonged duration has been associated with improved clinical response to rituximab.
- Additional B cell depletion therapies that enhance B cell depletion, reduce immunogenicity, delay relapse of B cell numbers or target memory B cells and plasma cells are under development, although trials comparing these therapies head to head are lacking.
- Innate and non-immune mechanisms that lead to B cell activation, as well as B cell-independent inflammation, might underlie resistance to B cell depletion therapy.
- Although enhanced B cell depletion improves clinical responses in patients with SLE, both B cell-driven mechanisms and innate or non-immune mechanisms might need to be targeted to achieve cure.

## Introduction

A role for B cells in the pathogenesis of systemic lupus erythematosus (SLE) has been recognized for decades, and the presence of autoantibodies is a feature of the disease<sup>1</sup>. Rituximab, a chimeric monoclonal IgG1 antibody that selectively targets B cells by binding to CD20 on their surface, was the first B cell depletion agent used in patients with SLE<sup>2,3</sup> (Fig. 1). As many other B cell depletion agents, rituximab was initially developed for the treatment of B cell-derived malignancies. In addition, B cell depletion with rituximab was efficacious in randomized trials in several rheumatic diseases, including patients with rheumatoid arthritis (RA) with insufficient response to TNF inhibitors<sup>4</sup>, and the induction and maintenance of remission in antineutrophil cytoplasmic antibody-associated vasculitis<sup>5–8</sup>.

Rituximab depletes B cells by several mechanisms (Fig. 1b). It crosslinks its targeted B cells with the activating Fcγ receptor III (FcγRIII) on effector cells such as natural killer (NK) cells and macrophages to induce death of the opsonized B cell through antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), respectively. In addition, B cell depletion can be induced independently of FcγRIII binding. The binding of type I monoclonal antibodies to CD20 on the B cell surface clusters CD20 into lipid rafts. This redistribution increases cell death by complement-dependent cytotoxicity (CDC) and apoptosis<sup>9</sup>. However, clustered complexes are more rapidly internalized, through interactions with inhibitory FcγRIIb on the B cell surface, which limits availability for ADCC and ADCP<sup>10</sup>. Some novel therapies include modifications to shift this balance between clustered complexes and non-clustered complexes.

CD20 is specifically expressed on B lineage cells, sparing progenitor cells, some plasmablasts and long-lived plasma cells (Fig. 1a). CD20 function is poorly understood, and its natural ligands remain unknown. In addition to CD20, key targets on B cells include CD19, CD38, B cell maturation antigen (BCMA) and the B cell-activating factor (BAFF) receptor (BAFF-R). These are expressed on different subpopulations of B cells during development (Fig. 1a). Compared with

CD20, CD19 has broader expression, including in plasmablasts and plasma cells. CD38 and the BAFF-R have more restricted expression that may direct biological effects towards subpopulations of B cells<sup>11–14</sup>.

In this review we examine the biological and clinical efficacy, as well as the limitations of off-label use, of rituximab in SLE and discuss the long-standing evidence showing that deep B cell depletion is important for clinical efficacy. In addition, we explore strategies currently employed to improve B cell depletion therapy, including enhanced monoclonal antibodies, chimeric antigen receptor (CAR) T cells and bispecific T cell engagers (BiTEs) and discuss how to manage long-term safety with B cell depletion. Finally, we examine how recent knowledge about B cell extrinsic mechanisms of SLE pathogenesis illuminate potential limitations of B cell depletion therapies in terms of achieving complete cure of SLE.

## B cell subsets in SLE

In established SLE, the B cell compartment displays several alterations compared to that of healthy individuals, including extrafollicular T cell-independent activation, reduced dependence on B cell receptor (BCR) signalling and B cell lymphopenia<sup>15–17</sup>. Lymphopenia preferentially affects naive B cells, whereas type I interferons (IFNs), which are commonly upregulated in SLE, promote the survival of transitional B cells (Box 1). This leads to an altered composition of the B cell pool with an increased proportion of memory B cells<sup>17–19</sup>. SLE has been described as a prototypic B cell-driven disease that is maintained by mature B cell clones that produce autoantibodies and cytokines and present antigens to autoreactive T cells. However, the extent to which SLE pathogenesis relies on complete and disrupted germinal centre reactions versus extrafollicular B cell maturation is not well clarified<sup>20</sup>. The idea of isolated and intrinsically autoreactive B cell clones that drive disease development might need revisiting as recent evidence points towards the involvement of polyclonally and extrafollicularly activated B cells in the pathogenesis of SLE<sup>15,21,22</sup>.

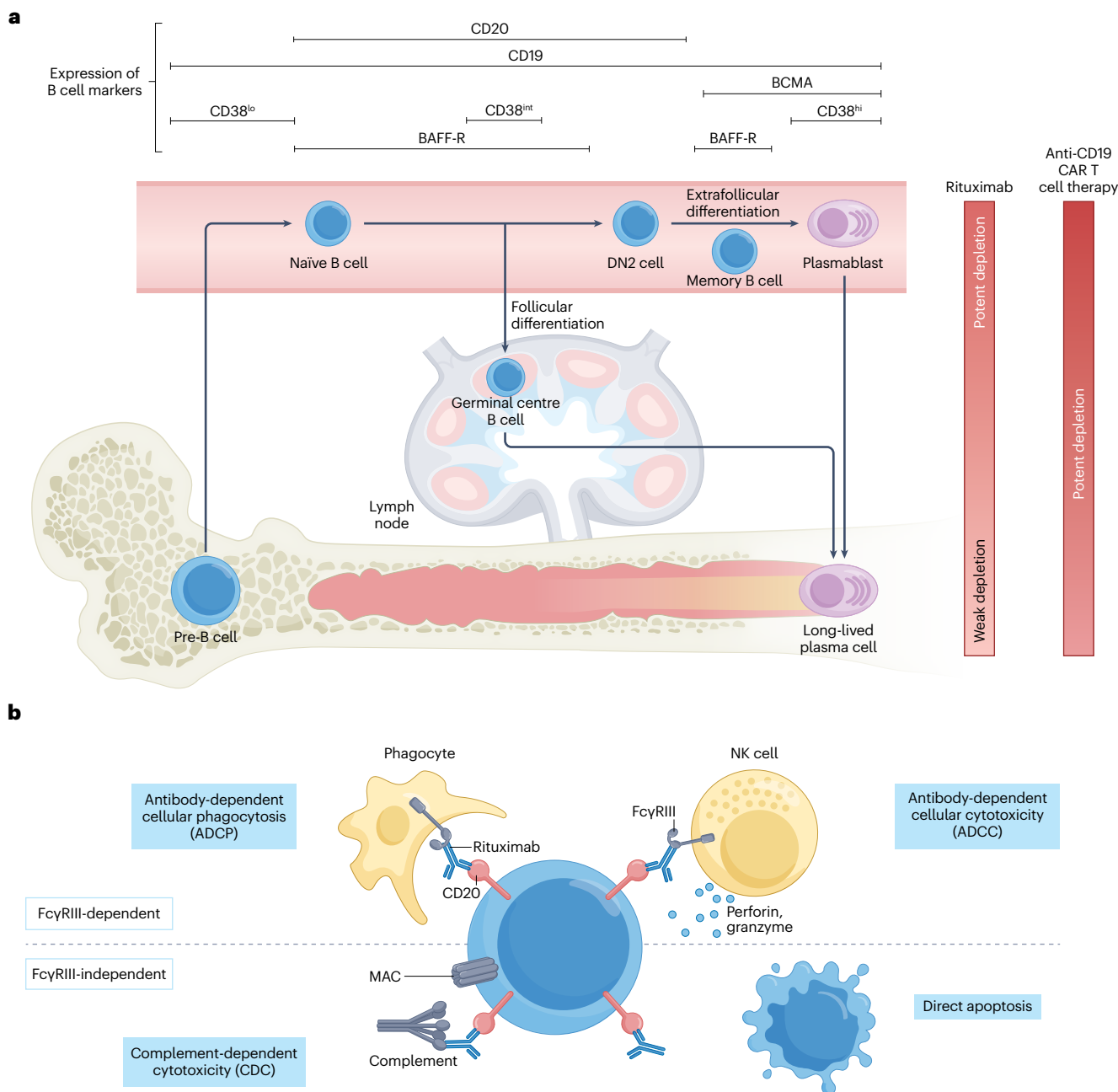
Central T cell tolerance is unlike the first B cell checkpoint in the bone marrow. In central T cell tolerance, expression of the autoimmune regulator protein in thymic epithelial cells ensures exposure of developing T cells to tissue-restricted autoantigens and thereby results in efficient elimination of autoreactive T cell clones. The first checkpoint of B cell tolerance is regulated by exposure to a narrower selection of antigens expressed in the bone marrow, and does not exclude autoreactivity in the naive B cell pool as efficiently. Therefore, an important second checkpoint of B cell tolerance is required to prevent the inappropriate activation of autoreactive naive B cells. Naive B cells, thus, have an elevated threshold for activation and require activation of both BCR signalling and co-stimulatory molecules for an optimal pro-inflammatory response<sup>23</sup>. The RNA-sensing TLR7 pathway is particularly relevant for SLE disease development. After a nuclear antigen has bound its cognate BCR on the B cell surface, the bound complex is internalized and delivered to endosomes containing TLR7 and TLR9 (refs. 23–25) (Box 2). These innate receptors are activated by nuclear and mitochondrial RNA and DNA, and their engagement in concert with BCR ligation provides a second signal that triggers B cell activation<sup>24,25</sup>. The activation of TLR7 is essential to develop an antibody response in SLE-prone mice and was required for the expansion of autoreactive B cells that bind to self nucleotides, including CD27<sup>+</sup>IgD<sup>+</sup> double-negative B cells (DN B cells) and plasmablasts<sup>26,27</sup>.

The DN B cell population is highly responsive to TLR signalling and shares some properties with murine age-associated B cells. DN stage 2 (DN2) B cells, which are marked by a higher expression of CD11c and T-BET than that of other DN B cell subsets, have increased

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levels of TLR7 but lack the TLR regulator TRAF5 (ref. 26). The DN2 B cell subset is expanded in both adults and children with SLE, especially in individuals with African American ancestry and nephritis,

and the percentage of DN2 B cells correlates with disease activity and Sm-specific or ribonucleoprotein-specific antibodies<sup>28,29</sup>. DN2 B cells are derived from activated naive B cells, are set to recognize nucleic



**Fig. 1 | Mechanisms of B cell depletion by rituximab. a**, B cell subsets express distinct surface markers depending on their differentiation stage. These surface markers can be targeted to direct biological effects towards the various stages of B cell maturation. For example, as rituximab uses CD20 as its target, biological effects are targeted towards B cells prior to the memory and plasma cell stages. B cell subpopulations of special interest for systemic lupus erythematosus (SLE) and their robust and characteristic expression of surface markers are depicted<sup>11–14</sup>. In addition to circulating B cells, which are efficiently targeted by rituximab, lymph node-resident and tissue-resident B cells are also likely to mediate pathology in SLE but are less efficiently depleted by

monoclonal antibodies. By virtue of their cellular mechanism of action, chimeric antigen receptor (CAR) T cells are expected to induce more potent depletion in peripheral tissue compared with monoclonal antibody-mediated depletion with rituximab. **b**, Rituximab induces B cell depletion by several different mechanisms, including Fcγ receptor III (FcyRIII)-mediated antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC), as well as complement-dependent cytotoxicity (CDC) and direct induction of apoptosis. BAFF-R, B cell-activating factor receptor; BCMA, B cell maturation antigen; DN2 cell, double-negative stage 2 B cell; MAC, membrane attack complex; NK, natural killer.

## Box 1 | Interconnections between autoantibodies and the type I interferon system

Studying the early phase of systemic lupus erythematosus (SLE), before the onset of any clinical symptoms, has revealed the early involvement of autoantibodies and the type I interferon (IFN) system in disease development. Loss of humoral tolerance develops progressively during the years preceding onset of disease, and most patients with SLE carry at least one antinuclear antibody (ANA) specificity 6 years before diagnosis<sup>143</sup>. Pathogenic ANAs, especially anti-double-stranded DNA antibodies, can form immune complexes, activate complement, bind apoptotic nucleosomes in the glomeruli and mediate neuronal apoptosis<sup>156,157</sup>. However, some ANAs are benign and can be present during remission<sup>158</sup>. Only a minority of healthy individuals who develop ANA progress to clinical SLE disease<sup>150</sup>. However, even among non-progressors without inflammatory symptoms, ANA positivity is transcriptionally a highly dysregulated state with several abnormalities, including upregulation of IFN production in blood and non-haematopoietic tissues, and the immunological profiles of individuals with ANA positivity are more similar to those of patients with SLE than to those of healthy controls<sup>148,159</sup>.

Extracellular nuclear material is a potent stimulus for both antibody production and the type I IFN response, and the progression from ANA positivity to an SLE flare can be predicted on the basis

of type I IFN pathway activation in blood and skin<sup>148,150,159</sup>. Most patients with established SLE exhibit sustained upregulation of the type I IFN system, and type I IFN activation has been associated with disease activity and distinct clinical features<sup>160–163</sup>. Local activation of the type I IFN pathway is evident in several organ systems and SLE can be ameliorated by the type I IFN receptor inhibitor anifrolumab<sup>64,159,164–166</sup>.

Elevated IFN $\alpha$  protein levels are associated with the presence of ANA and the number of specific autoantibodies is consistently associated with activation of type I IFN system in SLE and other rheumatic diseases<sup>167–169</sup>. Stimulation with type I IFNs upregulate TLR7 expression on B cells, promote BAFF production, and strongly promote B cell differentiation to plasmablasts<sup>170–172</sup>. In addition, exposure to IFN $\alpha$  imprints plasma cells to express high levels of ISG15 mRNA, accompanied by direct secretion of ISG15 protein in SLE<sup>149</sup>. ISG15 has a variety of immune modulatory effects both inside cells and as a soluble mediator. Thus, autoantibodies forming immune complexes can provide an interferonogenic stimulus<sup>173,174</sup>. In return, type I IFN stimulation imprint plasma cells to cause immunomodulatory effects that are independent of their antigen specificity, further contributing to the inflammatory vicious circle of SLE.

acids and seem to develop into antibody-producing cells<sup>26</sup>. This extrafollicular pathway of B cell differentiation seems to promote the development of autoreactive and antibody-producing plasma cells and plasmablasts in SLE.

Plasmablasts are a heterogeneous subset of short-lived, rapidly generated, antibody-producing B lineage cells, often defined on the basis of their CD19<sup>+</sup>/CD27<sup>+</sup>/CD38<sup>+</sup> surface marker profile, that markedly expand early during SLE disease<sup>30</sup>. In viral infections, TLR signalling seems to determine whether plasmablasts develop intrafollicularly or extrafollicularly, and in mice, overexpression of TLR7 increases both spontaneous germinal centre formation and plasmablast development<sup>27,31</sup>. Both a plasmablast gene signature and the proportion of peripheral plasmablasts have been associated with SLE disease activity<sup>32,33</sup>. In the absence of naive and memory B cells, the presence of plasmablasts in circulation might indicate ongoing B cell activity in other tissues. High-sensitivity flow cytometry is necessary to enumerate these rare cells, that are mostly located outside the lymphocyte region and have lower CD19 expression than that of other B cell populations.

Overall, TLR7 signalling has the potential to drive the extrafollicular and polyclonal differentiation of autoreactive B cell populations during SLE. As TLR7-activated B cell populations might contribute to the development of an autoantibody response, the presence of these cells might help explain both the lack of response to rituximab in some patients with SLE and the success of therapeutic strategies involving BAFF inhibition.

### Effectiveness and limitations of rituximab

#### Rituximab in SLE

Although early studies suggested efficacy in SLE, rituximab surprisingly failed to meet the primary and secondary end points in the LUNAR trial of renal SLE and the EXPLORER trial of non-renal SLE<sup>34,35</sup>. Results from both trials indicated biological effects, with rapid depletion of CD19<sup>+</sup>

B cells, improved serology, and normalization of complement factors. However, high doses of background corticosteroids and other immunosuppressive therapies, patient heterogeneity and trial design might have contributed to the failure to meet end points<sup>36</sup>. Regardless, rituximab has been used off-label in patients with SLE for over two decades on the basis of clinical effectiveness in case series, registries and two systematic reviews<sup>37–42</sup>. The clinical response to rituximab is variable and, although many patients need retreatment with rituximab to control disease, the time span before return of clinical symptoms differs considerably across these studies<sup>43,44</sup>. The pattern of relapse is bimodal and for approximately half the patients, relapse occurs within 18 months, whereas the remaining patients seem to benefit from longer periods of remission, sometimes exceeding 4 years<sup>43</sup>. Factors that limit the effects of B cell depletion therapy with rituximab include the presence of residual B cells in circulation and peripheral niches, non-response due to insufficient depletion or B cell-independent disease, and, in a proportion of patients, a sustained reduction in immunoglobulin that is associated with increased risk of serious infection.

#### Residual B cells in circulation

The level of B cell depletion by rituximab depends on the affinity of Fc $\gamma$ RIII to rituximab<sup>45</sup>. Fc $\gamma$ RIII allotypes have distinct receptor properties and influence the numbers of residual B cells after treatment with rituximab. In particular, the 158V polymorphism in *Fc $\gamma$ RIIIa* increases Fc $\gamma$ RIII affinity to IgG1. Patients who are homozygous for this polymorphism have enhanced NK cell-mediated degranulation and require lower serum rituximab levels than patients with other *Fc $\gamma$ RIIIa* haplotypes to achieve the same degree of B cell reduction<sup>45,46</sup>. In patients with RA, giving patients with incomplete B cell depletion an extra dose of rituximab 4 weeks after treatment initiation reduced residual B cells and clinical response without increasing adverse events<sup>47</sup>. Similarly, patients with SLE and *Fc $\gamma$ RIIIa* genotypes that confer lower affinity to

IgG1 are likely to need increased rituximab doses or B cell depletion by alternative agents to achieve equal biological and clinical responses as patients with higher IgG1 affinity *FcyRIIIa* genotypes.

B cell depletion by rituximab is transient, and many patients with SLE still have detectable B cell levels after two infusions of rituximab. Lower numbers of residual B cells are associated with improved clinical responses in both renal and non-renal SLE<sup>2,43,48–50</sup>. Moreover, the depletion of certain B cell subtypes is associated with clinical response, and transitional B cells are expanded in patients with prolonged clinical response to rituximab<sup>51</sup>. The number of plasmablasts at relapse after rituximab seems to correlate with the titres of double-stranded DNA (dsDNA)-specific autoantibodies, and repopulation by plasmablasts was found to be predictive of disease recurrence<sup>43</sup>. Using high-sensitivity flow cytometry, repopulation with plasmablasts can be identified early; individuals with detectable plasmablasts 6 months after rituximab treatment are at higher risk of clinical relapse within the following 6 months<sup>52</sup>. In these patients, retreatment on the basis of B cell repopulation might have the potential to prevent relapse.

## Residual B cells in peripheral niches

Most information about the B cell depletion efficiency of rituximab is based on counts of circulating B cells, which are more accessible than tissue-resident B cells. However, B cells in the blood comprise a minority of total B cell numbers and are not necessarily in homeostasis with tissue B cells<sup>53</sup>. Thus, residual B cells are likely to remain in peripheral niches even after B cell depletion therapy. The numbers of residual renal B cells have been evaluated after rituximab treatment in biopsies from patients with lupus nephritis. B cells were detected in most biopsies,

and the presence of renal B cells was associated with poor treatment response even when circulating B cells were low or undetectable<sup>54,55</sup>. In addition, although patients with SLE and a long-term response to rituximab showed an altered B cell composition in the tonsils compared with patients with untreated SLE, germinal centre reactions persisted in these samples<sup>51</sup>. In contrast to the very low levels of circulating memory B cells, this suggests that memory B cell depletion in tissue by rituximab is less effective than in blood.

Similar findings have been reported in other rheumatic diseases. In Sjögren syndrome, rituximab does not affect the presence of clonally related immunoglobulin-producing cells in salivary glands<sup>56</sup>. In RA, B cells remain detectable in the lymph nodes after rituximab treatment<sup>57</sup> and the presence of residual B cells in synovial tissue is associated with disease activity<sup>58–60</sup>. Taken together, although rituximab treatment decreases the number of B cells in secondary lymphoid organs and peripheral tissue, residual B cells often persist in these sites, even in the absence of circulating B cells, suggesting that B cells in secondary lymphoid organs change more slowly than peripheral B cells.

## Mechanisms of non-response

The chimeric composition of rituximab with murine variable regions increases immunogenicity and the risks of antidrug reactions. Up to a fifth of patients with SLE who receive rituximab have a good initial response but develop secondary non-depletion and non-response (2NDNR) with repeated use<sup>61</sup>. Symptoms and signs of 2NDNR include a severe infusion reaction that lasts >24 h during the second infusion of a cycle, failure to completely deplete B cells and lack of a clinical response<sup>52</sup>. This is suggestive of an immune reaction against rituximab,

## Box 2 | Autoantigens as drivers of B cell activation in SLE

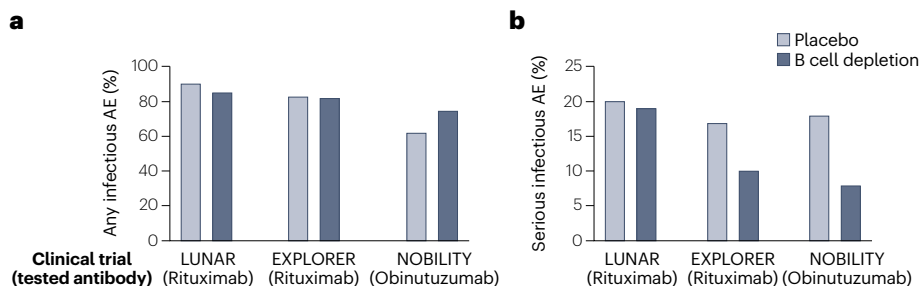
In its symptomatology, a systemic lupus erythematosus (SLE) flare resembles a viral infection, with fatigue, arthralgia and oral ulcers, as well as pleuritis and leukopenia, and both conditions involve immunological reactions directed against nucleotides<sup>175</sup>. However, instead of appropriately eliminating viral RNA and DNA, SLE involves an inappropriate reactivity induced by endogenous nucleotides that manifests in the presence of antinuclear antibodies (ANAs). If these autoantibodies were primarily caused by monoclonal B cell dysfunction (for example, by stochastically arising autoreactive B cells) and perpetuated by T cell help and epitope spreading in germinal centre reactions, then elimination of the resulting clones might be expected to induce a sustained drug-free remission. Cure through elimination of B cell clones has been suggested to be the aim of future B cell-targeted therapy<sup>99</sup>. However, several lines of evidence argue against this theory.

The ANAs observed in SLE comprise antibodies against a diverse mixture of intracellular antigens, including single-stranded and double-stranded DNA, histones and ribonuclear proteins that accumulate leading up to symptom onset<sup>143</sup>. These antigens are not connected by a shared molecular structure, but by their intracellular location and their persistence after defective apoptotic clearance<sup>144,176,177</sup>. SLE susceptibility can be conferred by loss-of-function mutations that increase the availability of these antigens, such as TREX1, which impairs their clearance during apoptosis, or gain-of-function mutations in loci that increase the innate sensing of these

antigens, such as STING<sup>145,146,176</sup>. Such antigen generation and sensing is a feature of all nucleated cells, not just the circulating immune system.

In addition, a monogenic TLR7 gain-of-function mutation was recently described leading to typical and severe SLE symptoms<sup>21</sup>. The carrier was a young girl who developed SLE by the age of 7 years with elevated ANA, hypocomplementaemia, inflammatory arthralgia and renal involvement<sup>21</sup>. Additional analyses revealed a further two patients with monogenic variants in TLR7 causing SLE disease<sup>21</sup>. Notably, the gain-of-function mutation in TLR7 caused SLE symptoms in mice by extrafollicular activation of B cells<sup>21</sup>. TLR7 escapes X chromosome inactivation leading to a higher expression in females than in males, and the endogenous X-inactive specific transcript (XIST) contributes a rich source of endogenous TLR7 ligands in female patients with SLE<sup>178</sup>. Thus, the alteration of the sensing of nuclear material by TLR7 is sufficient to induce typical and severe lupus, and may at least partly explain the female bias in SLE.

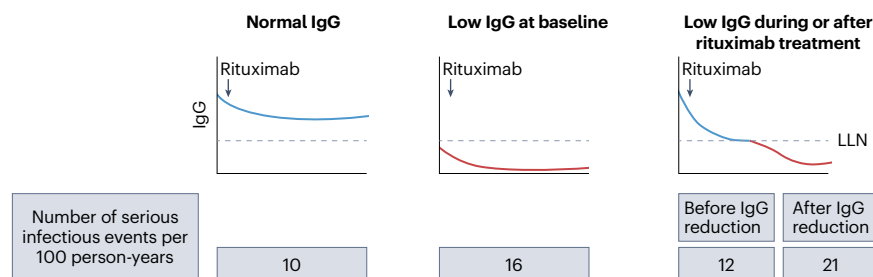
Hence, the antibody repertoire of SLE is not what one would predict from a series of stochastic B cell autoreactive events with epitope spreading leading to B cell clones as the primary cause of autoreactivity. Instead, these data collectively suggest that B cell autoreactivity is secondary to increased sensitivity to these antigens, and loss of function in clearing these antigens during apoptosis. These latter mechanisms would be predicted to return after any B cell-directed therapy, no matter what intensity of depletion or quality of remission is achieved.



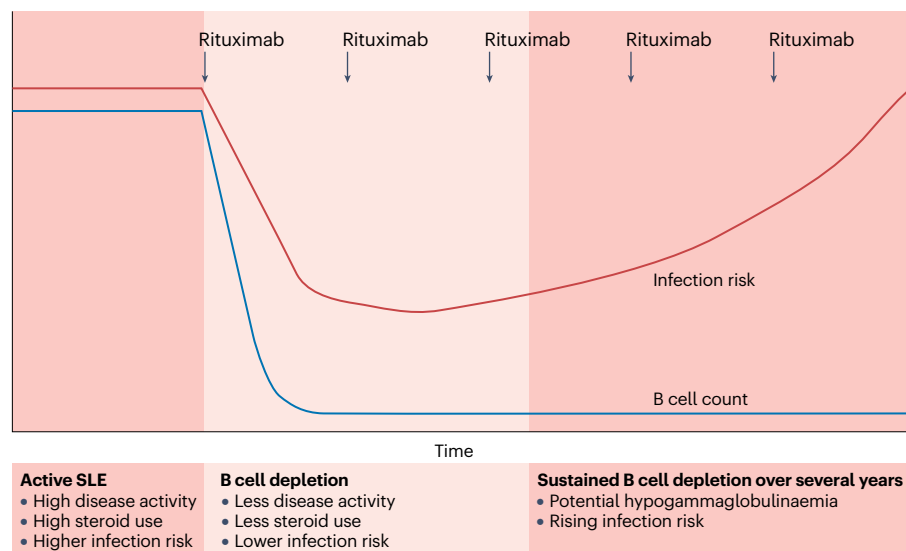
**Fig. 2 | No evidence for increased risk of infections by B cell depletion across multiple trials.**

Across multiple trials of patients with systemic lupus erythematosus (SLE), there is no evidence for increased risk of any (part **a**) or serious (part **b**) infections following B cell depletion therapy compared with placebo<sup>34,35,67</sup>. However, in a mixed cohort of patients with rheumatic diseases (part **c**), patients with low IgG levels at baseline or patients with hypogammaglobulinaemia during treatment had increased risk of serious infection after treatment with rituximab<sup>72</sup>. Active SLE is associated with increased risk of infection (part **d**), and this risk may be reduced by treatment. However, sustained B cell depletion is likely to cause hypogammaglobulinaemia, which is associated with increased risk of infection. AE, adverse event; LLN, lower limit of normal.

**c Multi-disease cohort**



**d Model of infection risk during B cell depletion in SLE**



and the presence of rituximab-specific antibodies is common in patients with SLE who develop 2NDNR. Thus, in primary responders to rituximab, subsequent treatment failure might occur because of insufficient B cell depletion, and tailored strategies to optimize depletion can restore clinical response.

Patients with mucocutaneous disease and, in particular, with chronic cutaneous lupus erythematosus (CCLE) have a low clinical response rate to rituximab<sup>52,62</sup>. In these patients, the level of B cell depletion is not associated with CCLE response rates, CCLE non-response is not associated with low response in other domains, and new cutaneous lesions erupt during the period of B cell depletion, suggesting that a primary non-response with B cell-independent inflammation is probably

involved in CCLE<sup>62</sup>. Indeed, CCLE is initiated by keratinocyte apoptosis, which leads to exposure of nuclear antigens and type I IFN production. Thus, patients with CCLE might be better suited to a between-class switch to type I IFN targeted therapy<sup>63,64</sup>

**Managing long-term safety**

A common misconception is that B cell depletion inherently increases the risk of infection<sup>65</sup>. Across multiple trials in SLE, no significant differences in the proportion of adverse or serious adverse events were found between placebo and B cell depletion agents, and infection rates were numerically lower in treated patients than in those receiving placebo (Fig. 2a,b). In registry data of patients with moderate-to-severe

SLE, rituximab, the BAFF inhibitor belimumab and standard immunosuppression were associated with similar rates of serious infection<sup>66</sup>. Although patients with SLE might be sometimes told that treatment with rituximab might increase the risk of infection, a general increase in serious infections has not been observed<sup>34,35,67</sup>. Patients with SLE are likely to have an increased risk of infection due to their disease and their treatment with glucocorticoids<sup>68</sup>. While suppressing immunity, specific targeting of B cells with rituximab can allow glucocorticoid tapering and normalization of other immune cells and molecules, which might potentially improve the endogenous defence against infections.

In the case of COVID-19, treatment with B cell depletion agents was initially associated with a higher risk of severe infection in patients with immune-mediated disease<sup>69</sup>. However, vaccination offered protection with every new dose received<sup>70</sup>. Together with the decreased immunogenicity of the virus, the risk of severe COVID-19 infection in patients with systemic rheumatic diseases abated towards the end of the pandemic<sup>71</sup>.

In around 15% of patients, repeated rituximab treatment leads to hypogammaglobulinaemia<sup>38</sup>. In a multi-disease cohort, low IgG at baseline was associated with an increased risk of serious infections, and a reduction in IgG levels occurring during or after treatment doubled the risk compared with normal IgG levels<sup>72</sup> (Fig. 2c). Immunoglobulin levels are probably sustained by long-lived plasma cells, and although targeting CD20-positive B cells does not affect plasma cells directly, it reduces the number of plasma cell precursors. Exposure to cyclophosphamide and glucocorticoids, as well as multimorbidity, also increase the risk of hypogammaglobulinaemia<sup>72–74</sup>. Thus, although B cell depletion is desirable for active disease and does not affect the short-term risk of infection,

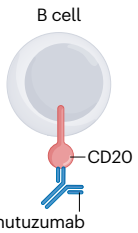
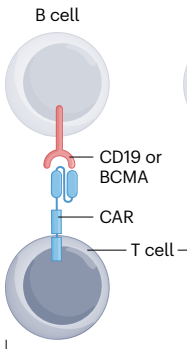
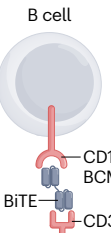
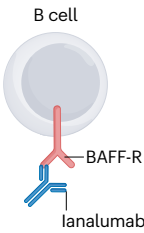
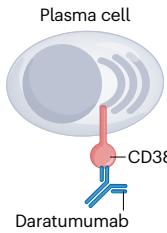

in a proportion of patients, repeated rituximab treatment leads to a reduction in immunoglobulin levels that is associated with an increased risk of serious infections (Fig. 2d). This calls for a tailored approach to B cell depletion. While acknowledging the infectious risks of alternative treatment options, monitoring of immunoglobulin levels before each cycle of rituximab allows an individualized risk–benefit analysis.

## B cell depletion therapies beyond rituximab

Several B cell depletion therapies are under development in SLE. These include two types of enhanced monoclonal antibodies that are specific to CD20: type I anti-CD20 antibodies have the ability to reorganize the CD20 molecule into lipid rafts, whereas type II anti-CD20 antibodies affect CD20 distribution in the plasma membrane to a lesser extent. Type I monoclonal antibodies include, in addition to rituximab, ocrelizumab and ofatumumab, which are humanized and fully human, respectively, to ensure reduced immunogenicity. The afucosylated type II monoclonal CD20-specific antibody obinutuzumab, as well as CAR T cells and BiTEs that redirect T cells towards B cell targets, have been developed to achieve deeper B cell depletion. In addition, combination treatment with BAFF inhibition using ianalumab, and plasma cell-directed treatment with the proteasome inhibitor bortezomib or the CD38-specific antibody daratumumab are treatment strategies designed to improve and direct B cell depletion. An overview of the mechanisms of emergent B cell depletion therapies is presented in Fig. 3.

## Type I anti-CD20 antibodies

The chimeric nature of rituximab occasionally induces immune reactivity, and the formation of antidrug antibodies often impairs clinical

	Deep depletion			Reduced relapse of B cell numbers		Addressing plasma cell pathology	
Mode of action	Obinutuzumab	CAR T cell	BiTE	Ianalumab	Daratumumab	Bortezomib	
							
Improvements compared with rituximab	<ul style="list-style-type: none"> <li>Humanized to reduce immunogenicity</li> <li>Afucosylated Fc region to improve FcγRIII affinity</li> <li>Improved direct cytotoxicity to B cells</li> </ul>	<ul style="list-style-type: none"> <li>Targeting CD19 or BCMA, that are more broadly expressed compared to CD20</li> <li>Potentially improved depletion by engagement of T cells</li> </ul>		<ul style="list-style-type: none"> <li>Fully human to reduce immunogenicity</li> <li>Afucosylated Fc region to improve FcγRIII affinity</li> <li>BAFF-R inhibition</li> </ul>		<ul style="list-style-type: none"> <li>Sparing other B cell subsets</li> </ul>	

**Fig. 3 | Emergent B lineage depletion therapies in SLE.** The emergent B cell depletion therapies use various modes of action that confer distinct advantages compared with rituximab for B cell depletion. Deep B cell depletion is attempted through the actions of type II monoclonal antibodies against CD20, such as obinutuzumab, or by chimeric antigen receptor (CAR) T cells and bispecific T cell engagers (BiTEs) that target CD19 or B cell maturation antigen (BCMA). A combination of B cell depletion and inhibition of B cell-activating factor

receptor (BAFF-R) with ianalumab could reduce the relapse of B cell numbers after B cell depletion and potentially prolong the duration of the treatment effect. In patients with evidence of plasma cell involvement and antibody-mediated inflammation, treatment with the CD38-specific monoclonal antibody daratumumab or a proteasome inhibitor such as bortezomib might be more targeted to the pathology of their individual systemic lupus erythematosus (SLE) disease. FcγRIII, Fcγ receptor III.

effects and reduces safety. One humanized (ocrelizumab) and one fully human (ofatumumab) type I monoclonal antibodies are, thus, currently under evaluation in SLE. Ofatumumab is a type I anti-CD20 IgG1 monoclonal antibody that targets both the large and the small external loops of CD20, recognizing epitopes that are distinct from those targeted by rituximab. Ofatumumab was shown to be well tolerated in a single-centre case series, and achieved B cell depletion in 12 out of 14 patients with SLE. In this setting, 6 months after treatment with ofatumumab six of the 12 patients with lupus nephritis had achieved renal remission<sup>75</sup>. Treatment with ofatumumab also showed clinical effects in smaller case series in patients with juvenile SLE or lupus nephritis<sup>76,77</sup>. However, this was not the case for ocrelizumab, a humanized IgG1 monoclonal antibody that recognizes the same epitope as rituximab on the large extracellular loop of CD20. A study evaluating ocrelizumab along with background immunosuppressive therapy in patients with lupus nephritis was terminated early owing to an increased risk of serious infections, some of which were fatal, in patients receiving background mycophenolate mofetil (MMF). At the time of study termination and despite efficient B cell depletion in peripheral blood, renal responses were not significantly better in patients receiving ocrelizumab plus MMF than in those receiving placebo<sup>78</sup>. Thus, following the results of these trials, alternative type I anti-CD20 antibodies have not yet demonstrated promise regarding efficacy.

## Type II anti-CD20 antibodies

Obinutuzumab is a humanized type II monoclonal antibody targeting CD20, and recognizes an overlapping epitope together with rituximab but at a different orientation<sup>79</sup>. Obinutuzumab is less efficient at clustering CD20 into lipid rafts than type I monoclonal antibodies such as rituximab. Although this limits cellular death through CDC, it also reduces internalization of the CD20–obinutuzumab complex, thereby increasing ADCC. In total, this leads to a net improvement in B cell depletion<sup>9,80–82</sup>. In addition, the Fc region of obinutuzumab is afucosylated to improve affinity to FcγRIII on effector cells. This enhances B cell depletion through ADCC<sup>80,82</sup>. In direct comparison with rituximab, obinutuzumab displays improved B cell depletion through FcγRIII-mediated activation of NK cells and ADCC, and obinutuzumab is also more potent in inducing direct cell death<sup>80,82</sup>.

In the NOBILITY phase II randomized controlled trial (RCT), obinutuzumab or placebo infusions were administered during weeks 1, 2, 24 and 26 together with standard-of-care<sup>83</sup>. The addition of obinutuzumab led to rapid depletion of peripheral B cells, including memory B cells, plasmablasts and naive B cells<sup>67</sup>. These biological effects were associated with an increased proportion of patients who reached complete renal response by week 52, together with significantly improved estimated glomerular filtration rate and proteinuria. In a post hoc analysis, obinutuzumab also decreased the risk of lupus nephritis flares<sup>84</sup>. The treatment was well tolerated with similar numbers of adverse events in the treatment group and the placebo group<sup>67</sup>. The REGENCY phase III RCT is a randomized double-blind placebo-controlled trial of the efficacy and safety of obinutuzumab together with MMF and glucocorticoids in lupus nephritis, including a total of 271 patients, that appears to have met its primary end point, as announced in the company's press release<sup>85,86</sup>. Here, a higher proportion of patients were reported to have achieved the primary end point of complete renal response when treated with obinutuzumab compared with patients receiving standard therapy. This indicates that improved B cell depletion may translate to clinical efficacy in SLE, and it may come down to the safety profile of obinutuzumab to determine its place as a novel B cell-targeted treatment strategy in SLE.

## CAR T cells

CAR-expressing T cells combine direct antigen recognition with the effector mechanisms of T cells. Thereby, ordinary immune checkpoints are bypassed, and the CAR T cell is activated in an MHC-independent manner<sup>87</sup>. Adoptive transfer of CAR T cells directed towards the CD19 antigen was approved in 2017 for the treatment of two types of refractory B cell-derived malignancies. Long-term effects vary depending on the type of malignancy, but CAR T cells induced durable remission for a proportion of the patients with the longest follow-up extending over a decade<sup>88</sup>. In SLE, CAR T cells targeting CD19 were first administered in 2021 as part of a compassionate use scheme to a woman with severe and refractory SLE despite several treatments, including rituximab and belimumab<sup>89</sup>. After leukapheresis, autologous T cells were activated and transduced with a lentiviral anti-CD19 CAR vector, expanded *in vitro*, and returned to the patient's blood. As the CAR T cells expanded, B cells were depleted, serology normalized, and clinical remission was achieved.

This first case report was followed by two publications detailing the treatment of a total of eight patients with refractory and multiorgan SLE, four of whom had previously failed rituximab<sup>90,91</sup>. In all patients, drug-free remission was achieved by 6 months after autologous CAR T cell delivery, and remission was accompanied by seroconversion, normalization of complement levels, and disappearance of proteinuria. CAR T cells rapidly expanded until day 9 after transfer and then rapidly declined. B cells were absent a few days after infusion but reappeared after about 100 days. The repopulating B cells were preferentially non-class-switched, whereas the numbers of memory B cells remained reduced and plasmablasts were low to absent. Despite the reappearance of B cells, no clinical relapse occurred during a follow-up period of 6–29 months.

In patients with B cell lymphoma and other malignancies that are treated with CAR T cells, adverse events are common and include cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome (ICANS), cytopenias, hypogammaglobulinaemia and infections<sup>88,92</sup>. In the patients with SLE who have received CAR T cells so far, toxic effects have been mostly mild, possibly attributable to a lower load of B cells in SLE than in B cell-derived malignancies<sup>89–91</sup>. It is noteworthy that long-term follow-up in cancer research has shown a non-trivial risk of non-relapse mortality. In a meta-analysis, around half of the non-relapse mortality cases were caused by infections, but almost 8% were attributed to the development of other malignant diseases<sup>93</sup>. In addition, case reports have described T cell-derived malignancies following CAR T cell therapy<sup>94</sup>. These observations highlight the need for long-term follow-up to ensure maintained remission and safety of CAR T cell therapies in SLE and other autoimmune diseases. The substantially lower mortality in SLE than in malignant disease increases the impact of long-term outcomes on the choice of therapy.

CAR T cells targeting CD19 are currently under evaluation in several phase I and II studies and some new technologies are under very early investigation in SLE, as described below. A case report described a patient with SLE and B cell lymphoma treated with CAR T cells designed to target both CD19 and BCMA<sup>95</sup>. This treatment would be expected to affect plasma cells to a larger degree, as BCMA is predominantly expressed on mature B cells. B cells were depleted but had recovered to normal levels by 9 months after infusion. The disease remained stable until follow-up 23 months after treatment. However, there was a marked reduction in total immunoglobulin levels, and the patient received prophylactic treatment with intravenous immunoglobulin to limit the risk of infection.

Various types of immune cells, including NK cells, can be transformed to produce CAR construct expressing therapies, as demonstrated by CAR regulatory T (T<sub>reg</sub>) cells targeting CD19 that improved serology and delayed lymphopenia in SLE-prone mice<sup>96</sup>. In addition, chimeric autoantibody receptor T cells could be designed to specifically target the B cells that produce autoantibodies involved in disease pathogenesis, a concept established in pemphigus vulgaris that could be applicable to autoantibodies in SLE, such as anti-dsDNA or anti-C1q<sup>97</sup>.

An argument for the use of CAR T cells in autoimmunity is that their cellular mechanism of B cell killing might be superior to that of monoclonal antibodies. In addition, CAR T cells might have improved penetrance to peripheral tissues and secondary lymphoid organs where pathogenic B cells reside<sup>98</sup>. In comparison with rituximab, depletion of peripheral B cells and immunoglobulin levels were similar, but a complete depletion of CD19<sup>+</sup> and CD20<sup>+</sup> B cells was observed in the lymph nodes of patients treated with CAR T cells but not in the lymph nodes of patients treated with rituximab<sup>98</sup>.

CAR T cell therapies have also been suggested to potentially achieve long-term clinical remission<sup>90</sup>. Across patients with malignant diseases that received CAR T cell therapy, at least a proportion have remained in remission for over a decade, despite the reduction in CAR T cell numbers and the B cell repopulation that occur over time<sup>88</sup>. However, this information cannot be directly applied to autoimmune diseases. B cell malignancies are initiated by single expanded B cell clones, and after elimination of such clonotypes it is less probable that the same disease-causing mutation would arise *de novo*. By contrast, SLE involves polyclonal B cell activation directed towards several distinct nuclear and intracellular antigens<sup>15</sup>. Even after initial depletion of autoreactive B cell clones, continuous exposure of such antigens is expected to drive new autoreactive B cell clones in the context of a susceptible host.

In addition, CAR T cell therapies differ from strategies that are based on monoclonal antibodies in terms of patient effort and economic costs. At present, CAR T cells are not produced at scale, and most protocols involve the use of autologous T cells<sup>99</sup>. Pretreatment conditioning, leukapheresis and initial follow-up is a time-consuming process requiring long periods of hospitalization for the patient and large initial costs for the healthcare system. These costs could be mitigated in the long term if CAR T cells survive and expand, creating a pool of T cells with the potential to respond even before disease is clinically evident. In addition, the development of allogeneic CAR T cells could reduce the logistic requirements and improve treatment availability<sup>100</sup>. The first available report of use of allogeneic anti-CD19 CAR T cells in rheumatic disease included one patient with severe myositis and two patients with systemic sclerosis<sup>101</sup>. To prevent rejection and improve persistence, genes for the T cell receptor and certain MHC molecules were knocked out using CRISPR-CAS9-based gene editing. The treatment was well tolerated, disease activity was reduced, and quality of life improved for the three participants. This is a rapidly developing field, and advances in T cell engineering and manufacturing of off-the-shelf CAR T cell products might increase availability and reduce the time requirement for future patients, as well as reduce costs for the healthcare system.

Overall, although the promise of CAR T cell therapy has generated much excitement, the therapy is still nascent, and it needs to be emphasized that no controlled studies have been reported so far. The obvious comparator arm in such studies would be monoclonal antibody-mediated B cell depletion with type I or type II antibodies.

Moreover, key questions must be answered before it can be concluded that CAR T cells are qualitatively better than monoclonal antibodies in terms of B cell depletion or a long-term cure in SLE. These include their comparative efficacy and added value both regarding immunological biomarkers and clinical outcome. In addition, a large, powered study with defined primary and secondary outcomes and long-term follow-up will be essential to understand the safety and efficacy of CAR T cells in SLE.

## Bispecific T cell engagers

Unlike monoclonal antibodies, which recognize only one antigen, T cell-engaging bispecific antibodies simultaneously engage two surface antigens: one on a target cell, and one on an endogenous T cell, which is redirected to the target. BiTEs are small, flexible bispecific antibodies that lack an Fc region. The first approved BiTE still in use, blinatumomab, was used in relapsed or refractory acute lymphoblastic leukaemia<sup>102</sup>. Blinatumomab depletes B cells by connecting two single-chain variable immunoglobulin fragments, recognizing CD19 and CD3ε, respectively<sup>102</sup>. As the B and T cells are brought into proximity, the T cell is activated in a polyclonal manner and secretes perforin that lyses the B cell<sup>103</sup>. Blinatumomab has since been administered in one patient with rapidly progressive systemic sclerosis, with swift improvement of symptoms<sup>104</sup>, as well as in a case series of six patients with severe resistant RA<sup>105</sup>. Circulating B cells, and particularly the CD27<sup>+</sup> memory B cell subset, were reduced and serology improved in all patients with RA who received blinatumomab. Clinically, the numbers of tender and swollen joints decreased, and remission was achieved. In two out of three patients who had a synovial biopsy, blinatumomab completely depleted synovial B cells. This deep depletion may be influenced by the broader range of expression of CD19, which probably leads to additional plasmablast and plasma cell killing with blinatumomab compared with approaches targeting CD20. In addition, the T cell engagement and killing mechanisms of BiTEs are likely to be superior to monoclonal antibody-mediated killing, although this has not yet been proven.

Teclistamab, a BiTE recognizing CD3 and BCMA, also showed efficacy in one patient with active lupus nephritis<sup>106</sup>, as well as in four patients with other rheumatic diseases, including systemic sclerosis, idiopathic inflammatory myositis, primary Sjögren syndrome and RA<sup>107</sup>. During the first few weeks after treatment, B cells were depleted, especially the plasmablast and plasma cell subsets, and serology and disease activity scores normalized. Several of the patients showed grade 1–2 cytokine release syndrome, infections and hypogammaglobulinaemia, side effects that have previously been reported with the use of teclistamab in patients with multiple myeloma<sup>108</sup>.

BiTEs are similar to CAR T cells in that they both require a functional T cell compartment, and several adverse reactions are shared, including cytokine-release syndrome and ICANS. However, similar to monoclonal antibodies, BiTEs are recombinant off-the-shelf proteins without interpatient variability. This gives the advantage of a short time from decision to infusion without the need for patient conditioning. The short half-life of BiTEs also means that continuous treatment might be required for long-term effects to be achieved. Overall, BiTEs have emerged as a promising therapeutic modality in SLE, and further investigations in SLE are planned.

## Combination with BAFF receptor blockade

B cell activation and survival is promoted by BAFF, and BAFF inhibition with belimumab has been shown to reduce disease activity in a

## Glossary

### B cell-activating factor

Also called B lymphocyte stimulator; a potent B cell activator and survival factor that promotes B cell maturation.

### Double-negative B cells

B cells that have class switched and lack expression of IgD but also the memory marker CD27. Of these, DN2 cells have higher expression of CD11c and T-BET and are increased in the circulation in patients with SLE.

### Fcγ receptor III

Activating Fc receptor that mediates interaction between the Fc domain of antibodies and FcγR-bearing effector cells.

### Plasmablasts

A heterogeneous subset of short-lived circulating antibody-producing cells that might lie outside a CD19<sup>+</sup> lymphocyte gate in flow cytometry and can be defined as CD3<sup>+</sup>CD14<sup>+</sup>CD19<sup>+</sup>CD38<sup>++</sup>CD27<sup>+</sup> mononuclear cells.

### Transitional B cells

B cells that have successfully recombined their surface receptor and exited the bone marrow but are not yet fully mature. Depending on their stage of transition, they can be CD24<sup>hi</sup>CD38<sup>hi</sup>.

proportion of patients with refractory SLE<sup>109,110</sup>. A proliferation-inducing ligand (APRIL) shares some cell surface receptors with BAFF and also has a key role in B cell maturation and survival<sup>111</sup>. The BAFF and APRIL inhibitors telitacept and atacicept have both shown some efficacy in phase II studies of SLE<sup>112–114</sup>.

The concentration of BAFF is elevated in patients with SLE after treatment with rituximab, and these increased BAFF levels have been associated with disease relapse and higher disease activity<sup>115–117</sup>. In mice, excess BAFF was able to rescue self-reactive early B cells from deletion and improved survival of new B cells emerging from the bone marrow<sup>118,119</sup>. The BAFF receptor (BAFF-R) is highly expressed on certain B cell subpopulations<sup>13,14</sup>, and combining B cell depletion with BAFF-R inhibition has the potential to delay B cell repopulation and provide longer duration of remission after B cell depletion.

Ianalumab is a human anti-BAFF-R IgG1 monoclonal antibody that combines B cell depletion with blockade of BAFF-R-mediated signaling. Ianalumab is afucosylated, thereby ensuring enhanced depletion through interaction with FcγRIIIb-bearing cells, and can be administered subcutaneously. Ianalumab was not internalized by acute leukaemia B cells, and it showed enhanced depletion of chronic leukaemia B cells by ADCC compared with monoclonal antibodies directed towards CD20 (refs. 120,121). In a phase IIb trial in Sjögren syndrome, ianalumab reduced disease activity and was overall well tolerated<sup>122</sup>. Preliminary interim data from the phase II SIRIUS RCT in SLE demonstrated both efficacy and safety, with a larger number of patients achieving SLE responder index 4 (SRI-4) response with sustained steroid reduction in the treatment arm compared with patients receiving placebo<sup>123,124</sup>.

The combination of B cell depletion and BAFF inhibition with belimumab has been evaluated in several trials. In the CALIBRATE, SYNBIOSE and BLISS-BELIEVE studies, B cell depletion and serology improved<sup>125–127</sup>. Although the CALIBRATE and BLISS-BELIEVE studies did not demonstrate clinical improvement by combination treatment, belimumab was found to reduce the risk of severe flare in the BEAT-LUPUS trial<sup>125,127,128</sup>. Thus, the results of the SYNBIOSE-2 phase III RCT that is currently in progress in patients with anti-dsDNA-positive, severe SLE are anticipated with interest<sup>129</sup>. Indeed, combination treatment

might be particularly relevant in patients who have tested positive for anti-dsDNA autoantibodies and, in particular, for anti-dsDNA antibodies of the IgA2 subtype, as these antibodies have emerged as a relevant predictor for clinical response of belimumab after rituximab<sup>115,130</sup>. In the BLISS-BELIEVE trial, the combination treatment seemed more effective in the subgroup that was anti-dsDNA-positive at baseline<sup>127</sup>. Thus, when there is biological response to rituximab but insufficient depletion, add-on therapy with belimumab might be beneficial, especially in patients with high anti-dsDNA autoantibody titres.

## Targeting plasma cells

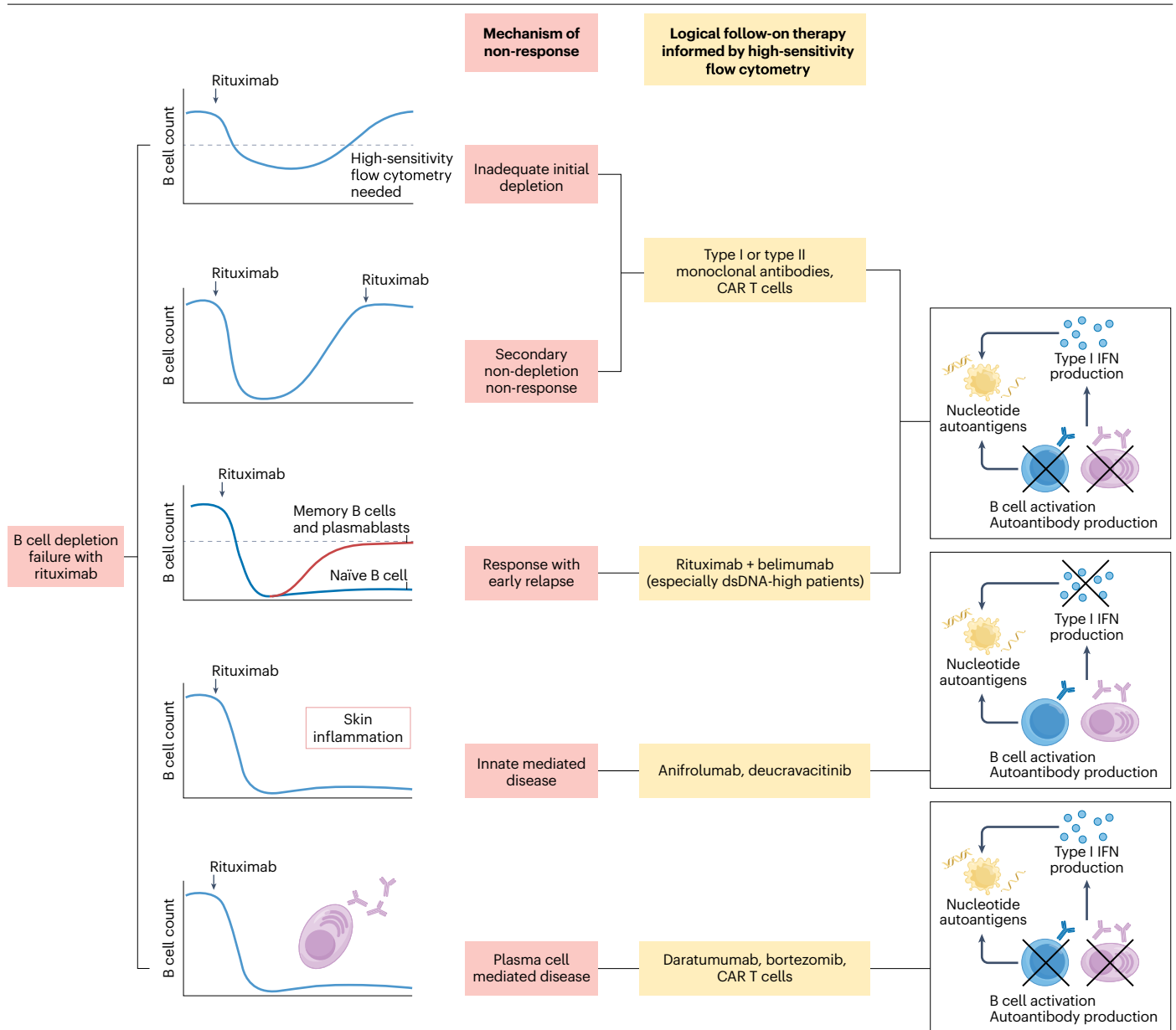
Plasma cells are important for autoantibody production but are unresponsive to anti-CD20-directed B cell depletion. In addition, CD19<sup>low</sup> antibody-secreting cell populations are abundant in patients with active SLE and correlate with disease activity<sup>131</sup>. The maintenance of vaccination responses after both rituximab and CAR T cell treatment suggests that memory B cells and long-lived plasma cells are likely to resist depletion strategies<sup>91,132</sup>. Treatment directed specifically towards plasma cells might be beneficial in certain patients, particularly when there is evidence for antibody-mediated inflammation. Daratumumab is a monoclonal antibody targeting CD38, which is highly expressed on plasmablasts and plasma cells<sup>11,12</sup>. After ligation of CD38, daratumumab induces cell death through ADCC, ADCP and CDC.

A few patients with SLE have been treated with daratumumab thus far. In two patients, daratumumab depleted plasma cells, reduced dsDNA-specific antibodies, and decreased SLE disease activity index scores<sup>133</sup>. In addition, daratumumab decreased type I IFN pathway activation in these patients. The titre of vaccine-induced response to tetanus toxoid declined. Responses were sustained at follow-up 3 years after treatment<sup>134</sup>. These initial results were followed by a case series of six patients with refractory lupus nephritis<sup>135</sup>. Three of these patients receiving daratumumab experienced a complete renal response, whereas two patients had a partial renal response during follow-up until 12 months. Commonly reported adverse events have been hypogammaglobulinaemia, infusion reactions and infections<sup>136</sup>.

Another way to target plasma cells is through proteasome inhibition. The proteasome destroys proteins marked for degradation, and its inhibition cause the accumulation of misfolded proteins which leads to apoptosis<sup>137</sup>. Plasma cells are especially vulnerable to proteasome inhibitors owing to their high rate of protein production during antibody synthesis<sup>137</sup>. Thus, treatment of patients with SLE with the proteasome inhibitor bortezomib led to rapid depletion of plasma cells while other B cells were mostly unchanged<sup>138</sup>. Bortezomib initially showed promise in case series; in one study in lupus nephritis, four out of five patients achieved a complete or partial response, and in three studies, each including 12 patients, disease activity declined and serology improved<sup>138–141</sup>. However, bortezomib displayed significant toxicity, with reduction in total immunoglobulins and vaccination responses, and has frequently been discontinued. In an RCT that included 14 patients, four out of eight in the bortezomib group discontinued treatment due to adverse effects, and although SRI was numerically higher in the treatment group, it was not significantly affected by treatment<sup>142</sup>. Thus, adverse reactions may limit the use of bortezomib, and its initial promise has not been corroborated by subsequent trials.

## Choice of therapy after rituximab failure

In case of treatment failure with rituximab, the choice of subsequent therapy can be guided by the residual B cell count (Fig. 4). This necessitates high-sensitivity flow cytometry and directs treatment towards

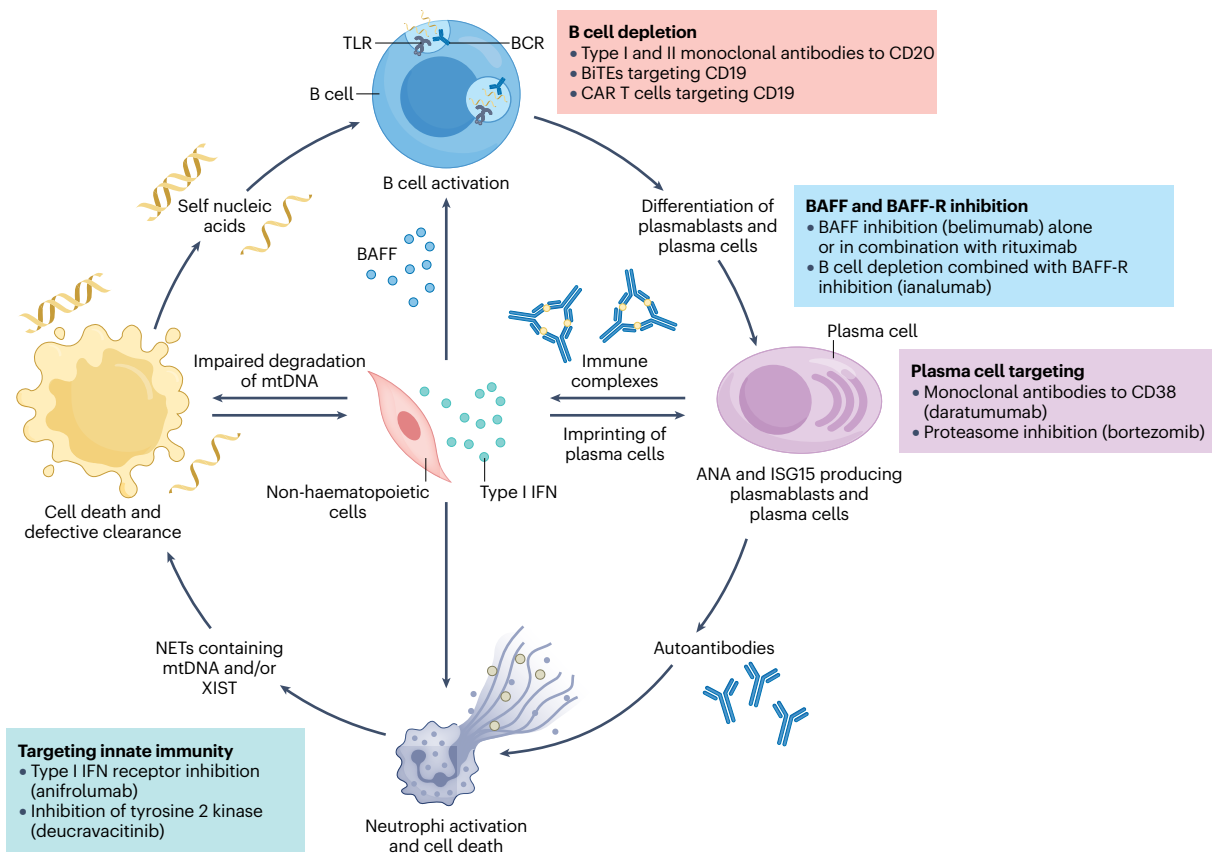


**Fig. 4 | Options after B cell depletion failure with rituximab with currently licensed or off-label drugs.** The choice of therapy after B cell depletion failure with rituximab can be guided by the B cell count. High-sensitivity flow cytometry is needed to measure rare B cell subpopulations and determine residual B cell counts, and might help stratify patients with systemic lupus erythematosus. In case of inadequate initial depletion or secondary non-depletion non-response, the logical follow-on therapy would be to use another type I or II monoclonal

antibody against CD20, such as obinutuzumab, or chimeric antigen receptor (CAR) T cells, to improve depletion. When there is a biological response but early relapse, add-on therapy with the B cell-activating factor receptor inhibitor belimumab might be beneficial, especially in patients with high anti-dsDNA antibody titres. When disease, particularly disease involving skin inflammation, is mediated by innate immune cells or by plasma cells, treatment could be designed to target these pathways instead. dsDNA, double-stranded DNA; IFN, interferon.

the active immunological mechanisms in the individual disease. In case of inadequate initial B cell depletion or 2NDNR, given the prior good response to rituximab, the logical follow-on therapy would be improved depletion with another type I or II monoclonal antibody against CD20. Switching to one of the alternative CD20-targeting monoclonal antibodies ocrelizumab, ofatumumab or obinutuzumab restored depletion and clinical response in patients with 2NDNR<sup>32</sup>. This within-class switch was

more effective than switching to a BAFF inhibitor<sup>61</sup>. In the future, CAR T cells and BiTEs might become options in this treatment arsenal. When there is B cell depletion with early relapse, add-on therapy with belimumab might be beneficial, especially in patients with high anti-dsDNA antibody titres. When B cells are depleted without clinical response, disease may be mediated not by B cells but by innate cells or plasma cells, and treatment could be designed to target these pathways instead.



**Fig. 5 | B cell-intrinsic and B cell-extrinsic mechanisms in SLE pathogenesis.**

Cell death, impaired degradation of extracellular nucleotides, impaired mitophagy of damaged mitochondria, and exposure to ultraviolet light have been associated with presentation of autoantigens to autoreactive B cells in systemic lupus erythematosus (SLE). After binding to their cognate B cell receptors (BCR), autoantigens are taken up in endosomes in the B cell. With simultaneous TLR7 stimulation, the B cell is activated and can differentiate intrafollicularly or extrafollicularly to become an antibody-producing cell. Extracellular nucleotides such as the X-inactive specific transcript (XIST) long non-coding RNA also induce type I interferon (IFN) production from non-haematopoietic cells. Mitochondrial DNA (mtDNA) is highly immunogenic and induces an interferon response, especially in its oxidized form. In most cells, damaged mitochondria are removed by mitophagy, through which an endophagosome sequesters mtDNA and fuses with a lysosome. However, neutrophils are constitutively unable to perform mitophagy and may instead

extrude complexes of mtDNA and protein in neutrophil extracellular traps (NETs)<sup>151,152</sup>. Ribonucleoprotein-containing immune complexes and TLR activation increase NET formation and the extrusion of mtDNA<sup>152–154</sup>. Type I IFN in turn impairs the degradation of mtDNA<sup>155</sup>. Type I IFN and its downstream mediators stimulate B cell-activating factor (BAFF) production and B cell activation, induce B cell differentiation to plasmablasts, and imprint plasma cells. Immune complexes are formed in the presence of sufficient amounts of autoantigen and add another interferonogenic stimulus. Both type I IFN and certain antinuclear antibodies (ANAs) induce neutrophil death through NETosis, through which intracellular autoantigens are exposed, further contributing to the inflammatory vicious circle. Treatments that specifically deplete B cells and plasma cells are depicted, as well as treatments that may supersede B cell depletion, including inhibition of BAFF and innate pathways using currently licensed or off-label drugs. BAFF-R, BAFF receptor; BiTEs, bispecific T cell engagers.

## Further limitations of B cell depletion in SLE

In the larger perspective, a limitation of B cell depletion therapeutic strategies in SLE involves the complex immunology underpinning the SLE disease. First, the autoreactivity of B cells in SLE does not develop in isolation but is induced by TLR7-triggered hyperresponsiveness of the immune system secondary to autoantigen exposure (Box 2). During progression to SLE, the autoantibody repertoire is expanded recognizing a broader range of various intracellular targets, including nucleotides and their binding proteins<sup>143</sup>. The recognized proteins are molecularly dissimilar but share an adjacent intracellular location<sup>144</sup>. Processes that increase the extracellular availability of these autoantigens are likely to precede the accumulating loss of B cell tolerance<sup>145,146</sup>. Accordingly, single-gene variants sufficient to cause severe and typical lupus mainly

affect the removal and sensing of extracellular nucleotides and waste<sup>147</sup>. These initiating and perpetuating processes would be expected to be maintained even after complete B cell depletion.

Second, lupus develops successively over a period of several years, and innate immune mechanisms have a prominent role during early disease development. Indeed, IFN-stimulated genes were found to be upregulated in antinuclear antibody (ANA)-positive individuals who later developed SLE but not in ANA-positive individuals who remained healthy<sup>148</sup>. In these individuals, as in patients with established SLE, autoantibody production is heavily interconnected with the type I IFN system (Box 1).

Together, these studies of the complex non-immune and innate involvement in SLE development are compatible with a model in which

increased availability, elevated sensing of nucleic autoantigens, or both, lead to potent TLR signalling that triggers type I IFN production and extrafollicular B cell differentiation. Once established, innate immune signalling and B cells will stimulate each other, leading to a vicious circle of immune activation and damage in SLE (Fig. 5).

This vicious circle might underly a major limitation of B cell depletion therapies. If polyclonally activated B cells function within a network of cellular interactions and are activated and imprinted by their microenvironment, these innate and non-immune mechanisms would remain present in patients with SLE even in a state of full depletion of B cells<sup>15,149</sup>. If so, this would mean that fundamental pathophysiological mechanisms of SLE are not dependent on B cells and might be unresponsive to B cell therapy.

If this is the case, the degree to which relapse would occur after complete depletion of B cells is unknown. Considering that only a small fraction of individuals with ANA positivity develop SLE, despite widespread immune abnormalities, it is conceivable that a similar small but substantial proportion of patients might relapse after deep B cell depletion with antibody-based or cellular therapy in the future, whereas the majority of patients receiving such treatments might be able to live with stable subclinical autoimmunity<sup>150</sup>. However, the genetic and immune abnormalities that once caused SLE might also eventually lead to relapse in most patients after a brief or longer period of remission. Although B cell depletion may lead to clinical quiescence, it is expected that patients with SLE will remain immunologically complex.

## Conclusions

Rituximab efficacy has been long known to depend on the depth and duration of B cell depletion. Improved B cell depletion mechanisms of therapeutics that are based on monoclonal antibodies, such as obinutuzumab and ianalumab, or on B cell targeting by T cells (for example using BiTEs or CAR T cells), are likely to translate into clinical efficacy. The multifaceted nature of SLE means that there will probably be occasion for several different treatment options and argues for personalized treatment according to the underlying immunological networks that are activated in individual patients with SLE. Predictive biomarkers that enable us to choose the treatment option with the highest likelihood of improvement for the clinical and immunological disease pattern of the individual patient, balancing short-term and long-term cost, toxicity and patient effort in the process, will be required for aiding clinical decisions.

However, the extent to which the various treatment options will be used remains unclear. So far, there are few direct comparisons between the treatment modalities regarding immunological and biological effects and no head-to-head trials comparing clinical efficacy. Planned studies of B cell depletion agents seldom compare them with existing monoclonal antibody-mediated B cell depletion regarding biomarkers or clinical outcome.

While awaiting head-to-head trials, targeting the active immunological pathways of the individual disease may have promise for optimizing treatment in each patient, given the complex nature of the SLE disease. Measuring B cell subpopulations might be relevant to evaluating disease activity, determining the immunological efficacy of B cell depletion and aiding in the decision to continue or discontinue B cell depletion therapy. To guide therapeutic B cell targeting in patients with SLE, a thorough understanding of the pathophysiology of B cells in relation to other immune abnormalities in SLE and of the limitations of B cell depletion therapies are required. The clinician's interpretation of how B cells are activated in SLE determines how we

predict if B cell depletion therapies will work. If B cells are intrafollicularly activated by T cell interactions resulting in isolated clones of intrinsically autoreactive cells, then depleting these clones deeply enough could cure the disease. However, if B cells are polyclonally and extrafollicularly activated, a long-term cure for SLE will probably need to equally target B cell-driven, innate and non-immune mechanisms.

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## Author contributions

All authors researched data for the article. All authors contributed substantially to discussion of the content. M.S. wrote the article. All authors reviewed and/or edited the manuscript before submission.

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