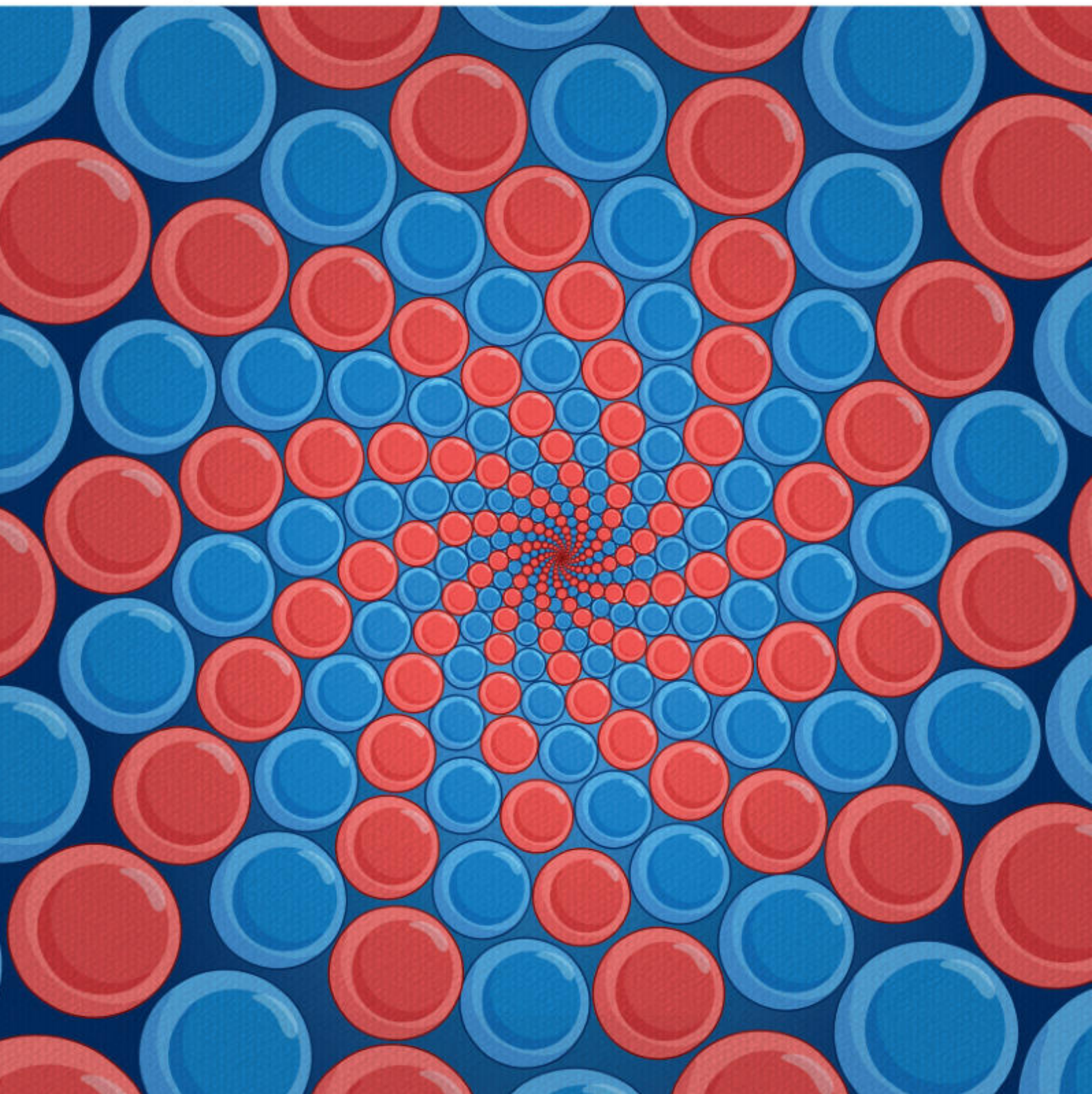


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Research highlights

Rheumatoid arthritis

A subset of ITGA5⁺ synovial fibroblasts alter the inflammatory niche in RA



Pro-inflammatory synovial fibroblasts contribute to chronic inflammation in joints in rheumatoid arthritis (RA); however, there are currently no approved therapies that target fibroblasts in RA. Therapeutic targeting of these cells requires an in-depth understanding of fibroblast heterogeneity in disease. A study by Zheng et al. provides a single-cell and spatial atlas of synovial cells in individuals with RA and identifies a subset of activated sub-lining fibroblasts that express integrin alpha 5 (ITGA5).

ITGA5⁺ fibroblasts had a distinct transcriptome signature (*POSTN*, *COL3A1*, *CCL5* and *TGFBI*), expressed podoplanin, and were enriched for numerous immunoregulatory pathways. The ITGA5⁺ fibroblast population was expanded in both active and lympho-myeloid type RA. The presence of ITGA5⁺ fibroblasts was more prominent in patients with treatment-refractory RA, indicating a potential link to multidrug resistance.

ITGA5⁺ fibroblasts promoted the migration of naive CD4⁺ T cells to the joint via the CCL5–CCR4 axis and induced the differentiation of these cells to PD-1^{hi}CXCL13⁺ T effector

cells via TGFβ1. PD-1^{hi}CXCL13⁺ T effector cells resembled a previously reported T helper cell subset that expressed CXCL13, IL-21, ICOS and MAF, and could promote B cell responses in individuals with RA. Therefore, the authors hypothesized that ITGA5⁺ fibroblasts might be promoting pathogenic T cell responses in the joint.

In a mouse model of collagen-induced arthritis, ITGA5⁺ fibroblasts were found at the early stages of disease and expressed mediators associated with cartilage degradation. Intra-articular injection of ITGA5⁺ fibroblasts led to higher disease activity and increased the differentiation of PD-1^{hi} CXCL13⁺ T cells compared with injection of ITGA5⁻ fibroblasts. A similar increase in disease activity occurred with intra-articular injection of TGFβ1.

When discussing the next steps for their research, Ziji Zhang, Puyi Sheng and Sui Peng, the corresponding authors of this study, note “Our future research will focus on the specific mechanisms that drive the differentiations of ITGA5⁺ synovial fibroblasts and will test targeting ITGA5⁺ synovial fibroblasts as a therapeutic intervention in RA”.

Holly Webster

Original article: Zheng, L. et al. ITGA5⁺ synovial fibroblasts orchestrate proinflammatory niche formation by remodelling the local immune microenvironment in rheumatoid arthritis. *Ann. Rheum. Dis.* <https://doi.org/10.1136/ard-2024-225778> (2024)

Related article: Rao, D. et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. *Nature* **542**, 110–114 (2017)

Osteoarthritis

Wnt-induced IGF1 drives OA

The results of a new study reveal that canonical β-catenin-dependent Wnt signalling induces the expression of insulin-like growth factor 1 (IGF1) in articular chondrocytes, and that IGF1 promotes the development and progression of osteoarthritis (OA). The findings suggest that reducing IGF1 could be a therapeutic strategy for OA.

The link between Wnt signalling and IGF1 was identified through analysis of genome-wide transcriptome datasets of human articular chondrocytes and validation experiments. The researchers then showed that Wnt signalling activation induced IGF1 gene and protein expression in articular chondrocytes, and that *Igf1* is a direct target gene of Wnt signalling in these cells.

In vivo, cartilage-specific deletion of *Igf1* protected male mice from osteoarthritic joint damage downstream of Wnt signalling hyperactivation. *Igf1* deletion in cartilage was also protective in a mouse model of human post-traumatic OA in which Wnt signalling is upregulated in the joint after injury.

In articular chondrocytes from patients with OA, in which increased Wnt signalling activation is observed, silencing of *IGF1* using small interfering RNA restored a healthy molecular profile, marked by increased expression of *COL2A1* and *ACAN* and decreased expression of the disease-associated markers *MMP13*, *ADAMTS4* and *ADAMTS5*.

Sarah Onuora

Original article: Escribano-Núñez, A. et al. IGF1 drives Wnt-induced joint damage and is a potential therapeutic target for osteoarthritis. *Nat. Commun.* **15**, 9170 (2024)

Epidemiology

DADA2 prevalence in China

The rare autosomal recessive autoinflammatory disease DADA2 was first described in 2014, and 116 pathogenic and likely pathogenic variants of the causal gene *ADA2* have been identified since. Yan et al. have now screened available exome sequencing data for *ADA2* variants to estimate the frequency of genetic carriers and the prevalence of DADA2 in China.

Analysis of data from 69,413 individuals, excluding persons with a DADA2 diagnosis, identified 30 exon variants that had been previously associated with DADA2, as well as 8 yet unknown loss-of-function mutations and 12 missense mutations that were newly shown in vitro to reduce the *ADA2* enzymatic activity by over 25%.

Moreover, the authors estimated an *ADA2* variant carrier frequency of 1.05% and a DADA2 prevalence of 1 in 92,251 in the Chinese population, both values being higher than those estimated in other populations. Future studies with improved regional representation and larger sample sizes will help further elucidate the epidemiology of DADA2 in China. However, the authors suggest that their estimated DADA2 prevalence in China already supports screening for *ADA2* variant carriers to facilitate early diagnosis and effective therapeutic targeting in paediatric patients. **Maria Papatriantafyllou**

Original article: Yan, L. et al. Carrier frequency and incidence estimation of deficiency of adenosine deaminase 2 in the Chinese population based on massive exome sequencing data. *Clin. Immunol.* **269**, 110394 (2024)

Related article: Goldbach-Mansky, R., Alehashemi, S. & de Jesus, A. A. Emerging concepts and treatments in autoinflammatory interferonopathies and monogenic systemic lupus erythematosus. *Nat. Rev. Rheumatol.* <https://doi.org/10.1038/s41584-024-01184-8> (2024)

Rheumatoid arthritis

A lung–joint axis in smokers with RA?

Cigarette smoking is a risk factor for rheumatoid arthritis (RA), especially in individuals who share variants of the hyper-variable region of HLA-DR β chain that are known as ‘shared epitope’ alleles. An enhancement of protein citrullination in smokers has been suggested as underlying the emergence of anti-citrullinated protein antibodies (ACPAs) and, potentially, of RA, but the mechanisms by which smoking might break T cell tolerance to drive joint inflammation remain unclear.

Venken et al. addressed this by analysing the single-cell T cell receptor (TCR) repertoires and phenotypes of T cells isolated from lung and synovial samples of treatment-naïve individuals newly diagnosed with ACPA positivity and early RA. “This was achieved by a joint coordinated effort between the rheumatology and pneumology departments in a one-day procedure including bronchoalveolar lavage upon bronchoscopy, venipuncture and ultrasound guided synovial biopsy sampling of inflamed joints”, notes first author Koen Venken.

TCR repertoire analyses in samples from 17 patients – 10 smokers and 7 non-smokers – indicated that the TCR sequences of both CD4 $^+$ and CD8 $^+$ T cells are more likely to be shared between the lung and the joint in smokers than in non-smokers, and this effect was even more prominent across smokers with shared epitope alleles. Use of the GLIPH clustering algorithm further predicted that shared lung and synovium clonotypes might recognize the same antigens across smokers, indicating that smoking might initiate the expansion of T cells that respond to public epitopes.

But how would T cell clones that are primed in the lungs as a result of cigarette smoking initiate an autoimmune response in the joints? Bulk TCR β sequencing of matched blood samples suggested that lung-primed T cell clones pass through the circulation before reaching the joints. Moreover, phenotypic analysis of shared T cell clones from the lung and synovium of smokers with RA indicated that these cells acquire an effector phenotype in the joints that is characterized by expression of CD57 – a biomarker previously associated with RA severity – and high expression of CD137 (also known as 4-1BB) and low expression of PD1 in CD4 $^+$ T cells and high expression of CD38 and HLA-DR in CD8 $^+$ T cells. “One of the most striking observations was that shared clonotypes exhibited a particular phenotype” notes Dirk Elewaut, last author of the article.

“This is an amazing study showing strong evidence of lung–joint linkage in the pathogenesis of RA, and suggestive evidence of epitope-driven expansions”, notes Matthew Brown, who also investigates TCR repertoires in rheumatic diseases. “By studying patients with early disease and reducing experimental heterogeneity through a focus on small joint samples and patients with a common environmental exposure, the investigators have shown a way forward to potentially identifying RA-associated TCR expansions and the mechanism by which HLA-antigens drive the disease”, Brown adds. **Maria Papatriantafyllou**

Original article: Venken, K. et al. Shared lung and joint T cell repertoire in early rheumatoid arthritis driven by cigarette smoking. *Ann. Rheum. Dis.* <https://doi.org/10.1136/ard-2024-226284> (2024)

Inflammatory arthritis

Impaired adipocyte-mediated regulation of fibroblasts in inflammatory arthritis

In the healthy synovium, in which fibroblasts maintain tissue homeostasis, adipocytes are abundant; however, in inflammatory arthritis, adiposity is lost and fibroblasts mediate joint inflammation and degradation. A study now identifies a link between adipocytes and fibroblasts in the healthy synovium that is lost in disease.

Single-cell RNA sequencing of synovial tissue from healthy individuals and those with rheumatoid arthritis (RA) or osteoarthritis (OA) revealed a gene signature in healthy synovial samples, but not in RA or OA samples, that was characterized by enhanced lipid transport and fatty acid metabolism. Interestingly, treating fibroblasts from individuals with RA with fat-conditioned medium upregulated the gene signature observed in healthy fibroblasts.

The authors then sought to identify the factors in the fat-conditioned medium that could drive this upregulated gene expression. Analysis revealed that the endogenous glucocorticoid cortisol was present in the fat-conditioned medium, and stimulating RA fibroblasts with cortisol (but not other related steroids) could induce the expression of the gene signature in these cells. Inhibiting glucocorticoid signalling using a glucocorticoid receptor antagonist in fibroblasts treated with fat-conditioned medium caused downregulation of the gene signature, which indicates that cortisol and glucocorticoid signalling are required for the gene signature associated with healthy fibroblasts.

In a mouse model of inducible adipocyte depletion, loss of adipocytes correlated with loss of expression of *Hsd11b1* (which encodes the enzyme that converts cortisone into cortisol); in addition, fibroblasts in the adipocyte-depleted joint became activated, indicating that adipocytes generate cortisol in the joint and regulate fibroblast activation. Mice lacking *Nr3c1* (which encodes the glucocorticoid receptor NR3C1) were also more susceptible to inflammatory arthritis than genotype control mice. However, in a serum transfer mouse model of inflammatory arthritis, depletion of adipocytes did not confer protection against arthritis development.

The addition of fat-conditioned medium could abrogate TNF- and TGF β -mediated extracellular matrix remodelling in vitro. By contrast, the addition of high concentrations of TNF, TGF β or IFN γ (cytokines that are upregulated in OA and RA) to fibroblasts treated with fat-conditioned medium reduced cortisol signalling; IFN γ and TGF β could also suppress adipocyte differentiation.

Overall, data from this study demonstrate that in inflammatory arthritis, loss of adipocytes and subsequent loss of cortisol signalling could contribute to the activation and pathogenic phenotype of fibroblasts in the joint.

Holly Webster

Original article: Faust, H. J. et al. Adipocyte associated glucocorticoid signaling regulates normal fibroblast function which is lost in inflammatory arthritis. *Nat. Commun.* **15**, 9859 (2024)

Defining axial involvement in juvenile SpA

Denis Poddubnyy

 Check for updates

New classification criteria for axial disease in juvenile spondyloarthritis aim to enhance the identification and study of this condition in affected youth, offering a tool for future non-interventional studies and interventional trials. Better understanding of the efficacy of various interventions in the axial domain could help tailor treatment strategies.

REFERS TO Weiss, P. F. et al. Classification criteria for axial disease in youth with juvenile spondyloarthritis. *Arthritis Rheumatol.* <https://doi.org/10.1002/art.42959> (2024).

An international clinical expert panel has now introduced the first validated classification criteria designed specifically for axial disease in youth with juvenile spondyloarthritis (SpA)¹. This important advancement addresses the critical need for well-defined criteria to accurately classify and study axial involvement in juvenile SpA, a condition that often progresses to adult axial SpA with substantial long-term consequences. The development of these criteria was necessitated by the lack of data supporting the efficacy of non-pharmacological and pharmacological treatments, including biologic and targeted synthetic DMARDs, in patients with juvenile SpA and axial manifestations. Importantly, the outcomes experienced by patients with axial involvement and juvenile arthritis are often worse than the outcomes of those without axial involvement^{2–4}, which highlights the need for specialized criteria for use in clinical trials.

The response of axial disease might be discordant with the response of peripheral manifestations (such as arthritis, enthesitis and dactylitis) and extra-musculoskeletal manifestations (such as acute anterior uveitis, inflammatory bowel disease and psoriasis). This lesson has been learned from studies in adult indications, including negative results of IL-23 blockade in axial SpA^{5,6} and of IL-17 blockade in Crohn's disease⁷ suggesting that efficacy in one disease manifestation does not necessarily mean efficacy in all other domains. Notably, in adult rheumatology, despite the existing general criteria for axial SpA and psoriatic arthritis, an initiative related to the development of criteria specifically for axial involvement in psoriatic arthritis is ongoing⁸. However, data from adult studies cannot be directly translated to juvenile SpA. Furthermore, adult classification criteria for SpA are unsuitable for juvenile arthritis, owing to differences in clinical presentation and imaging features.

To develop these new criteria for axial disease in juvenile SpA, an international panel of experts worked through several phases, including item generation, reduction and validation. The final criteria are grounded in seven weighted domains (Box 1), with a particular emphasis on imaging, which includes both active inflammation and structural lesions as the most heavily weighted factors. Other domains reflect pain chronicity, pain pattern and location, the presence of morning

stiffness and the presence of genetic predisposition. These criteria are designed for use in research settings to ensure that study populations are more homogeneous, thereby improving the reliability of data from clinical trials. Importantly, although imaging features are necessary to classify patients – by providing an objective confirmation of axial disease – they are not sufficient without any clinical parameters. This distinction is crucial from a clinical perspective, as it ensures that classification relies on both imaging and relevant clinical features.

The criteria are applicable to youth with a physician diagnosis of juvenile SpA with axial involvement. They incorporate key features of SpA, including symptoms suggestive of inflammatory back pain, a good response to NSAIDs, family history, HLA-B27 status and MRI signs of inflammatory involvement of sacroiliac joints. As part of the criteria development process, MRI criteria for inflammatory and structural lesions in sacroiliac joints were developed⁹ that represent an important step towards unified definition of imaging features of axial involvement in juvenile SpA. Radiographic sacroiliitis received a substantially lower weight than the structural changes on MRI, given the challenges associated with interpreting sacroiliac radiographic images in children¹⁰.

The relevance of this work extends beyond immediate applications to studies. By providing a robust tool for classification, these criteria pave the way for targeted therapeutic studies in juvenile populations, addressing the current gap in clinical trials for this group. The methodology of this study, which leverages multi-criteria decision analysis and consensus among experts, highlights the complexity of classifying juvenile SpA, the phenotypic presentation of which can differ substantially from adult-onset disease. This work is a crucial step forward in understanding and treating juvenile SpA, with the potential to influence clinical practice and research.

Although the criteria had high specificity (97.5%) and moderate sensitivity (64.3%) in validation cohorts, the authors acknowledge some limitations, including the potential for misclassification in clinical practice, owing to the stringent requirements for imaging evidence of axial involvement in juvenile SpA. Similarly to adult SpA, there is a need for the education of specialists dealing with paediatric SpA in terms of imaging interpretation. Nonetheless, the balance between sensitivity and specificity of the criteria discussed here is acceptable, given that the primary aim of classification criteria is to build a homogeneous group of patients for research purposes. The authors correctly underscore the importance of differentiating between diagnostic criteria and classification criteria, particularly in the context of clinical trials, in which the goal is to include participants with a high likelihood of having the disease.

Future research might result in the further refinement of these criteria as new data emerge (similar to the current process in the classification criteria for adult axial SpA), particularly data on axial involvement in juvenile SpA derived from non-interventional and interventional cohort studies. The integration of these criteria into clinical trials will help identify the most effective treatments for this challenging condition and could ultimately improve outcomes for young patients. Overall, this development marks an important step towards better treatment strategies; studies using these criteria are eagerly anticipated.

Box 1 | Domains and weighted criteria for axial juvenile SpA

Seven domains and weighted criteria are included in the final classification set. A total score of 55 or higher is the threshold described for classification as axial juvenile spondyloarthritis (SpA).

Presence of active inflammation on imaging

- Maximum score of 23 based on the presence of active lesions in the sacroiliac joints.

Presence of structural lesions on imaging

- Maximum score of 23 and can vary based on evidence of sacroiliitis or sacroiliitis associated lesions in the presence or absence of MRI.

Chronic pain

- Maximum score 9 and this can be lower based on the duration of pain.

Pattern of pain

- Maximum score of 10 depending on factors such as sleep disruption, pain onset and severity, activity related pain improvement and pain relief from NSAIDs.

The location of pain

- Maximum score of 12 and can vary depending on the affected area(s) of the body.

Morning stiffness

- Maximum score of 9 and varies depending on the duration of morning stiffness.

Genetic predisposition

- Maximum score of 11 and varies depending on the presence of and factors associated with HLA-B27 and SpA.

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Competing interests

D.P. has received research support from AbbVie, Eli Lilly, MSD, Novartis, Pfizer, consulting fees from AbbVie, Bristol-Myers Squibb, Eli Lilly, Janssen, Moonlake, Novartis, Pfizer and UCB, and speaker fees from AbbVie, Canon, DKSH, Eli Lilly, Janssen, MSD, Medscape, Novartis, Peervoice, Pfizer and UCB, and is a member of the Assessment of SpondyloArthritis International Society executive committee and of the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis steering committee.

EULAR and PReS bridge the age gap in Still's disease

Qiongyi Hu & Chengde Yang

 Check for updates

EULAR and the Paediatric Rheumatology European Society (PReS) now view systemic juvenile idiopathic arthritis and adult-onset Still's disease as a single disease – Still's disease – given their overlapping biomarkers, clinical manifestations and complications. This consensus provides valuable insights into Still's disease diagnosis and management across age groups, and also highlights research priorities.

REFERS TO: De Matteis, A. et al. Systemic juvenile idiopathic arthritis and adult-onset Still's disease are the same disease: evidence from systematic reviews and meta-analyses informing the 2023 EULAR/PReS recommendations for the diagnosis and management of Still's disease. *Ann. Rheum. Dis.* <https://doi.org/10.1136/ard-2024-225853> (2024) & Fautrel, B. et al. EULAR/PReS recommendations for the diagnosis and management of Still's disease, comprising systemic juvenile idiopathic arthritis and adult-onset Still's disease. *Ann. Rheum. Dis.* <https://doi.org/10.1136/ard-2024-225851> (2024).

Systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still's disease (AOSD) are rare polygenic autoinflammatory disorders that present with polyarthritis and systemic inflammation and have severe complications, including macrophage-activation syndrome (MAS) and lung disease. sJIA and AOSD have distinct age ranges of onset, and their diagnosis and management have traditionally been performed according to separate criteria¹. As with other rare disorders, the identification of diagnostic biomarkers and targeted therapeutics for sJIA and AOSD has been constrained by the limited sizes of patient cohorts, and patient enrolment in clinical trials has been challenging. Importantly, the substantial clinical heterogeneity and diverse prognostic and treatment response profiles that are observed in cohorts of patients with sJIA or AOSD further complicate clinical research.

In September 2024, EULAR and PReS published – on the basis of evidence from systematic reviews and meta-analyses – the consensus that sJIA and AOSD are manifestations of the same disease: Still's disease². Concurrently, EULAR and PReS presented a consensus-based set of recommendations for the diagnosis and management of Still's disease, including the management of difficult-to-treat Still's disease or any Still's disease-associated life-threatening complications (collectively called the 2023 EULAR and PReS recommendations). Moreover, this consensus set priorities in the Still's disease research agenda³ (Fig. 1).

The previous classification of sJIA and AOSD as two distinct entities within the spectrum of autoinflammatory diseases has been debatable, especially in the absence of studies that compare large patient cohorts.

The most recent systematic review performed by members of EULAR and PReS indicates that there are no substantial differences between sJIA and AOSD in their clinical manifestations, except for the fact that myalgia, sore throat and weight loss are more common in AOSD than in sJIA², possibly because such symptoms are less likely to be accurately reported in children. Moreover, the Yamaguchi criteria, which had been initially proposed for the diagnosis of AOSD, have also shown high sensitivity in patients with sJIA⁴, which indicates that sJIA and AOSD might be eligible for common diagnostic criteria. Thus, a future set of common classification criteria might include both the Yamaguchi criteria and clinical manifestations that can be recognized in a timely way in paediatric patients.

EULAR and PReS recommend the use of IL-18, S100A8 and S100A9 as biomarkers for the diagnosis of Still's disease. However, caution is needed if relying solely on these biomarkers, as IL-18, S100A8 and S100A9 are also detectable in patients with sepsis and monogenetic autoinflammatory disease. In addition, the 2023 EULAR and PReS recommendations suggest the use of IL-18 and CXCL9 as diagnostic biomarkers for MAS secondary to Still's disease, as recommended in the 2022 EULAR and ACR management guidelines for hemophagocytic lymphohistiocytosis (HLH) or MAS⁵, which were based mainly on data from patients with sJIA-associated MAS. Detailed studies comparing the levels of IL-18 and CXCL9 in AOSD-associated MAS are still needed to exclude any age-specific fluctuations. Other biomarkers that were recommended by EULAR and PReS in 2023, such as adenosine deaminase 2 activity, activated CD38^{high} or HLA-DR⁺ CD8⁺ T cells and CD4^{dim}CD8⁺ T cells, have not yet undergone multi-centre validation or sufficient testing against disease controls, and further studies are needed before they are established as biomarkers for the diagnosis and monitoring of Still's disease.

The 2023 EULAR and PReS recommendations are the first to suggest potential therapeutic targets in Still's disease, with a specific focus on achieving clinically inactive disease and remission. Additionally, these recommendations emphasize the need for patient and caregiver involvement in the decision-making process, and the crucial role of expert centres in assessing and treating refractory patients. The recommended use of biologic DMARDs such as IL-1 or IL-6 inhibitors in early-stage disease might help achieve rapid disease inactivation and remission after medication. In addition, EULAR and PReS recommend the use of high-dose glucocorticoids, inhibitors of IL-1 or interferon- γ signalling, and cyclosporin in patients who develop MAS³. However, the frequency of relapse after treatment with the IL-6 receptor-blocking antibody tocilizumab seems to be higher in patients with paediatric-onset Still's disease than in those with adult-onset Still's disease⁶. Thus, the drugs available that target IL-18, interferon- γ or JAK, which have all emerged as being involved in disease pathophysiology, might hold therapeutic promise in Still's disease^{7,8}.

Given the phenotype heterogeneity of Still's disease, including the heterogeneity of clinical symptoms, complications, disease clusters and prognoses, early diagnosis and timely intervention remain challenging,

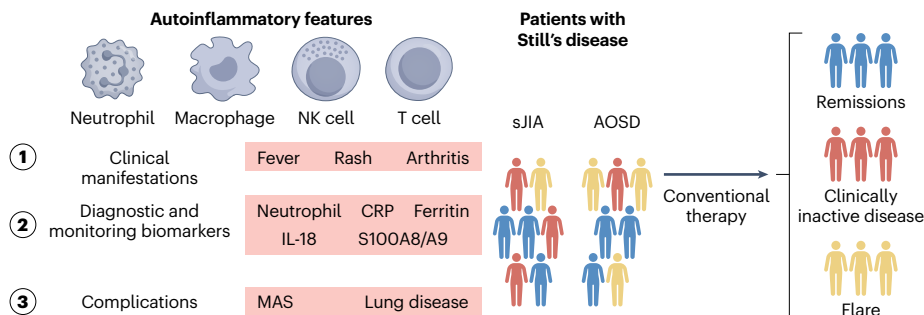


Fig. 1 | Summary of EULAR and PReS recommendations for sjIA and Still's disease. AOSD, adult-onset Still's disease; CRP, C-reactive protein; MAS, macrophage-activation syndrome; NK cell, natural killer cell; S100A8/A9, inflammatory biomarkers; sjIA, systemic juvenile idiopathic arthritis.

particularly in patients who develop MAS, as the early diagnostic effectiveness of the primary diagnostic criteria of MAS, MAS-2016 and HLH-2004 criteria⁵ is suboptimal. The biomarkers included in the HLH-2004 criteria, such as natural killer cell activity and soluble levels of CD25 in the blood, are time-consuming and complex to measure. Moreover, leukopenia and hemophagocytic phenomena sometimes become apparent only in the later stages of the disease. In the early stages, patients often do not exhibit typical symptoms, yet they can deteriorate rapidly. This complexity is compounded by the fact that the underlying pathogenesis is still partly unclear.

The consideration of sjIA and AOSD as a single disease entity is expected to help clinicians and researchers include increased numbers of patients in future studies. Only if a large team of paediatric and adult rheumatologists joins forces to investigate the common mechanisms underlying disease occurrence and progression can the development of unified classification and diagnostic criteria, as well as the application of new targeted therapeutics, be accelerated to ensure both early diagnosis and effective patient management.

In addition to accelerating diagnosis and targeted treatment, the recommended consideration of a Still's disease continuum might help expand translational research, as the joint genetic analysis of patients with sjIA and patients with AOSD might elucidate genetic predispositions associated with Still's disease. Although the genetic backgrounds of sjIA and AOSD seem to differ at present, both disease entities share risk loci associated with HLA class II (including risk variants of HLA-DRB1, HLA-DQA1 and HLA-DQB1)⁹. Given the polygenic involvement of Still's disease, the contribution of non-familial and non-genetic factors to the risk of disease also needs to be considered in the future. Transcriptomic, proteomic, metabolomic and genomic analyses of combined cohorts of patients with sjIA and AOSD might help identify new molecular targets in disease pathogenesis, which might potentially also enable personalized treatment strategies in Still's disease.

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Competing interests

The authors declare no competing interests.

Revisiting the heterogeneity of interferon-related autoimmune diseases

Guillermo Barturen & Marta E. Alarcón-Riquelme



The identification of shared molecular mechanisms across systemic inflammatory autoimmune diseases with overlapping clinical manifestations has prompted research into the underlying genetics that could be driving these manifestations; elucidating these genes could aid in the diagnosis, treatment and outcome prediction of these complex diseases.

REFERS TO Bianchi. M. et al. Unraveling the genetics of shared clinical and serological manifestations in systemic inflammatory autoimmune diseases. *Arthritis Rheumatol.* <https://doi.org/10.1002/art.42988> (2024).

Since the advent of the genome-wide association studies (GWAS) era, researchers in the fields of inflammation and autoimmune diseases have recognized the shared genetic predisposition across various clinical diagnoses¹. In fact, molecular advances in the past 3 years have highlighted the extent to which the molecular mechanisms underlying inflammatory conditions are inter-related and could be triggered by similar pathogenic pathways in different subgroups of patients, even from distinct clinical entities². Furthermore, an increasing number of publications are pointing to the molecular heterogeneity within these clinical entities, which might help explain the limited success rates of prescribed treatments. A publication from Bianchi et al.³ investigates the interplay between autoantibodies and genetic factors across various clinical entities, including systemic lupus erythematosus (SLE), primary Sjögren syndrome and idiopathic inflammatory myopathies (also known as myositis); all three diseases share a molecular signature that is driven by type I interferon, known as the interferon-stimulated gene molecular signature.

Notably, although drugs target particular molecular processes, they are often prescribed on the basis of clinical and laboratory findings, and many studies that aimed to unravel the molecular heterogeneity of these diseases have failed to establish a clear relationship between specific molecular signatures and clinical outcomes^{1,4}. For example, it remains unclear how the same inflammatory pathways – such as neutrophil-related signatures found in subgroups of patients with SLE – manifest in different organs. In lupus nephritis, the kidneys are affected, whereas in cutaneous lupus, the skin is the primary target, but patients from both subgroups of lupus present with the same neutrophil-related signature⁵.

To some extent, molecular quantitative trait loci approaches have begun to elucidate the relationship between genetics and other molecular omics⁶. However, these analyses are primarily designed to

identify genetic factors that predispose individuals to the activation of certain molecular mechanisms involved in autoimmunity, rather than determining which organs are likely to be affected by the infiltration of autoreactive immune cells, which leads to irreversible tissue damage. Thus, the study by Bianchi et al.³ is important, as the authors aimed to understand how autoantibodies and genetic factors contribute to a predisposition for organ-specific damage. Moreover, the study used a targeted DNA-sequencing approach, a much deeper sequencing approach than the genotyping and imputation approaches that have been used in previous studies. Direct sequencing of genes encoding key immunoregulatory molecules and their *cis*-regulatory regions enabled the researchers to investigate low-frequency variants, for which imputation accuracy is typically low, as well as non-coding regulatory regions of crucial immune genes. This method enables the study of genetic regions often overlooked in standard GWASs and could help address the missing heritability of these diseases.

The study included analysis of three well-characterized systemic autoimmune diseases in a Scandinavian cohort of 2,292 patients with SLE ($n = 935$), primary Sjögren syndrome ($n = 906$) or idiopathic inflammatory myopathies ($n = 451$) and 1,252 healthy individuals. Overall, case-control gene-based aggregate association analyses stratified by autoantibody specificity and positivity and clinical manifestations confirmed the established contributions of numerous genes within the MHC region, as well as well-known non-MHC interferon-related genes, to both autoantibody production and the involvement of clinical domains. The case-case analyses were more interesting, as they validated the triad of interferon, MHC and antinuclear antibody positivity that has been very well described and contrasted in the three clinical entities included in the analysis^{7,8}, and also reported two additional key findings. First, the authors identified associations between eight non-MHC genes (*CXCL5*, *IL17REL*, *ISGL1*, *JUN*, *PRM3*, *RXRA*, *TH* and *TOGARAM2*) and the presence of anti-double-stranded DNA (dsDNA) antibodies. Second, Bianchi et al. observed that several non-MHC genes, particularly *DUSP1*, were exclusively associated with the involvement of the musculoskeletal and cutaneous domains, whereas no statistically significant associations were found between MHC genes and these domains³. Notably, this is the first time that genetic associations with anti-dsDNA antibody positivity have been identified, although previous attempts have been made with no clear validation⁹. As the presence of anti-dsDNA antibodies is regarded as a biomarker of disease activity, the question arises of whether this biomarker should be considered a genetic association in patients with active disease.

When discussing the lack of MHC associations with anti-dsDNA antibody positivity, the authors suggest that it could be attributed to the predominance of patients with SLE within the anti-dsDNA antibody-positive subgroup and the inherent heterogeneity of this population. The authors also propose that anti-dsDNA antibody positivity could be driven, at least in part, by genetic factors outside the MHC genes or even by environmental factors, but no clear interactions



What determines which organs will be targeted by autoimmune processes? Could environmental factors such as lifestyle, diet, or exposure to contaminants be responsible for this predisposition? Alternatively, as suggested by Bianchi et al.³, could the key lie in the in-depth study of genetic variant combinations? One clear limitation is that because of the cost of sequencing and the large sample sizes required for GWAS, genetic analyses are often limited to common alleles and rely on predictions (imputation) rather than actual sequencing of most variants. Therefore, it seems necessary to revisit genetic factors at the whole-genome sequencing level, with larger, well-characterized clinical cohorts. This approach, which includes rare variants and regulatory regions, could offer a deeper and previously unexplored avenue for understanding the genetic determinants of organ-specific targeting in autoimmune diseases.

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were reported between environmental factors and the eight non-MHC associated genes. A similar explanation could apply to the associations observed in the cutaneous and musculoskeletal domains, which are also enriched in patients with SLE; however, the limited sample size in clinical domains that are predominant in SLE, such as renal disorder, is a major constraint of this study, which makes it difficult to rule out pleiotropic effects in the comparisons. Despite these limitations, the findings from this study offer valuable insights. Notably, albeit not totally unexpectedly, there seems to be a broad involvement of MHC genes in the production of most autoantibodies, and this study provides an initial indication of the role of genetic predisposition in organ involvement, which might occur independently of the specific autoimmune pathways underlying disease pathogenesis.

Molecular heterogeneity at the levels of proteomics, transcriptomics and epigenomics is currently a major focus in autoimmunity research; however, this variability does not seem to be directly linked to predisposition for organ-specific damage. Moreover, given that neither autoantibody positivity nor MHC genes seem to dictate organ-specific autoimmunity targets, the following questions remain.

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Competing interests

The authors declare no competing interests.

The 'T_{reg} paradox' in inflammatory arthritis

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Abstract

Classic regulatory T (T_{reg}) cells expressing CD4 and the hallmark transcription factor FOXP3 are integral to the prevention of multi-system autoimmunity. However, immune-mediated arthritis is often associated with increased numbers of T_{reg} cells in the inflamed joints. To understand these seemingly conflicting observations, which we collectively describe as 'the T_{reg} paradox', we provide an overview of T_{reg} cell biology with a focus on T_{reg} cell heterogeneity, function and dysfunction in arthritis. We discuss how the inflamed environment constrains the immunosuppressive activity of T_{reg} cells while also promoting the differentiation of T_H17-like T_{reg} cell, exT_{reg} cell (effector T cells that were formerly T_{reg} cells), and osteoclastogenic T_{reg} cell subsets that mediate tissue injury. We present a new framework to understand T_{reg} cells in joint inflammation and define potential strategies for T_{reg} cell-directed interventions in human inflammatory arthritis.

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An introduction to regulatory T cell biology

Regulatory T cells in inflammatory arthritis

Regulatory T cells as inadequate immune suppressors

Regulatory T cells as pathogenic contributors

Regulatory T cells as therapeutic targets

Conclusions

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Key points

- Regulatory T (T_{reg}) cells are abundant in the synovial fluid of patients with active arthritis, yet inflammation persists, a paradox that we term the T_{reg} paradox.
- T_{reg} cell-intrinsic constraints include genetic variants affecting T_{reg} cell function and restriction of the T_{reg} cell T cell receptor repertoire.
- T_{reg} cell-extrinsic constraints include the cytokine milieu and hypoxia of the inflamed joint.
- Activated synovial effector T cells and other inflammatory mechanisms within the joint can exhibit resistance to suppression by T_{reg} cells, even without T_{reg} cell dysfunction.
- T_{reg} cells can contribute to synovial inflammation by becoming pathogenic exT_{reg} cells, or by remaining T_{reg} cells but expressing pro-inflammatory cytokines or RANKL.
- Several current disease-modifying antirheumatic drugs might act in part by supporting T_{reg} cell function, and new treatments are in development that use T_{reg} cells as therapeutic agents, identifying a new direction for treatment of inflammatory arthritis.

Introduction

Since their discovery in the 1990s, regulatory T (T_{reg}) cells have been intensively studied as a key modulator of immunity and as a promising tool to alleviate pathogenic inflammation^{1,2}. For rheumatologists, these cells are of particular interest because individuals with genetic defects that affect FOXP3 – the transcription factor central to T_{reg} cell development and function – develop multisystem autoimmunity^{3–5}. However, T_{reg} cells are abundant in inflamed joints from patients with juvenile and adult arthritis, posing a ‘T_{reg} paradox’ as to why these cells fail to control synovitis. Recent studies further complicate the picture by showing that some synovial T_{reg} cells even acquire the ability to promote joint injury^{6,7}.

In this Review, we suggest a revised understanding of T_{reg} cells in arthritis as immune regulators that can, under defined conditions, become either ineffective or even pathogenic. We first provide a broad introduction to T_{reg} cell biology and T_{reg} cell plasticity, and then examine the abundance and phenotypes of T_{reg} cells in arthritis. Finally, we discuss how the protective and potentially harmful roles of synovial T_{reg} cells should be considered when developing T_{reg} cell-based therapeutics in arthritis.

An introduction to regulatory T cell biology

T_{reg} cells were described initially as a subpopulation of CD4⁺ T cells characterized by expression of the IL-2 receptor α -chain (CD25) and the ability to maintain self-tolerance¹. T_{reg} cell dysfunction translates into widespread immune-mediated tissue injury^{8–10}. Through interaction with multiple binding partners, FOXP3 drives the expression of hallmark T_{reg} cell proteins such as cytotoxic T lymphocyte-associated protein 4 (CTLA4) and the high-affinity IL-2 receptor chain CD25, suppresses expression of competing effector T cell pathways and contributes to metabolic transition from glycolysis to oxidative phosphorylation, thus orchestrating the T_{reg} cell phenotype^{2,11}.

Most T_{reg} cells develop in the thymus, arising from immature precursors expressing $\alpha\beta$ T cell receptors (TCRs) with intermediate affinity for native antigens¹². These thymus-derived T_{reg} cells are supplemented by a smaller population of peripherally derived T_{reg} cells that develop outside the thymus as they encounter their antigens under the influence of tolerogenic dendritic cells or, in a mechanism termed infectious tolerance, other T_{reg} cells^{13,14}. Thymic and peripherally derived T_{reg} cells share surface expression of CD4 and CD25 and low expression of the IL-7 receptor α -chain CD127, as well as expression of FOXP3; thymus-derived T_{reg} cells preferentially, but not exclusively, express the transcription factor Helios and, in mice, the surface protein neuropilin 1 (refs. 15–20). In the peripheral blood of healthy human donors, T_{reg} cells typically represent 2–4% of the CD4⁺ T cell population, of which 60–70% express Helios and are plausibly considered to be thymus-derived T_{reg} cells, although Helios expression is also induced by T cell activation^{21–24}. T_{reg} cells that are in a naive or resting state can be distinguished in humans based on CD45RA and lower FOXP3 expression, whereas T_{reg} cells that have encountered antigen express CD45RO and higher levels of FOXP3, as well as other effector molecules². T_{reg} cells generated *in vitro*, typically from naive CD4⁺ T cells in the presence of IL-2 and TGF β , are termed induced T_{reg} cells, and more closely resemble peripherally derived T_{reg} cells in that they lack expression of Helios and neuropilin-1 (ref. 14).

Regulatory T cell functions

T_{reg} cells carry out their immunoregulatory activities through five main pathways: secretion of anti-inflammatory cytokines; cytokine deprivation; metabolic disruption; modification of antigen-presenting cells (APCs); and cytotoxicity.

Anti-inflammatory cytokines. Activated T_{reg} cells secrete the cytokines IL-10 and IL-35, which promote the expansion of FOXP3⁺ T_{reg} cells while suppressing effector T cell proliferation and activation^{25–27}. T_{reg} cells also release TGF β , a cytokine that is important in the control of T helper 17 (T_H17) cells (both negatively and positively) and for the maintenance of T_{reg} cells at mucosal interfaces^{28,29}.

Cytokine deprivation. IL-2 is a crucial growth factor for effector CD4⁺ T cells. T_{reg} cells express higher levels of the high-affinity IL-2 receptor CD25 than activated T cells. As a result, T_{reg} cells limit the availability of IL-2 to effector T cells^{30,31}. T_{reg} cells expressing additional cytokine receptors might also consume the pro-inflammatory and the T cell differentiation cytokines IL-1 and IL-6 (refs. 32,33).

Metabolic disruption. T_{reg} cells generate high internal levels of cyclic AMP (cAMP) and transfer this metabolite to targeted effector T cells via gap junctions. As a result, inducible cAMP early repressor (ICER) accumulates in the nucleus of effector T cells and attenuates the transcription of IL-2 that is necessary for T cell proliferation^{34,35}. Further, through serial activity of the ectonucleases CD39 and CD73, mouse T_{reg} cells convert extracellular ATP to adenosine, which acts via the A2A adenosine receptor on activated effector T cells to suppress their proliferation^{36,37}. In humans, CD39 is expressed by some but not all T_{reg} cells, and CD73 is rarely expressed, possibly being induced during activation. Human T_{reg} cells are therefore likely to generate adenosine largely in cooperation with neighbouring CD73⁺ cells³⁸.

Antigen-presenting cell modification. Effector T cell activation requires antigen presentation to the TCR together with a costimulatory

signal delivered through the interaction of CD28 with CD80 and CD86 in APCs. T_{reg} cells constitutively express the co-inhibitory molecule CTLA4, which competes with CD28 for CD80 and CD86 binding, and strips CD80 and CD86 from the APC membrane through trans-endocytosis^{39,40}. T_{reg} cells further limit APC efficacy by altering APC metabolism and by removing their surface antigen-presenting complexes, while promoting development of a tolerogenic APC phenotype through the production of IL-10, the surface co-inhibitory molecule TIGIT and other mechanisms^{11,41,42}.

Cytotoxicity. T_{reg} cells can terminate immune responses by inducing apoptosis in T cells, B cells, monocytes and dendritic cells through the secretion of granzyme and perforin^{43–46}.

Beyond their immunosuppressive functions, T_{reg} cells participate in homeostasis and repair of non-lymphoid tissues. These so-called tissue T_{reg} cells migrate from secondary lymphoid organs to tissue sites in response to specific homing signals, recirculating in a tissue-agnostic manner after transient tissue residence^{47–49}.

Regulatory T cell plasticity

The $CD4^+$ T cell compartment is characterized by remarkable phenotypic plasticity, allowing T cells to adapt to the specific circumstances encountered by each lymphocyte⁵⁰. T_{reg} cell plasticity enables them to target their immunosuppressive signals with remarkable precision; however, T_{reg} cells can also acquire potentially pathogenic effector functions and even lose regulatory capacity all together. With respect to their immunosuppressive ‘mission’, T_{reg} cell plasticity can thus be characterized as adaptive or maladaptive; most changes could be either depending on the circumstances.

Adaptive regulatory T cell plasticity. T_{reg} cells respond to the same environmental cues that programme effector T cell differentiation; as a result, they express chemokine receptors similar to those of their target cells, enabling co-localization and so lineage-specific effector T cell suppression. For example, effector T_H17 cells differentiate in response to IL-6, IL-21 and IL-23, which upregulate the transcription factors STAT3 and ROR γ t and thereby induce expression of the chemokine receptor CCR6, the receptor for CCL20. Mouse T_{reg} cells respond similarly to IL-6, IL-21 and IL-23, upregulating CCR6 to enable migration to CCL20-rich tissues where they can suppress T_H17 cells^{33,51,52}. Ablation of the T_H2 -associated transcription factor IRF4 in T_{reg} cells leads to autoreactive T_H2 responses as T_{reg} cells lose expression of key effector molecules as well as of the chemokine receptor CCR8, which is believed to enable T_{reg} cells to co-localize with T_H2 cells at inflamed sites⁵³. In the presence of IL-12 and interferon- γ (IFN γ), T_{reg} cells upregulate T-bet, the hallmark transcription factor of T_H1 cells, leading to expression of CXCR3; deficiency of T-bet-expressing T_{reg} cells thus impairs control of T_H1 autoimmunity^{54,55}. These studies highlight the importance of T_{reg} cell plasticity for optimal control of T helper cell-specific immune responses.

Maladaptive regulatory T cell plasticity. Under some circumstances, T_{reg} cells shed their suppressive capacity in favour of an effector T cell phenotype. This so-called T_{reg} cell instability has been identified in mice using lineage tracing to mark all cells that ever expressed FOXP3, as well as by transfer of sorted FOXP3⁺ cells into recipient animals^{56–59}. Some T_{reg} cells lost FOXP3 expression and upregulated pro-inflammatory cytokines, mostly IFN γ and IL-17; these cells have been termed ex T_{reg} cells. A proportion of ex T_{reg} cells, called latent

T_{reg} cells, later re-expressed FOXP3 and regained suppressive capacity upon stimulation via the TCR or by cytokines^{57,60–62}. However, in mice, most ex T_{reg} cells remain effector T cells and have the potential to contribute to autoimmune tissue injury, potentially even more than conventional effector T cells because T_{reg} cells are selected in the thymus on the basis of their self-reactive TCR repertoire^{6,56,58,59}. Ex T_{reg} cell-like populations have been recently identified in humans, as $CD3^+CD4^+CD16^+CD56^+$ cells that exert direct cell–cell cytotoxicity and produce tumour necrosis factor (TNF) and IFN γ , or as IL-13-expressing T_H2 -like effector T cells with demethylated *CNS2* regions among FOXP3-negative T cells in patients with IPEX with hypofunctional variants in FOXP3 but preserved FOXP3 protein expression^{63,64}.

Whether a particular T_{reg} cell exhibits instability depends critically on epigenetic programming⁶⁵. The T_{reg} cell lineage identity is defined by two key components: a T_{reg} cell-specific epigenome, which is established prior to FOXP3 expression; and the expression of FOXP3, which interacts with accessible chromatin to generate the full T_{reg} cell phenotype^{66,67}. The FOXP3 locus contains several conserved non-coding sequences (*CNS*) that are essential for the development and maintenance of the T_{reg} cell phenotype^{68–72}. *CNS2*, also known as T_{reg} cell-specific demethylated region (*TSDR*), has the key role in T_{reg} cell stability. FOXP3 binds to *CNS2* in a demethylation-dependent manner, rendering T_{reg} cells particularly sensitive to IL-2 and facilitating FOXP3 expression in a positive feedback loop that allows T_{reg} cells to retain their regulatory phenotype through multiple cell divisions. As a result, *CNS2* demethylation is a hallmark of stable T_{reg} cells^{69,73}. Accordingly, ex T_{reg} cells that have pathogenic potential have been shown in mice to lose *CNS2* demethylation, at least to some degree, whereas ex T_{reg} cells that regain suppressive function exhibit maintained *CNS2* demethylation^{56,60}.

All endogenous T_{reg} cells exhibit *CNS2* methylation, although peripherally derived T_{reg} cells display lower levels of *CNS2* demethylation than thymus-derived T_{reg} cells, which potentially explains why peripherally derived T_{reg} cells might be more prone than thymus-derived T_{reg} cells to becoming pathogenic ex T_{reg} cells under inflammatory conditions^{59,60,74}. In vitro-induced T_{reg} cells do not display T_{reg} cell-specific epigenetic patterns, and might, thus, be particularly susceptible to pathogenic T_{reg} cell instability, a fact relevant to their therapeutic application to inflammatory diseases, as discussed below^{75,76}.

Regulatory T cell plasticity with dual consequences. T_{reg} cells can respond to environmental cues by retaining their immunosuppressive capacity while also acquiring pro-inflammatory features. For example, some mouse and human T_{reg} cells with preserved FOXP3 expression and immunosuppressive function also produce IFN γ and IL-17 (refs. 77–80). These cytokines have known immunomodulatory roles; for example, IFN γ inhibits lymphocyte trafficking and T cell proliferation, whereas IL-17 protects the intestine from experimental colitis by enhancing epithelial barrier function and IL-10 production^{81–83}. However, IFN γ and IL-17 are also the hallmarks of T_H1 cells and T_H17 cells, respectively, and promote inflammation via macrophage activation and increased antigen presentation in the case of IFN γ , or via neutrophil expansion, recruitment and activation in the case of IL-17. In addition, some T_{reg} cells acquire RANKL expression in an IL-1-rich microenvironment and have been termed osteoclastogenic T_{reg} cells because they promote osteoclast development and bone erosion in a mouse model of arthritis⁷. Although such a phenotype would generally be considered maladaptive, RANKL-expressing ex T_{reg} cells are also known to contribute to osteoclast-mediated loss of infected teeth and,

thereby, to speed recovery from experimental gingivitis, suggesting that there may be contexts in which osteoclastogenic T_{reg} cells might have a homeostatic role⁸⁴.

Regulatory T cells in inflammatory arthritis

The term inflammatory arthritis describes a set of diseases featuring immune-mediated destruction of joints. Rheumatoid arthritis (RA) is characterized by aggressive synovitis and bone erosion, whereas spondyloarthropathies such as ankylosing spondylitis and psoriatic arthritis (PsA) feature both synovitis and enthesitis. Arthritis beginning before the 16th birthday, historically termed juvenile idiopathic arthritis (JIA), is largely a continuation of the same spectrum but includes a distinctive population of young children with oligoarticular presentation, as well as overrepresentation of Still disease, a syndrome characterized by arthritis, fever, rash and multisystem inflammation (also called systemic JIA in children and adult-onset Still disease in adults)⁸⁵.

Compelling observational evidence implicates T_{reg} cells in arthritis biology. In RA, lineage-specific epigenetic marks suggest that T_{reg} cells are a key population through which genetic variants drive risk of incident disease^{86–90}. Risk of arthritis, including RA and the spondyloarthropathies, is associated with genetic polymorphisms in T_{reg} cell-associated genes, including *CTLA4* and *BACH2* (refs. 87,91,92). This evidence is not directional and could conceivably reflect either impairment of T_{reg} cell abundance or function or, for example, enhanced T_{reg} cell instability. Of note, arthritis is reported in FOXP3 deficiency, though this manifestation is uncommon^{93,94}.

Compared with healthy individuals, patients with RA exhibit an abundance of T_{reg} cells in peripheral blood that is variably reported to be increased^{95,96}, decreased^{97–100} or unchanged^{101–103}. Similar variability in T_{reg} cell numbers has been observed in patients with PsA¹⁰⁴. In JIA, studies report comparable to reduced frequency of T_{reg} cells compared with paediatric controls^{105–108}. A careful review of published data

concluded that the proportion of circulating CD25^{hi}FOXP3⁺ T_{reg} cells among CD4⁺ T cells averages slightly lower in patients with RA than in healthy individuals, and is further reduced in active disease compared with remission²¹. The proportion of Helios⁺ T_{reg} cells in the circulation is either comparable with or reduced in comparison with healthy controls, though given the limitations of this marker, no reliable data are available detailing the relative abundance of thymic T_{reg} cells and peripheral T_{reg} cells in arthritis^{109–111}.

By contrast, in inflamed synovial fluid, T_{reg} cells are clearly enriched. In patients with RA, T_{reg} cells typically represent 10–20% of synovial CD4⁺ T cells, compared with 3–5% in peripheral blood^{21,112}. Comparable results have been observed in patients with JIA, and in PsA and other spondyloarthropathies^{105,106,113–116}. Similarly, in mouse models of experimental arthritis, T_{reg} cells accumulate in inflamed joints^{7,117}. Thus, arthritis presents a T_{reg} paradox: persistent inflammation despite an increased abundance of T_{reg} cells in the joints.

The phenotypes of synovial fluid T_{reg} cells are summarized in Table 1. Similar to circulating T_{reg} cells, those from human arthritic joints express FOXP3 and CD25 and exhibit *CNS2* demethylation; levels of FOXP3, CD25 and CTLA4 are generally higher in T_{reg} cells from inflamed joints than in T_{reg} cells from peripheral blood^{95,109,114,116,118}. A higher proportion of synovial fluid T_{reg} cells than circulating T_{reg} cells carry the memory marker CD45RO⁺, and most are CD69⁺CD62L⁻, indicating activation; by contrast, blood T_{reg} cells are largely CD69⁻CD62L⁺, even in patients with arthritis¹¹⁹. Further, 4–10% of synovial fluid T_{reg} cells express Ki67, a nuclear marker of cells that are actively dividing^{115,120}. The proportion of FOXP3⁺CD4⁺ T cells that express Helios is variably reported; in oligoarticular JIA it is reduced in the affected joints compared with peripheral blood, suggesting that some synovial fluid T_{reg} cells were derived peripherally, although admixture of recently activated FOXP3⁺ effector T cells is difficult to exclude^{109,116}. Compared with peripheral blood T_{reg} cells from healthy donors, T_{reg} cells in synovial

Table 1 | Phenotypes of synovial fluid regulatory T (T_{reg}) cells in arthritis

T_{reg} cell subset	Phenotypic markers	Functional characteristics	Frequency	Refs.
Recently recruited T_{reg} cells	CCR7 ⁺ , LEF1 ⁺ , low clonality	Similar to peripheral blood T_{reg} cells, not fully activated	30–40% of T_{reg} cells in the synovial fluid of patients with JIA	123
Activated effector T_{reg} cells	CD25 ^{hi} , FOXP3 ⁺ , CD45RO ⁺ , CD69 ⁺ , CD62L ⁻ , CTLA4 ^{hi} , some Ki67 ⁺	Higher expression of activation markers (CD69, OX-40, GITR, ICOS, CXCR3) and inhibitory molecules (CTLA4, GZMB, IL-10, TIM3, PD-1, CD39)	Activated effector T_{reg} cells are shown in the synovial fluid of patients with RA, JIA, OA, PsA and SpA	95,109, 113–119,121
Subphenotypes within activated effector T_{reg} cells				
T_{H1} -like T_{reg} cells	CXCR3 ⁺ , T-bet ⁺ , IL-12Rβ ⁺ ; some express IFNγ	Adapted to T_{H1} environment, maintain suppressive function and T_{reg} cell-specific demethylation patterns	~40–80% of T_{reg} cells in the synovial fluid from patients with RA and JIA	116,122
T_{H17} -like T_{reg} cells	CCR6 ⁺	Expression of CCR6, IL-10 and LAG3	~24% of T_{reg} cells in the synovial fluid of patients with RA. T_{H17} -like T_{reg} cells were also discovered in synovial fluid and blood of patients with SpA using transcriptional analysis (no frequency data)	122,126,248
Cytotoxic T_{reg} cells	Expression of genes with cytotoxic function: granzyme A, granzyme K, granzyme B, granzyme H, perforin and granulysin	Undefined	Cytotoxic T_{reg} cells were observed in the peripheral blood and SF of patients with SpA	116,126
T_{PH} -like T_{reg} cells (T_{PR} cells)	CD161 ⁺ , GPR56 ⁺ , CXCL13 ⁺ , PD-1 ^{hi} , LAG3 ^{hi} , CXCR5 ⁺ , ICOS, HLA-DR	Potentially regulate T_{PH} cell–B cell interactions	5–20% of T_{reg} cells in the synovial fluid of patients with JIA	123–125,127

CTLA4, cytotoxic T lymphocyte-associated antigen 4; GZMB, granzyme B; HLA-DR, human leukocyte antigen DR isotype; ICOS, inducible T cell co-stimulator; IFNγ, interferon-γ; JIA, juvenile idiopathic arthritis; OA, osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SF, synovial fluid; SpA, spondyloarthritis; T_{H1} , T helper 1; T_{PH} , T peripheral helper.

fluid from patients with JIA show increased expression of genes associated with T_{reg} cell activation and potentially suppressive effector function, including *GZMB*, *ICOS*, *IL10*, and of the suppressive proteins GTR, TIM3 and PD-1 (refs. 116,121). Synovial fluid T_{reg} cells exhibited enhanced expression of T_H1 -associated proteins T-bet, IL-12R β and CXCR3; few expressed IFN γ , suggesting that these features potentially reflect adaptation to a T_H1 -skewed environment rather than conversion into T_H1 -like effector cells, as supported further by intact in vitro suppressive function and preserved *CNS2* demethylation^{116,121}. In RA, CXCR3-expressing T_{reg} cells (T_H1 -like T_{reg} cells) represented almost 15% of all synovial fluid T_{reg} cells, whereas 7% expressed CCR6 (T_H17 -like T_{reg} cells)¹²².

Single-cell RNA sequencing of arthritic synovial fluid T_{reg} cells has identified considerable heterogeneity^{116,123–126}. Approximately one third express genes associated with a resting state in T_{reg} cells, such as *CCR7* and *LEF1*, and little clonal expansion, suggesting that this population of T_{reg} cells is newly recruited to the inflamed joint and not yet activated¹²³. The remaining two thirds of T_{reg} cells exhibit a gene signature consistent with activation, together with elevated clonality, suggesting local expansion¹²³. Subpopulations of synovial fluid T_{reg} cells exhibit expression of granzymes and other cytotoxic genes and evidence of activation by type I interferons^{116,126}. Approximately 10–20% of synovial fluid T_{reg} cells express genes that are otherwise unusual in T_{reg} cells, including *CXCL13*, *GPR56* and *KLRB1* (which encodes CD161)^{116,123,127}. In patients with JIA, CD161⁺GPR56⁺ T_{reg} cells are more common in synovial fluid than in blood (18% versus 5% in one study), and include CXCL13⁺ T_{reg} cells that express high levels of PD-1 and LAG3 (refs. 123,126). These T_{reg} cells, termed T peripheral regulatory (T_{PR}) cells, are hypothesized to be the regulatory counterpart of CXCL13⁺PD-1⁺CXCR5⁺ T peripheral helper (T_{PH}) cells, and to be involved in the regulation of T_{PH} cell–B cell interactions in inflamed joints^{124,128,129}.

Yet, if T_{reg} cells are abundant, why does joint inflammation persist? Taking a cue from T_{reg} cell biology, the two broad possibilities are that T_{reg} cells are insufficient to restrain the inflammatory process and that T_{reg} cell plasticity directs arthritic T_{reg} cells towards potentially pathogenic phenotypes.

Regulatory T cells as inadequate immune suppressors

The functional competence of T_{reg} cells in arthritis remains controversial. Multiple studies have found that T_{reg} cells from patients with arthritis effectively suppress effector T cells in in vitro suppression assays, with their suppressive capacity sometimes exceeding that of T_{reg} cells isolated from healthy blood^{95,97,105,116,121} (Supplementary Table 1). However, other investigations document either global impairment of suppressive function in T_{reg} cells from arthritic peripheral blood and synovial fluid^{103,122,130,131} or intact suppression of effector T cell proliferation but poor control of effector cytokine production^{132–134} (Supplementary Table 2). Some of this divergence might reflect disease activity, as T_{reg} cell function in RA has been observed to vary with disease state, with high disease activity correlating with impaired suppressive capacity of T_{reg} cells^{134,135}. Divergence in results might also depend on what cell population was studied; for example, CD4⁺CD25⁺ cells include both CD4⁺CD25⁺CD127^{low} T_{reg} cells and CD4⁺CD25⁺CD127^{high} activated effector T cells.

The hypothesis that T_{reg} cells contribute to controlling arthritis is supported by observational and experimental evidence. The presence of an expanded population of CD25^{bright} T_{reg} cells in synovial fluid has been associated with a favourable course of JIA¹⁰⁵, and normalization

of peripheral blood T_{reg} cell populations has been observed in patients with JIA who improved after bone marrow transplantation^{136,137}. In mice, T_{reg} cell deficiency accelerates the onset and severity of arthritis, whereas, in some models, adoptive transfer of T_{reg} cells ameliorates arthritis in an antigen-specific manner^{6,117,138–141}. Interestingly, although T_{reg} cells can reduce autoantibody production in K/BxN arthritis and collagen-induced arthritis (CIA), disease amelioration correlates incompletely with antibody titres, suggesting that T_{reg} cells might exert additional, as yet unexplored, protective functions in synovial tissues^{117,139,140}.

Yet the persistence of joint inflammation despite the presence of abundant synovial T_{reg} cells implies that these cells fail to fully control disease. This failure could reflect intrinsic defects in T_{reg} cells, extrinsic factors causing T_{reg} cell dysfunction and target factors rendering arthritis resistant to suppression by T_{reg} cells (Fig. 1).

Cell-intrinsic constraints on T_{reg} cells could include dysfunction related to genetic variation. Genome-wide association studies have found that many common genetic variants associated with risk of RA and JIA, as well as other autoimmune diseases, are localized to areas of chromatin that are epigenetically accessible in T_{reg} cells, including in regions encoding hallmark genes such as *CTLA4* (refs. 87,91,92). It is therefore plausible that common genetic variation could predispose some individuals to arthritis risk through perturbation in T_{reg} cell abundance or function, though to date no examples have been established at a mechanistic level. A further intrinsic constraint is associated with the TCR repertoire of T_{reg} cells. In healthy individuals, the diversity of the TCR repertoire of T_{reg} cells is comparable with that of naive and effector T cells, contributing to optimal control of autoimmunity^{142–144}. However, in patients with arthritis, both peripheral blood and synovial fluid T_{reg} cells exhibit a restricted repertoire, as reflected in skewed usage of TCR β chains and overuse of gene segments associated with autoimmunity, presumably related to clonal expansion. This skewed TCR repertoire of T_{reg} cells might limit their ability to control a diverse effector T cell population^{115,145}. A substantial fraction of T_{reg} cell clonotypes detected in the synovial fluid of patients with JIA are not found in healthy individuals or in patients with other forms of arthritis. Moreover, the clonotypes of the most abundant synovial T_{reg} cell clones are also found in the peripheral blood of these patients, and are shared across disease flares within an individual patient^{115,145}. A restricted TCR repertoire has been reported for T_{reg} cells in mouse models of autoimmunity^{146–148}. A skewed T_{reg} cell TCR repertoire could be particularly problematic if the inflamed synovium expresses autologous neoantigens recognized by effector T cells but not by T_{reg} cell populations.

The synovial environment offers multiple extrinsic constraints on T_{reg} cells. The inflammatory mediators TNF and IL-6 can impair immunosuppression by T_{reg} cells^{103,130,134,149–151}. Reduced IL-2 production by CD4⁺ T_H1 cells could impair T_{reg} cell survival and function¹⁵². The hypoxia of the rheumatoid joint could reduce *FOXP3* expression through the hypoxia-inducible factor 1 α (HIF1 α), although the in vivo relevance of this mechanism remains uncertain, because in the tumour context, hypoxia has been observed to amplify T_{reg} cell function^{153–155}. PD-1-expressing T_{reg} cells could be suppressed by cells expressing PD-1 ligand, including synovial fluid neutrophils, a possibility supported by the finding in other disease contexts that mouse PD-1-deficient T_{reg} cells exhibited enhanced proliferation and suppressive function^{156–158}.

Finally, target resistance probably limits the efficacy of T_{reg} cells in the joint. When removed from the inflammatory environment and

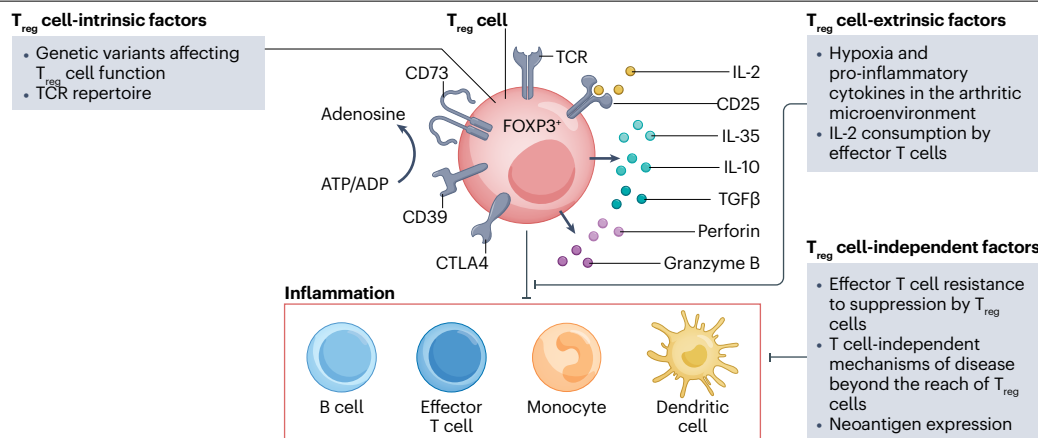


Fig. 1 | Potential constraints on the control of inflammatory arthritis by synovial T_{reg} cells. The persistence of joint inflammation despite the presence of abundant regulatory T (T_{reg}) cells might reflect multiple constraints on T_{reg} cell activity. Cell-intrinsic factors that reduce T_{reg} cell function include a limited T_{reg} cell T cell receptor (TCR) repertoire and genetic variants that lower the T_{reg} cell numbers or impair T_{reg} cell function. Cell-extrinsic factors that limit

T_{reg} cell function include the presence of inflammatory cytokines and hypoxia as well as consumption of IL-2 by CD25^{hi} effector T cells. T_{reg} cell-independent factors mediating the inefficacy of suppression include resistance of effector T cells to suppression, inflammatory mechanisms such as immune complex deposition that are beyond the reach of T_{reg} cells and potentially expression of neoantigens that T_{reg} cells do not recognize. CTLA4, cytotoxic T lymphocyte-associated protein 4.

tested in vitro, synovial fluid T_{reg} cells generally suppress replication of effector T cells isolated from peripheral blood but not from synovial fluid^{95,159,160}. This resistance reflects, at least in part, the activated phenotype of effector T cells, and can be reversed by effector T cell-specific inhibition of protein kinase B (PKB, also known as AKT), downstream of TNF and IL-6 (ref. 160). In mice, effector T cell resistance to suppression by T_{reg} cells can be induced by IL-1 through MYD88 signalling¹⁶¹. Some forms of inflammatory arthritis might involve pathogenetic mechanisms that are simply beyond the reach of T_{reg} cells. For example, one common route to joint inflammation is synovial immune complex accumulation followed by complement fixation¹⁶². The ability of T_{reg} cells to suppress effector lymphocytes would be irrelevant to such a process, and indeed transferred T_{reg} cells do not ameliorate disease in a mouse model of immune complex-induced arthritis⁷.

Regulatory T cells as pathogenic contributors

The persistence of arthritis despite abundant synovial fluid T_{reg} cells could reflect not only the inability to suppress inflammation but also conversion into cells that contribute to disease, either as ex T_{reg} cells or through acquisition of characteristics that promote tissue injury (Fig. 2).

The plasticity between T_{reg} cells and T_{H17} cells is of particular relevance in arthritis¹⁶³. Despite the striking divergence in function between pro-inflammatory T_{H17} cells and immunomodulatory T_{reg} cells, their differentiation pathways from naive CD4⁺ T cells both require TGF β , which upregulates FOXP3 and ROR γ t^{164,165}. IL-2 and TGF β drive FOXP3⁺ROR γ t⁺ precursor cells to become T_{reg} cells by promoting FOXP3 expression over ROR γ t, whereas combined IL-21, IL-6 and TGF β favour a T_{H17} fate and the expression of ROR α and ROR γ t over FOXP3 (refs. 29,166,167). Even after differentiation, T_{H17} cells and T_{reg} cells can transdifferentiate, with TGF β and IL-2 shifting the balance towards the T_{reg} cell lineage and IL-1 β , IL-6 and IL-21 promoting T_{H17} identity^{167–169}. Studies in mice indicate that T_{H17} cells can in turn transdifferentiate into IL-10-expressing but FOXP3-negative regulatory cells, thereby contributing to resolution of T_{H17} -mediated inflammation¹⁷⁰.

An altered balance between T_{reg} cells and T_{H17} cells has been described in multiple autoimmune conditions, including RA and JIA^{100,103,171,172}. Both peripheral and synovial fluid T cells producing IL-17 are reported in arthritic peripheral blood and synovial fluid, and their numbers correlate with disease activity¹⁷³. In RA, a modestly higher proportion of peripheral blood T_{reg} cells produce IL-17 upon stimulation, although the abundance of these cells correlates negatively with markers of systemic inflammation¹⁷⁴. Synovial fluid from patients with JIA is enriched in CD161⁺ T_{reg} cells that secrete pro-inflammatory cytokines, including IL-17, despite retained FOXP3 expression and suppressive function^{127,175}. IL-17-producing CD161⁺ T_{reg} cells have been identified in RA as well¹⁷⁴. In systemic JIA, T_{H17} -like polarization of T_{reg} cells is observed in patients with highly inflammatory new-onset disease, a transcriptional signature that becomes detectable in effector T cells as the disease develops into chronic arthritis; this transition is abrogated by early IL-1 blockade, probably reflecting the role of IL-1 β in T_{H17} polarization¹⁷⁶.

The relevance of these correlational observations is underscored by studies in experimental models. In CIA, CD25^{lo}FOXP3⁺CD4⁺ T cells were found to transdifferentiate into IL-17-expressing ex T_{reg} cells, a process enabled by IL-6 from synovial fibroblasts, at least in vitro⁶. These T_{H17} -like ex T_{reg} cells accumulate in joints, exhibit decreased suppressive capacity and induce osteoclast differentiation through IL-17 and RANKL. Whereas definitive identification of comparable ex T_{reg} cells in humans remains difficult, IL-17⁺FOXP3⁺ cells were identified in synovial fluid from patients with RA during a phase of high disease activity, potentially representing a related population⁶. Interestingly, development of cytokine-producing ex T_{reg} cells was not identified in a second mouse system, in which labelled T_{reg} cells from arthritic K/BxN mice were transferred into congenic K/BxN animals; in this setting, donor T_{reg} cells localized to the inflamed joint but retained expression of FOXP3 and did not produce IL-17 (ref. 74).

T_{reg} cells characterized by high expression of RANKL have been identified in a mouse model of arthritis mediated through genetic deletion of the IL-1 receptor antagonist⁷. These IL-1-driven RANKL^{hi} osteoclastogenic T_{reg} cells induced differentiation of osteoclasts and

contributed to bone erosion. Correspondingly, IL-1 β blockade attenuated disease more effectively in mice with early disease than in mice with established arthritis, especially with respect to bone erosion. Unlike the exT_{reg} cells observed in CIA, IL-1 β -derived osteoclastogenic T_{reg} cells remained suppressive and did not produce IL-17. In RA, osteoclastogenic T_{reg} cells represent up to 10% of all synovial fluid T_{reg} cells⁷.

Dysfunctional T_{reg} cells expressing IFN γ have been identified in multiple sclerosis and type 1 diabetes^{65,78,177}. CD161⁺ T_{reg} cells expressing T-bet and IFN γ have also been observed in arthritis, although their role remains undefined^{116,127}.

Regulatory T cells as therapeutic targets

The ability of T_{reg} cells to control antigen-directed immune response suggests that enhancing T_{reg} cell function might help to control inflammation without otherwise impairing immune function, a so-called T_{reg}-up strategy². Indeed, some drugs used to treat arthritis have an impact on T_{reg} cells, and this potentially accounts, at least in part, for their therapeutic efficacy. Alternatively, the direct targeting of endogenous T_{reg} cells to enhance their function, or the amplification of immune suppression via the adoptive transfer of exogenous T_{reg} cells, might hold therapeutic potential.

Arthritis therapeutics and regulatory T cells

Multiple drugs currently in use for inflammatory arthritis affect T_{reg} cell function (Table 2). Best studied have been methotrexate (MTX); blockers of TNF, IL-6 and IL-1; the CTLA4-Ig fusion protein abatacept; and Janus kinase (JAK) inhibitors.

Methotrexate. The cornerstone of arthritis therapy, MTX acts via blockade of purine and pyrimidine synthesis and enhancement of anti-inflammatory adenosine production¹⁷⁸. Treatment with MTX has been associated with restoration of previously impaired suppressive function of T_{reg} cells in patients with RA, including increased FOXP3 by

T_{reg} cells¹⁷⁹. Correspondingly, T_{reg} cells treated in vitro with MTX also exhibited increased FOXP3 expression, potentially related to enhanced demethylation of *CNS2* (ref. 179). In CD4 T cells from patients with RA, MTX treatment is associated with downregulation of TAp63, a T_H17 cell-associated transcription factor that suppresses FOXP3 expression; TAp63 inhibition improved arthritis in mice and increased FOXP3 expression in human T_{reg} cells¹⁸⁰. T_{reg} cells from patients responding to MTX exhibit increased levels of adenosine-producing exonuclease CD39, and depletion of T_{reg} cells or blockade of CD39 abrogated the anti-arthritic impact of MTX in a mouse model of arthritis. Thus, MTX might function in part through the enhancement of adenosine production by T_{reg} cells^{181–183}. MTX therapy has also been associated with an increase in T_{reg} cell abundance in RA¹⁸⁴. In JIA, however, treatment with MTX did not alter T_{reg} cell abundance or phenotype¹⁸⁵.

TNF blockade. Multiple studies have found that TNF impairs T_{reg} cells and, correspondingly, that TNF inhibitor therapy can restore or even enhance T_{reg} cell function. Several mechanisms have been suggested. TNF enhances recruitment of the inhibitor protein kinase C θ (PKC θ) to the immune synapse of T_{reg} cells, thereby enabling inhibitory signals downstream of the TCR^{103,134}. TNF has been found to decrease FOXP3 expression in CD4⁺CD25^{hi} T cells, or alternatively to dephosphorylate FOXP3, impairing the function but not the expression of this hallmark transcription factor^{103,130}. In humans and in mice, treatment with the monoclonal anti-TNF infliximab was associated with improved T_{reg} cell function and with the appearance of a population of suppressive CD62L^{neg} T_{reg} cells^{130,186,187}. More generally, TNF inhibitor therapy has been associated with an increase in T_{reg} cell abundance in RA peripheral blood¹⁸⁴. TNF inhibition also reverses the resistance of CD4⁺ and CD8⁺ effector T cells to T_{reg} cell suppression¹⁸⁸.

Interestingly, TNF also exerts protective effects on T_{reg} cells, acting through its receptor TNFRII to promote their expansion and stability^{189–193}. This role for TNFRII may explain the observation that the monoclonal TNF inhibitor adalimumab, but not the chimeric soluble

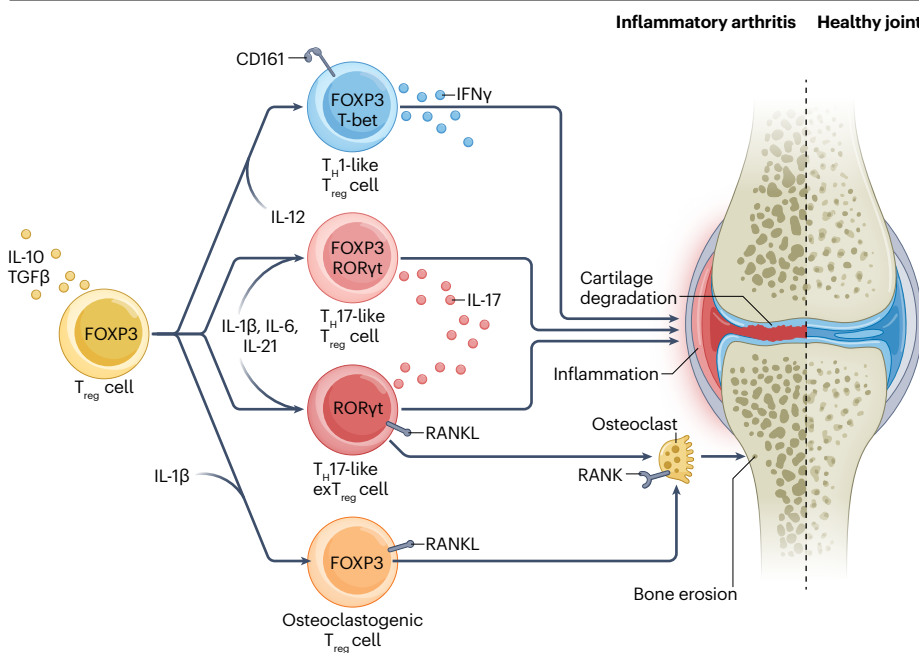


Fig. 2 | Potential pathways by which synovial T_{reg} cells become pathogenic contributors to arthritis. Synovial regulatory T (T_{reg}) cells have the potential to augment inflammation by acquiring the ability to produce pro-inflammatory cytokines including interferon- γ (IFN γ) and IL-17, or by acquiring expression of surface RANKL that drives the differentiation of osteoclasts to promote bone erosion (osteoclastogenic T_{reg} cell). Synovial T_{reg} cells can also lose FOXP3 expression to become exT_{reg} cells, expressing pathogenic cytokines and surface RANKL while losing all suppressor function. T_H1, T helper 1.

Table 2 | Impact of inflammatory arthritis treatments on regulatory T (T_{reg}) cells

Treatment	Mechanism of action	Effect on T _{reg} cells	Refs.
Methotrexate	Inhibition of dihydrofolate reductase, blockade of purine and pyrimidine synthesis, increased adenosine production	RA: increased T _{reg} cell abundance; enhanced suppressive function; upregulation of CD39 and FOXP3 JIA: no effects on T _{reg} cell abundance or function	179–185
Sulfasalazine	Not fully understood	Reduction of FOXP3 expression and suppressive function in T _{reg} cells isolated from healthy donors	249
Leflunomide	Inhibition of dihydroorotate dehydrogenase, blockade of pyrimidine synthesis	Reduction of FOXP3 expression and suppressive function in T _{reg} cells isolated from healthy donors Mouse model of arthritis: enhanced T _{reg} cell differentiation and TGFβ production	249–251
Infliximab, adalimumab, etanercept	TNF blockade	RA: enhanced T _{reg} cell abundance and function; induction of a population of suppressive CD62L ^{neg} T _{reg} cells JIA: reduction of the resistance of effector T cells towards T _{reg} cell suppression	103,130, 184,186–188, 194–197
Tocilizumab	IL-6 receptor antagonist	RA: increased T _{reg} cell–T _H 17 cell ratio in the blood	100,137,184
Anakinra	IL-1 receptor antagonist	<i>IL-1rn</i> ^{-/-} mouse model of arthritis: arthritis and bone erosion blocked, including through reduced development of osteoclastogenic T _{reg} cells	7
Abatacept	CTLA4–Ig fusion protein, inhibition of T cell activation	Mouse model of collagen-induced arthritis: increased T _{reg} cell abundance RA: no effect on T _{reg} cell abundance	208–211
Rituximab	CD20-dependent B cell depletion	RA: reduced T _H 17 cell numbers but no effect on T _{reg} cells SLE: increased mRNA levels of <i>FOXP3</i> , <i>CD25</i> , <i>GITR</i> , <i>CTLA4</i>	252–254
Tofacitinib	JAK inhibition	Transient loss of FOXP3 expression from healthy humans and mice Kidney transplant patients: suppression of effector T cells and preserved function of T _{reg} cell function Murine hepatitis: restored T _{reg} cell–T _H 17 cell balance	213–215

CTLA4, cytotoxic T lymphocyte-associated protein 4; JAK, Janus kinase; JIA, juvenile idiopathic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; T_H17, T helper 17.

receptor etanercept, increases the abundance of circulating T_{reg} cells in patients with RA¹⁹⁴. As etanercept does not recognize surface-bound TNF, one proposed mechanism is that the monoclonal antibody stabilizes membrane-bound TNF in monocytes, enabling monocytes to promote T_{reg} cell survival via TNFR1^{193,195–197}.

IL-6 blockade. In mice, IL-6 contributes to the differentiation of arthritogenic IL-17-expressing exT_{reg} cells, and more generally to a skew towards T_H17 cells and away from T_{reg} cells^{6,198}. IL-6 can also inhibit T_{reg} cell function^{150,151}. In RA, treatment with the IL-6 inhibitor tocilizumab has been associated with a shift of the T_{reg} cell–T_H17 cell balance in favour of T_{reg} cells, as well as with an increased frequency of CD39⁺ T_{reg} cells in the blood, where T_{reg} cell frequency correlates with clinical response^{100,137,184,199}.

IL-1β blockade. IL-1β inhibits TGFβ-driven FOXP3 expression and favours the differentiation of T_H17 cells, as well as the conversion of T_{reg} cells to T_H17 cells^{200–202}. This effect is likely to contribute to the observed expansion of T_H17 cells in systemic JIA, a highly inflammatory arthritis that often responds briskly to IL-1β blockade early in the disease course^{176,203,204}. In both mice and humans, exposure of T_{reg} cells to IL-1β favours expression of RANKL and differentiation towards an osteoclastogenic T_{reg} cell phenotype⁷.

CTLA–Ig fusion protein. The CTLA4–Ig fusion protein abatacept binds CD80 and CD86 on APCs and thereby interrupts the delivery of an essential costimulatory signal to CD28 on effector T cells, thus attenuating T cell activation and favouring anergy. However, T_{reg} cells also

depend on stimulation through CD28 for their development in the thymus and their proliferation and maintenance^{205–207}. In a mouse model of CIA, CTLA4–Ig induced tolerogenic dendritic cells, resulting in an increased number of T_{reg} cells in the joints and spleen²⁰⁸. However, in patients with RA, treatment with abatacept has been associated with only modest and variable effects on the abundance of T_{reg} cells in peripheral blood^{209–212}.

JAK inhibitors. Signalling of IL-2 through the JAK3–STAT5 pathway is crucial for T_{reg} cell lineage stability and is associated with demethylation of *CNS2* (ref. 68). Treatment of human CD4⁺ T cells with inhibitors of the JAK3–STAT5 pathway resulted in a loss of FOXP3 expression, which was accompanied by a loss of STAT5 binding to *CNS2*; demethylation of *CNS2* was, however, not affected, and loss of FOXP3 expression was reversible²¹³. In both humans and mice, JAK inhibition with tofacitinib inhibited effector T cell function more than T_{reg} cell function, leading to a net anti-inflammatory impact^{214,215}. JAK inhibition is not associated with a change in T_{reg} cell abundance¹⁸⁴.

Enhancing the function of endogenous regulatory T cells

T_{reg} cells express a high-affinity IL-2 receptor that contains IL-2Rα (CD25), IL-2Rβ and IL-2Rγ, and require IL-2 to survive and proliferate normally²¹⁶. Treatment with low-dose IL-2 expands T_{reg} cells with minimal impact on effector T cells, as the latter generally express an IL-2 receptor that is lower affinity because it lacks CD25 (refs. 217–220). In RA and PsA, low-dose IL-2 has been shown to be well tolerated, increase T_{reg} cell abundance and correlate with lower disease activity^{221–224}. A similar approach has been shown to be effective in a mouse model of arthritis²²⁵.

To overcome the short half-life of IL-2, multiple strategies have been applied, including the use of IL-2–anti-IL-2 complexes, a IL-2 fusion protein or muteins that exhibit more favourable binding or persistence in circulation^{226,227}.

Another strategy to enhance T_{reg} cell function is mTOR inhibition. By selectively blocking the expansion and proliferation of effector T cells, the mTOR inhibitor rapamycin has been shown to selectively favour expansion of T_{reg} cells in vitro and in mouse models of autoimmunity^{228–233}. Rapamycin also prevents T_{reg} cell destabilization and maintains or enhances their suppressive functions by antagonizing the effector T cell programmes and by modulating their metabolism^{234,235}. In patients with RA or systemic lupus erythematosus, rapamycin increases the abundance of circulating T_{reg} cells and restores the T_H17 cell–T_{reg} cell balance, when used either as monotherapy or in combination with low-dose IL-2 (refs. 236–238). Correspondingly, rapamycin helps to control autoimmunity and restore T_{reg} cell-suppressive function in patients who are deficient in FOXP3 (refs. 239,240). Thus, rapamycin is an attractive option for enhancing T_{reg} cell function while suppressing autoreactive effector cells and related pro-inflammatory pathways, a suggestion confirmed in a mouse model of arthritis resulting from deficiency of the IL-1 receptor antagonist²⁴¹.

Whatever strategy is used to boost T_{reg} cell function, efficacy might improve when such strategies are further combined with therapies that suppress activated effector T cells, or, potentially, when they are applied during a period of remission. Such approaches might help to reduce the risk of relapse, considering that effector T cells can develop resistance to suppression by T_{reg} cells within an inflammatory environment¹⁸⁸.

Adoptive regulatory T cell therapy

In mouse models of T cell-mediated arthritis, transfer of T_{reg} cells can ameliorate disease, an approach that is especially effective if the transferred T_{reg} cells are antigen specific^{6,117,138–141,242}. In humans, such treatment would be particularly appealing if the therapeutic response were durable, either through persistence of the engrafted cells or through the establishment of infectious tolerance, whereby transferred T_{reg} cells render endogenous T cells suppressive. Adoptive T_{reg} cell therapies have not yet been explored in arthritis, but their advantages are well documented in the context of organ transplantation²⁴³.

Extensive academic and commercial effort is being devoted to optimizing both the efficiency and safety of adoptive T_{reg} cell therapies. Important variables will include the source of T_{reg} cells, be it autologous peripheral blood or allogenic umbilical cord blood, thymus, or peripheral blood; the protocol for T_{reg} cell culture and expansion; the screening methods used to ensure that the engrafted cells do not inadvertently include autoreactive effector T cells that express CD25 through activation; and the use of ancillary therapies such as low-dose IL-2 or rapamycin to favour T_{reg} cell over effector T cell expansion²⁴⁴. Mouse studies suggest that transferred T_{reg} cells could potentially be ‘purged’ of subpopulations at a higher risk of conversion to exT_{reg} cells⁵⁹. Fortunately, ex vivo expanded T_{reg} cells have proven to be at least as potent immune suppressors as endogenous T_{reg} cells, and conversion to pathogenic exT_{reg} cells has not yet been observed²⁴⁴.

Another frontier in T_{reg} cell therapeutics is the use of engineered T_{reg} cells. One approach has been to enforce FOXP3 expression through genetic editing, yielding a readily expandable population of functionally stable T_{reg} cells, potentially including antigen-specific T_{reg} cells able to suppress effector T cells in vitro and in vivo^{245,246}. A related approach is to genetically introduce a chimeric antigen receptor (CAR) or other

targeting receptor into T_{reg} cells to generate cells that selectively suppress or delete specific pathogenic T cells with a lower risk of systemic toxicity than effector cell-based CAR T cells²⁴⁷.

Conclusions

T_{reg} cells are crucial for the maintenance of immune homeostasis and protection against autoimmunity, yet, paradoxically, they are abundantly present in inflamed joints. Genetic, observational and experimental evidence suggest that this ‘T_{reg} paradox’ might reflect multiple distinct factors, including intrinsic T_{reg} cell dysfunction, extrinsic restrictions on T_{reg} cell efficacy, target resistance, and conversion of T_{reg} cells into pathogenic contributors. T_{reg} cell-directed therapeutics hold promise but their ability to overcome effector T cell resistance in the inflamed joint and to avoid the perils of T_{reg} cell instability, through which transferred cells might accelerate rather than suppress disease, will need to be established. Ultimately, clinical trials will be required to determine the safety and efficacy of T_{reg} cell-directed intervention as an effective therapeutic approach in arthritis.

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Emerging concepts and treatments in autoinflammatory interferonopathies and monogenic systemic lupus erythematosus

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Abstract

Over the past two decades, the number of genetically defined autoinflammatory interferonopathies has steadily increased. Aicardi–Goutières syndrome and proteasome-associated autoinflammatory syndromes (PRAAS, also known as CANDLE) are caused by genetic defects that impair homeostatic intracellular nucleic acid and protein processing respectively. Research into these genetic defects revealed intracellular sensors that activate type I interferon production. In SAVI and COPA syndrome, genetic defects that cause chronic activation of the dinucleotide sensor stimulator of interferon genes (STING) share features of lung inflammation and fibrosis; and selected mutations that amplify interferon- α/β receptor signalling cause central nervous system manifestations resembling Aicardi–Goutières syndrome. Research into the monogenic causes of childhood-onset systemic lupus erythematosus (SLE) demonstrates the pathogenic role of autoantibodies to particle-bound extracellular nucleic acids that distinguishes monogenic SLE from the autoinflammatory interferonopathies. This Review introduces a classification for autoinflammatory interferonopathies and discusses the divergent and shared pathomechanisms of interferon production and signalling in these diseases. Early success with drugs that block type I interferon signalling, new insights into the roles of cytoplasmic DNA or RNA sensors, pathways in type I interferon production and organ-specific pathology of the autoinflammatory interferonopathies and monogenic SLE, reveal novel drug targets that could personalize treatment approaches.

Sections

Introduction

Interferon production and signalling

Intracellular oligonucleotidopathies

STINGopathies

20S proteasomopathies

The IFNAR1 signalopathies


Monogenic SLE

Complex diseases with variable interferon signatures

The role of type I interferons: cause, amplification or bystander?

Treatment challenges

Conclusion

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Key points

- Genetic defects impairing intracellular nucleic acid and protein processing and/or STING activation cause autoinflammatory interferonopathies, indicating that intracellular sensor-mediated type I interferon production exhibits disease-specific and disease-overlapping clinical features.
- Identification of the genetic causes of monogenic systemic lupus erythematosus (SLE) reveals molecular mechanisms triggered by extranuclear nucleic acid that distinguish monogenic SLE from the autoinflammatory interferonopathies.
- Monogenic inborn errors of immunity caused by NF- κ B dysregulation, mitochondrial dysfunction or impaired DNA damage responses unravel sources of interferogenic nucleic acids that drive type I interferon production in the context of complex developmental and/or immune-dysregulation.
- Treatments that block interferon signalling and interferon-stimulated genes continue to elucidate the role of type I interferon in driving systemic and organ-specific inflammation.
- Novel drugs targeting cytoplasmic sensors in autoinflammatory interferonopathies and endosomal TLRs in paediatric-onset SLE provide new treatment opportunities for a broader spectrum of diseases with presumed interferon-mediated pathology.
- Advances in modelling tissue and organ inflammation start to unravel the effect of disease-causing mutations on critical tissues. These insights herald a new era of precision medicine in rare monogenic diseases.

Introduction

Although interferons are protective against numerous infections¹, they can also be pathogenic. Pathological processes are illustrated in early childhood-onset systemic autoinflammatory diseases (SAIDs) and are characterized by a persistent type I interferon response gene signature in the blood², which led to their categorization as type I interferonopathies². Overlapping and disease-specific organ involvement can now be linked to molecular mechanisms that provide insights into the diverse triggers of interferon production. These insights facilitate investigation of the role of type I interferon and cell-intrinsic dysregulation in causing organ inflammation and damage that define pathogenic subgroups of type I interferonopathies. Syndromes caused by increased signalling of cytoplasmic interferogenic self-nucleic acids characterize a group of ‘intracellular oligonucleotidopathies’ that encompass the spectrum of Aicardi–Goutières syndrome (AGS)^{3,4}. Constitutive or increased stimulator of interferon genes (STING) activation and overlapping pathology characterize individuals with STING-associated vasculopathy with onset in infancy (SAVI)^{5,6} and COPA syndrome^{7–9} as ‘STINGopathies’. Diseases caused by loss-of-function (LOF) mutations in components of the 20S proteasome, including proteasome-associated autoinflammatory syndromes (PRAAS), link proteostasis defects to interferon production and can be grouped as ‘20S proteasomopathies’^{10–13}. In 2014, syndromes caused by increased interferon- α/β receptor (IFNAR) and/or signal transducer and activator

of transcription 2 (STAT2) signalling, presenting as ‘pseudo-TORCH syndromes’ (resembling congenital TORCH infection), were grouped as ‘IFNAR signalopathies’^{14–18} (Fig. 1). The distinct pathology of systemic lupus erythematosus (SLE) is revealed by the discovery of monogenic causes of paediatric-onset SLE. With the advent of emerging therapies that target both interferon signalling and production, it is becoming increasingly important to understand the pathogenic mechanisms of interferon production and its subsequent effect on clinical manifestations in autoinflammatory interferonopathies, monogenic SLE and other diseases that present with complex immune dysregulation and heightened interferon signalling. In this Review, we summarize pathways leading to increased type I interferon production and signalling in autoinflammatory interferonopathies and monogenic SLE, we propose a pathogenesis-based classification of autoinflammatory interferonopathies (Box 1) and provide updates on interferon-dependent and -independent disease mechanisms that drive disease-specific organ inflammation and damage.

Interferon production and signalling

Interferons confer resistance to viral and bacterial infections and regulate immune responses, the cell cycle, survival and cell differentiation. Dysregulation of interferon therefore results in pathological processes¹⁹ (such as reduced interferon signalling, which causes infections and increased interferon production, leading to autoinflammation and autoimmunity) thus illustrating the need to tightly regulate type I interferon production and signalling in health and disease.

Interferon production

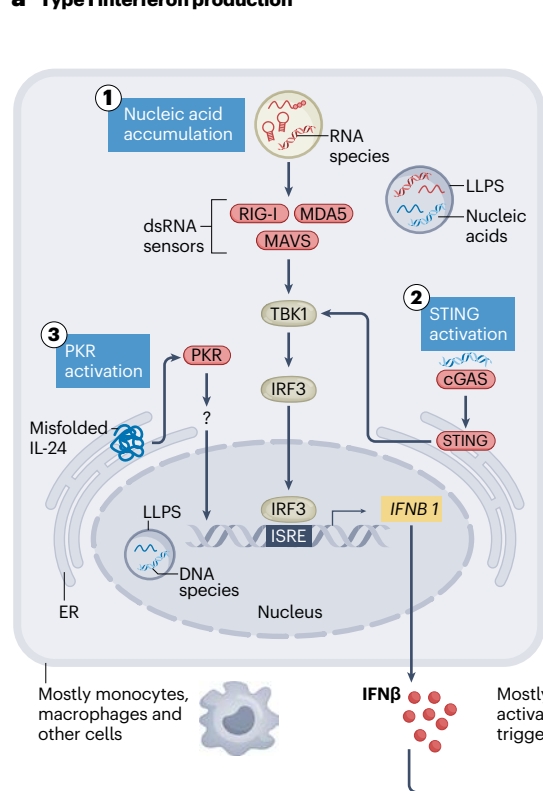
The type I interferon cytokine family consists of 13 human IFN α genes, along with single genes encoding IFN β , IFN κ , IFN ω and IFN ϵ , which are produced by both immune and non-immune cells²⁰. Immune cells, including macrophages and dendritic cells (DCs), produce type I interferon upon detecting pathogen components using pattern-recognition receptors²¹ (Fig. 1). Four Toll-like receptors (TLRs) are predominantly located on endosomal membranes in the endosome (including TLR3, TLR7, TLR8 and TLR9), others (including RIG-I-like receptors (MDA5, RIG-I and MAVS) along with cGAS and STING reside in the cytoplasm^{22,23}, all of which are linked to shared and tissue-specific activation pathways. Viral infections and cytoplasmic accumulation of foreign and self-double-stranded DNA (dsDNA) oligonucleotides are sensed by cGAS and trigger the synthesis of 2'3' cyclic GMP-AMP (cGAMP), which activates STING, and subsequent downstream pathways involving phosphorylation of TBK1 and dimerization of IRF3, nuclear translocation and transcription of *IFNBI*; the mechanisms that lead to STING-mediated NF- κ B activation, which peaks later than TNF-induced NF- κ B activation, have not fully been elucidated²⁴. Foreign and/or self-double-stranded RNA (dsRNA) trigger the MDA5 (encoded by *IFIH1*) and RIG-I (encoded by *RIGI*, also known as *DDX58*) pathway and TBK1-dependent type I interferon and NF- κ B activation²⁴ (Fig. 1). Gain-of-function (GOF) mutations in STING can lead to its constitutive activation⁵. Research in PRAAS identified misfolded IL-24 as an indicator of impaired proteostasis and activator of the PKR kinase and the PKR-mediated interferon stress response²⁵. In plasmacytoid DCs (pDCs), which have high basal levels of IRF7 and IRF8, activation of endosomal TLRs (TLR3, TLR7 and TLR9) leads to IRF7-mediated IFN α production^{21,26}. GOF mutations in *TLR7* that cause paediatric SLE^{27–29} are thought to trigger type I interferon production from pDCs through increased activation of endosomal TLR7 (which is highly expressed in these cells) (Fig. 1).

Interferon signalling

Type I interferons signal through IFNAR and activate the Janus kinase (JAK)–STAT signalling pathway. Activation of this pathway leads to nuclear translocation of the interferon-stimulated gene factor 3 (ISGF3) signalling complex, composed of phosphorylated STAT1 and STAT2 dimers bound to IRF9 (ref. 30). The ISGF3 complex binds to conserved DNA elements called interferon-sensitive response elements³⁰ and upregulates IFN α genes, ISGs or interferon-regulated genes; ISGs include antiviral proteins, chemokines and molecules involved in antigen presentation³¹ (Fig. 1). A trimeric complex consisting of STAT2, USP18 and ISG15 has emerged as a clinically important negative regulator of interferon signalling that functions by directly suppressing JAK1, JAK2 and TYK2 (refs. 32–34) (Fig. 1).

Under physiological conditions, nucleic acids released during cell death and interferon modulation by gut microbiota during trauma and everyday stresses are thought to be the main ‘triggers’ of a tonic, low-dose type I interferon production^{35,36}, which provides homeostatic control over stem cell maintenance and bone remodelling, and ensures that innate immune cells remain ready to respond to environmental challenges^{35,37–39}. Interferons prime immune responses by shaping the epigenome⁴⁰, amplifying immune responses⁴⁰ and preventing TNF-induced cross-tolerance^{30,41}. Interferons prime chromatin by depositing STAT1, IRF1 and positive histone marks and by increasing chromatin accessibility (which can last for days to weeks) and confer transcriptional memory and sustained expression of inflammatory genes, including ISGs^{30,42}. In autoimmune interferonopathies,

a Type I interferon production



b Type I interferon signalling and ISG production

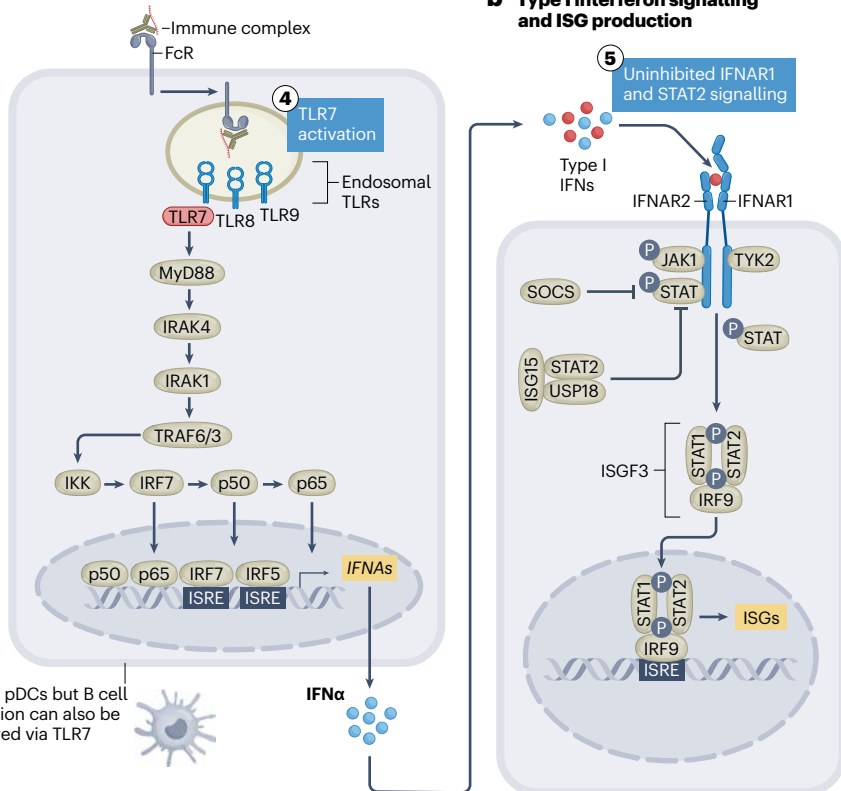


Fig. 1 | Pathway dysregulation in autoimmune type I interferonopathies and monogenic SLE. Distinct pathways involved in the production (a) and signalling (b) of type I interferon (IFN) are dysregulated in the interferonopathies and monogenic systemic lupus erythematosus (SLE). **a**, Left: cytosolic sensors of RNA (RIG-I and MDA5) and of DNA (cGAS) signal via the adaptors MAVS and STING, respectively, upon phosphorylation by TANK-binding kinase 1 (TBK1). IRF3 translocates into the nucleus and drives the transcription of *IFNB1* in monocytes, macrophages and other cell types, including endothelial cells, epithelial cells and fibroblasts. (1) Oligonucleotideopathies that comprise the Aicardi–Goutières syndrome (AGS) spectrum are caused by interferogenic nucleic acid accumulation that activates cytoplasmic nucleic acid sensors. (2) STINGopathies are caused by constitutive and/or increased activation of stimulator of interferon genes (STING). (3) The 20S proteasomopathies (proteasome-associated autoimmune syndromes (PRAAS) type) trigger interferon production via protein kinase R (PKR) activation. **a**, Right: plasmacytoid dendritic cells (pDCs) produce large amounts of IFN α in

response to nucleic-acid-containing immune complexes that are endocytosed via Fc receptors (FcR) and cross-presented to TLRs in the endosome (mostly TLR7 and TLR8 for types of RNA, or TLR9 for types of DNA). The activation of MyD88-dependent IRF7 phosphorylation leads to nuclear translocation and predominant transcription of *IFNA* genes. (4) Monogenic SLE is caused by Toll-like receptor 7 (TLR7) activation. **b**, Following type I interferon binding, the interferon- α/β receptor (IFNAR) complex initiates signalling through the adaptor kinases Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), leading to the activation and formation of a heteromultimer of signal transducer and activator of transcription 1 (STAT1, STAT2 and IRF9, known as the ISGF3 complex. This complex translocates into the nucleus and induces the transcription of interferon-stimulated genes (ISGs). IRFs mediate transcription of type-1 interferon genes and host defence genes by binding to interferon-sensitive response elements (ISREs) within *cis*-regulatory promoters and enhancers. (5) IFNAR signalopathies caused by loss of negative regulatory molecules result in increased IFNAR signalling. ER, endoplasmic reticulum.

Box 1 | Genetic causes and pathomechanisms of diseases with interferon signatures

Monogenic causes of diseases presenting with interferon signatures have revealed cytoplasmic nucleic acid and protein sensing pathways that lead to type I interferon production in diseases clinically characterized as autoinflammatory interferonopathies, monogenic systemic lupus erythematosus (SLE) and several complex inborn errors of immunity. These insights, coupled with disease-specific similarities and differences in organ manifestations, enable grouping of these diseases by type I interferon, producing pathomechanisms and clinical similarities.

- Autoinflammatory interferonopathies can be divided into diseases where the interferon response is triggered through activation of intracellular or cytoplasmic nucleic acid sensors, by constitutive activation of stimulator of interferon genes (STING) and/or by protein kinase R (PKR) activation, causing intracellular oligonucleotidopathies presenting with leukodystrophy (mostly Aicardi–Goutières syndrome (AGS)), STINGopathies with characteristic lung and vascular disease and 20S proteasomopathies presenting with panniculitis and hepatosteatosis, respectively). Lastly, conditions caused by increased or prolonged signalling through the interferon- α/β receptor (IFNAR) (*IFNAR1* signalopathies) present with prominent central nervous system disease that resembles AGS.
- The discovery of monogenic defects that cause paediatric-onset monogenic SLE point to dysregulation of clearance of

extracellular nucleic acid-containing microparticles and activation of autoreactive B cells that generate autoantibodies. These autoantibodies form immune complexes with microparticles, which drive IFN production in plasmacytoid dendritic cells and autoantibody and/or immune complex-mediated clinical features of SLE that are distinct from autoinflammatory interferonopathies.

- Several genetically defined, complex, immunodysregulatory and metabolic diseases that present with interferon signatures are being characterized. Monogenic defects that impair the DNA damage response or cause mitochondrial dysfunction point to leaked nuclear and mitochondrial nucleic acids as triggers of type I interferon production in the context of the complex pathology caused by these defects; in other complex diseases, pathomechanisms that lead to type I interferon signatures remain poorly characterized.
- Technical advances in generating better models of disease-associated tissue inflammation and damage, such as organoids, are poised to generate insights into the still poorly understood organ-specific vulnerability inflicted by disease-causing mutations and will continue to improve the molecular characterization and classification of these diseases.

epigenetic mechanisms could contribute to chronic elevation of ISGs, thus serving as a biomarker for ongoing disease activity. Many overlapping clinical features of autoinflammatory interferonopathies can be a direct result of increased tissue type I interferon concentrations (Fig. 2).

Intracellular oligonucleotidopathies

The initial definition of the AGS disease spectrum was based on clinical features resembling sporadic TORCH-like encephalopathies and increased type I interferon activity in the cerebrospinal fluid early in infancy⁴³. Subsequently, a subset of AGS-like disorders and familial Cree encephalitis were found to be caused by autosomal-recessive LOF mutations in the DNA exonuclease *TREX1* (refs. 3,44). Further investigation identified additional ‘AGS-causing’ genes: *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR*, *IFIH1*, *LSM11* and *RNU7-1* (refs. 3,4,45–48). Disease onset after infancy with distinct clinical phenotypes caused by specific mutations in these genes has also been described⁴⁹. These discoveries have spurred research aimed at unravelling the biology of dysregulated self-nucleic acid homeostasis and type I interferon signalling across the spectrum of AGS.

Clinical presentation

AGS can present in utero and is associated with fetal loss⁴³. Individuals with AGS can present in the first few months post-partum with psychomotor retardation, irritability, seizures, dystonia, spasticity or spastic paraparesis, resembling transplacentally acquired TORCH infections and described as pseudo-TORCH encephalitis⁴³. Typical brain MRI and CT findings include intracranial white matter calcifications, predominantly in the basal ganglia and thalami, ischaemic or haemorrhagic strokes and white matter loss resulting in cerebral atrophy and microcephaly⁴³. Dystonia and striatal necrosis are seen

in individuals with *ADAR* mutations^{50,51}, and progressive lower limb spastic paraparesis with normal imaging can occur in individuals with *ADAR*, *IFIH1* and *RNASEH2B*⁵² mutations; the latter mutation, *RNASEH2B*, causes over half of AGS cases⁴³. Furthermore, the interferon signature can be absent in individuals with *RNASEH2B* mutations⁵³. *SAMHD1* mutations lead to progressive cerebral artery stenosis and tangled vessels, known as moyamoya, predisposing to aneurysms, stroke and intracerebral haemorrhage^{54–56}. Approximately one-third of individuals with AGS develop chilblain lesions in cold-exposed acral regions⁴³. Autoimmune features include the presence of anti-nuclear antibodies (ANAs) and anti-phospholipid antibodies in one-third of individuals⁵⁷, autoimmune hepatitis^{43,58}, insulin-dependent diabetes mellitus, haemolytic anaemia^{43,59,60} and thyroiditis, which occurs in 4% of individuals⁴³. Individuals with *RNU7-1*-related disease can develop thrombotic microangiopathy and have the highest mortality among individuals with AGS, often associated with concurrent liver and kidney disease⁶¹.

Pathophysiology

The discovery of high IFN α activity in the cerebrospinal fluid led to the hypothesis that IFN α has a pathogenic role in central nervous system (CNS) disease⁴⁴. Mice engineered to overexpress IFN α in astrocytes develop progressive inflammatory encephalopathy, neurodegeneration and cerebellar calcium deposits⁶², resembling human disease and confirming the critical role of type I interferon in CNS inflammation and damage. Cellular models have identified multiple sources of cytoplasmic self-DNA and RNA species that, if not processed, accumulate in the cytoplasm; these can include transposable elements, mitochondrial, ribosomal and nuclear DNA or RNA, all of which can stimulate type I interferon production in vitro^{3,4,45–47,63,64}.

Tissue-specific mechanisms

In contrast to basal ganglia calcifications, which are present in many interferonopathies, leukoencephalopathy is a hallmark feature of AGS. However, identifying interferogenic nucleic acids that drive human brain pathology has proven challenging. Mouse knockout or LOF models of nuclease defects that are known to cause AGS in patients do not typically result in brain pathology. Seminal studies in *Trex1*-knockout mice identified endogenous transposable elements with reverse-transcribed DNA from long interspersed nuclear element 1 (L1, also known as LINE1) and long terminal repeat endogenous retroviruses that accumulated in the cytoplasm and triggered interferon production and myocarditis but not CNS disease⁶³. Subsequent *in vitro* studies showed that two additional AGS-associated genes, *ADAR* and *IFIH1*, mutations that cause AGS6 and AGS7, respectively, encode proteins that regulate sensing of short interspersed nuclear elements (SINEs) most of which are Alu elements. Alu elements are highly abundant primate-specific, around 300-bp long oligonucleotides that account for ~10% of the human genome⁶⁵ and are absent in mice. Together with L1s, they make up over 30% of the human genome^{66,67}. Alu elements form hairpin structures and are the primary self-ligands for the intracellular dsRNA sensor MDA5 (encoded by *IFIH1*); MDA5 molecules form filaments along the length of double-stranded Alu (dsAlu) duplexes; these Alu-containing filaments oligomerize and induce MAVS and IRF3-dependent type I interferon production⁶⁷. Alu elements regulate mRNA stability⁶⁸ and are thought to contribute to a low, tonic,

homeostatic type I interferon response that primes and prepares the CNS for possible infections⁶⁹. Both Alu and L1 elements can be reverse transcribed, a process that produces RNA:DNA intermediates and cDNA that accumulates in the nucleus and/or cytoplasm^{70,71}. The abundant expression of L1 and primate-specific Alu elements, particularly in the CNS, and their tight regulation, make them potential interferogenic triggers in AGS-mediated CNS disease⁶⁵. The contribution of mutant AGS-causing nucleases to interferon production is described later in this Review and focuses on RNA, particularly on L1 and Alu processing.

The dsRNA-binding enzyme ADAR, encoded by *ADAR*, edits Alu duplexes by catalysing the hydrolytic deamination of adenosine to inosine, known as A-to-I RNA editing (Fig. 3). This editing makes the Alu duplexes no longer recognizable to MDA5 (encoded by *IFIH1*) and prevents type I interferon production^{72–74}. However, mutant MDA5 containing AGS7-causing *IFIH1* mutations can bind edited Alu–dsRNA and generate signalling-competent filaments that trigger type I interferon production. The ability of edited and unedited Alu elements to activate mutant *IFIH1*, which encodes mutant MDA5, points to a common pathology of AGS-causing mutations that involve the accumulation of interferogenic oligonucleotides, which drive all forms of AGS, including those caused by mutant MDA5. Conversely, AGS-causing *ADAR* LOF mutations in the catalytic domain^{46,51} that result in loss of the editing function enable accumulation of unedited dsAlu duplexes (also known as inverted Alu repeats or Alu:Alu hybrids)^{75,76} that assemble signalling-competent wild type MDA5 filaments and mediate

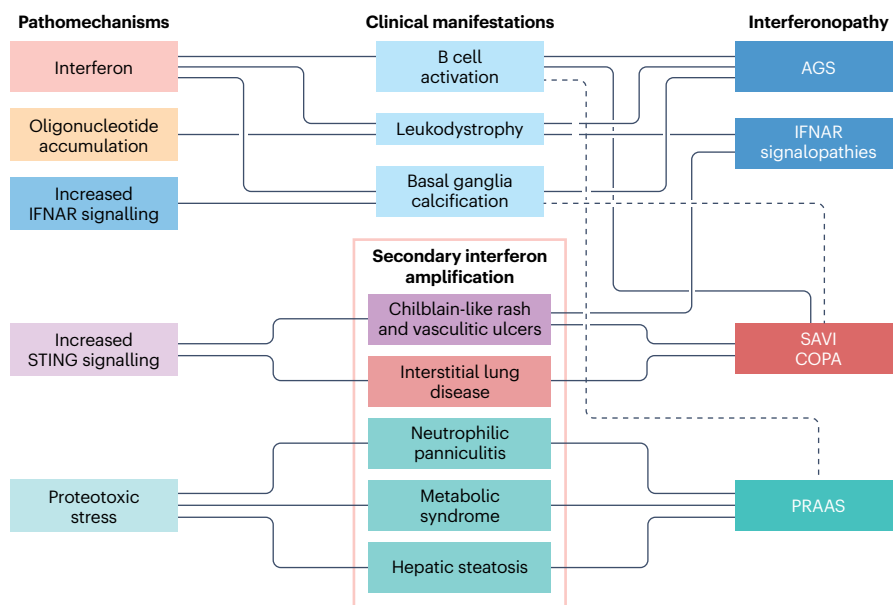


Fig. 2 | Pathomechanisms causing distinct and overlapping clinical manifestations in the interferonopathies. Interferon-dependent and other clinical features are listed. On the left, the pathomechanisms causing monogenic IFNopathies are shown and include intracellular oligonucleotide accumulation, increased interferon- α/β receptor (IFNAR) signalling, increased cGAS–STING (cyclic GMP–AMP synthase–stimulator of interferon genes) signalling and proteotoxic stress. All pathomechanisms (left column) lead to increased type I interferon production but can cause different clinical manifestations (middle column). Type I interferon directly drives a subset of clinical manifestations (shown in blue in the middle column), including B cell activation, leukodystrophy and basal ganglia calcifications. The rose box outline (in the middle column)

indicates the clinical manifestations that type I interferon can aggravate. Leukodystrophy, chilblain-like rash and vasculitic ulcers, interstitial lung disease, neutrophilic panniculitis, metabolic syndrome and hepatic steatosis are disease-specific and are assigned to the diseases they occur in. Clinical syndromes are listed in the right column and include Aicardi–Goutières syndrome (AGS), IFNAR1 signalopathies (limited to STAT2, ISG15 and USP18), STING-associated vasculopathy with onset in infancy (SAVI), COPA syndrome and 20S proteasome-associated autoinflammatory syndromes (PRAAS). Known overlapping features are depicted by solid lines that represent direct connections between pathomechanisms, clinical manifestations and clinical syndromes. Dotted lines represent possible connections or a rare manifestation.

MAVS–IRF3-dependent *IFNBI* transcription. These findings expose a unique vulnerability of the human brain to the accumulation of unprocessed interferogenic RNA metabolites⁶⁹, which could have evolved

with the expansion of Alu elements in the brains of humans and other primates. The relative contribution of Alu elements, L1 and other RNA and DNA oligonucleotides as interferogenic oligonucleotide sources

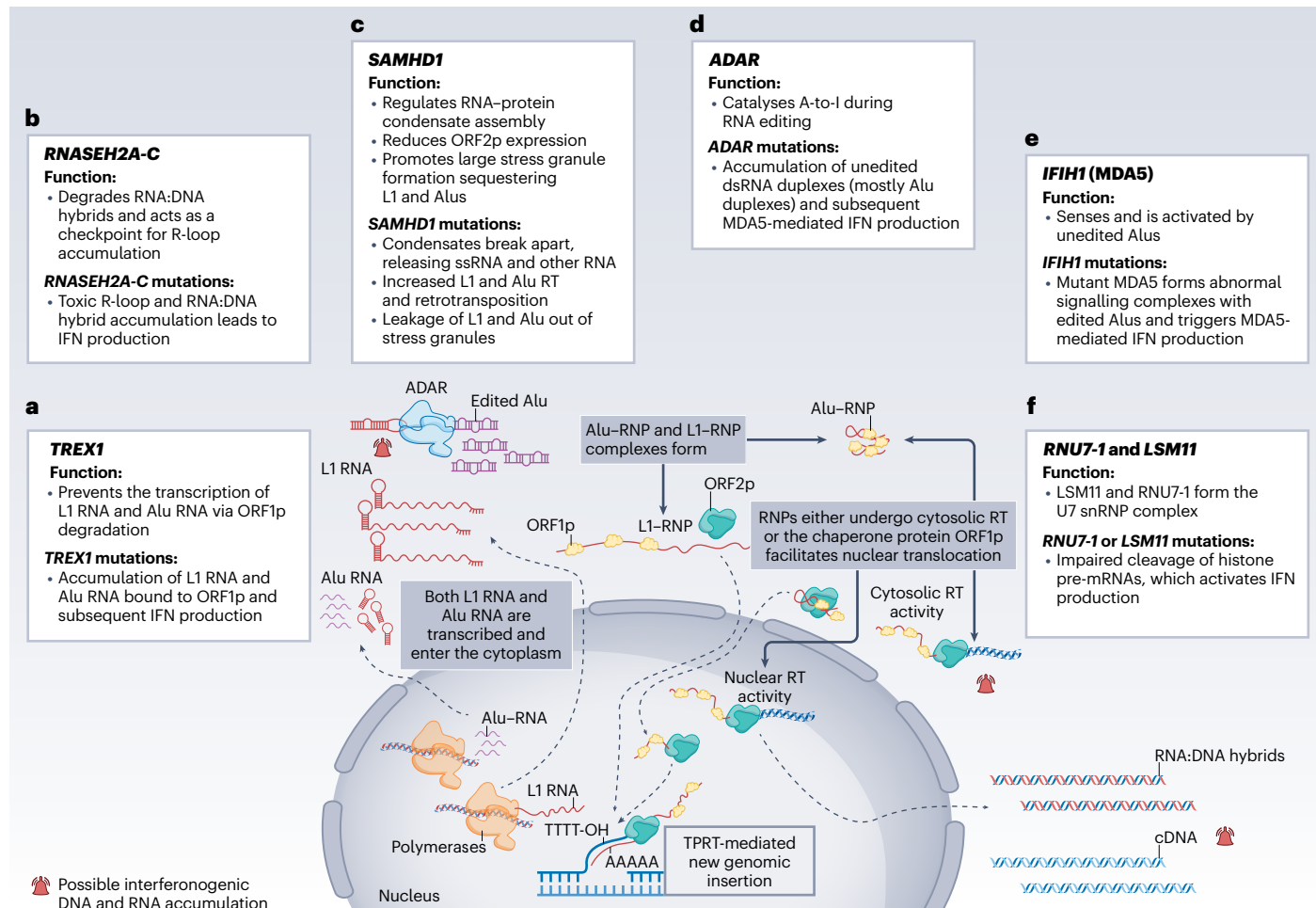


Fig. 3 | Pathogenesis of intracellular oligonucleotideopathies. Nucleases that cause Aicardi–Goutières syndrome (AGS) variably affect DNA and RNA processing. This figure depicts the functional effect of the AGS-causing endonuclease mutations on RNA processing (in boxes) with a focus on transposable retroelement (long interspersed nuclear element 1 (L1) and Alu elements) processing (middle section). Alus are mainly located in the intronic 3' UTR; both Alus and L1 elements are transcribed in the nucleus and enter the cytoplasm. Inverted Alus can form duplexes that need to be edited by ADAR (deaminase acting on RNA-1), which renders them non-immunogenic. Two L1-encoded polypeptides, the chaperone protein open-reading frame 1 (ORF1p) and ORF2p, an enzyme that has reverse transcriptase and endonuclease activity, are required for L1 retrotransposition^{71,327}. L1 and/or Alu RNA bind to ORF1p to assemble L1 or Alu-ribonucleoprotein (RNP) complexes. Upon recruitment of ORF2p, L1 or Alu elements can be reverse transcribed in the cytoplasm or they can translocate to the nucleus, where they can undergo reverse transcription by a process known as target-primed reverse transcription (TPRT); this process integrates the L1 or Alu cDNA into the genome (which is a rare event). **a**, Loss-of-function mutations in *TREX1*, a cytosolic DNA nuclease, results in cytoplasmic accumulation of interferogenic single-stranded and double-stranded DNA (ssDNA and dsDNA), which can trigger aberrant innate immune activation via cytosolic DNA sensors such as cGAS and STING. *TREX1* also restricts the nuclear reverse transcription of single-stranded L1 (ssL1) RNA and Alu RNA by facilitating proteasome-mediated degradation of ORF1p. ssL1 and Alu RNA

accumulation can activate RNA sensors. **b**, RNASEH2 controls R-loop homeostasis. Impairment of RNASEH2 leads to the accumulation of toxic R-loops that cause replication stress by stalling replication forks, causing DNA damage and increasing unprocessed RNA:DNA hybrids that lead to type I interferon production through the cGAS–STING pathway. **c**, SAMHD1 regulates cellular concentrations of single-stranded RNA (ssRNA). SAMHD1 deficiency leads to the disintegration of RNA-protein condensates that leak immunostimulatory double-stranded RNA (dsRNA) sequestered therein, causing aberrant RIG-I and MDA5-dependent type I interferon production. SAMHD1 serves as a negative regulator of L1 and Alu retrotransposition by reducing ORF2p expression through unclear mechanisms. SAMHD1 promotes the formation of large stress granules that sequester L1s and prevents L1 retrotransposition. **d**, ADAR edits dsRNAs, including Alu duplexes, by catalysing A-to-I RNA editing, which makes Alu duplexes no longer recognizable to MDA5 (encoded by *IFIH1*) and prevents type I interferon production. ADAR dysfunction leads to the accumulation of unedited dsRNA Alu molecules and MDA5-dependent interferon production. **e**, Mutant MDA5 forms abnormal signalling complexes with edited, 'imperfect' Alu duplexes that trigger type I interferon production. **f**, Mutations in *LSM11* and *RNU7-1* prevent the formation of the U7–snRNA complex and endonucleolytic cleavage of replication-dependent histone pre-mRNAs, which activates intracellular sensors (such as RIG-I-like receptors and Toll-like receptors (TLRs)) to cause type I interferon production. Supplementary Fig. 3 shows curated disease-causing mutations. RT, reverse transcription.

in the CNS remains unresolved, but redundancy of most AGS-causing genes in processing not only DNA but also transposable elements and RNA accumulation could indicate the need for a combined effort in processing transposable elements to restrict type I interferon production in the CNS (Fig. 3). Although TREX1 functions as a DNA processing exonuclease⁷⁷ that degrades DNA repair metabolites and reverse-transcribed DNA⁷⁸, selected disease-causing *TREX1* mutations that retain single-stranded DNA (ssDNA) exonuclease activity fail to suppress L1 retrotransposition to the nucleus^{79,80}. These observations led to the discovery of an expanded role of TREX1 in restricting nuclear translocation and reverse-transcription of ssL1 RNA by facilitating proteasome-mediated degradation of the open-reading frame 1 protein (ORF1p) that chaperones L1 nuclear translocation⁷⁹. Accumulation of ssL1 bound to ORF1p (L1-RNP) in the cytoplasm can trigger RIG-I-dependent and MDA5-dependent interferon production⁸¹.

Disease-causing *RNASEH2A*, *RNASEH2B* or *RNASEH2C* mutations destabilize the RNaseH2 enzyme complex⁸² and impair degradation of RNA:DNA hybrids^{83–85}. RNaseH2, a DNA–RNA helicase, degrades RNA moieties (including DNA hybrids and RNA:DNA primers of Okazaki fragments) and functions as a ribonucleotide excision repair enzyme that excises ribonucleotides erroneously incorporated into DNA during transcription⁸⁶. A critical role for RNaseH2 in regulating R-loop homeostasis is emerging⁸⁵. R-loops are generated during homeostatic transcription when nascent RNA reanneals with its template DNA duplex, but transcription–replication conflicts can occur when unresolved, damaging or toxic R-loops lead to DNA instability (owing to dsDNA breaks or stalling of replication forks)⁸⁷. In the absence of RNaseH2, dsDNA breaks and replication stress occur when DNA replication encounters gene transcription in a head-on collision that can result in the inability to repair stalled replication forks⁸⁵ and in the accumulation of toxic RNA:DNA hybrids that trigger an interferon signature in a cGAS–STING-dependent manner⁸⁸. Whether these processes cause CNS disease in individuals with AGS with mutations in *RNASEH2* subunits and whether a role for increased DNA damage caused by R-loop toxicity (which has been linked to neurodegenerative disorders⁸⁹) contribute to AGS pathology remain unclear (Fig. 3).

The enzyme SAMHD1 (encoded by *SAMHD1*) degrades cellular 2'-deoxynucleoside-5'-triphosphate (dNTP) and regulates the cytoplasmic dNTP pool that restricts viral replication^{45,90}. SAMHD1 also has single-stranded RNA (ssRNA) 3' exonuclease activity that regulates membraneless RNA–protein assemblies in cytoplasmic liquid-phase condensates^{91,92}. Dissolution of RNA–protein condensates in the absence of SAMHD1 releases interferogenic dsRNAs into the cytoplasm, triggering RIG-I and MDA5 (encoded by *IFIH1*) and subsequent type I interferon production⁹². SAMHD1 also serves as a negative regulator of L1 and Alu retrotransposition by reducing ORF2p expression through unclear mechanisms (Fig. 3). Last, SAMHD1 disrupts formation of the “eIF4F translation initiation complex”⁹³ and promotes large stress granule formation, sequestering L1s and preventing L1 retrotransposition⁹³. Pathomechanisms that cause moyamoya manifestations remain unresolved. A single homozygous AGS8-causing mutation (p.G211S) in *LSM11* and compound heterozygous AGS9-causing LOF mutations in *RNU7-1* (refs. 48,61) highlight a role of the nucleus as a subcellular source of interferogenic DNA in AGS⁴⁸. LSM11 binds to the U7 small nuclear ribonucleoprotein (RNP) encoded by *RNU7-1* to form a large replication-dependent histone pre-mRNA processing complex. Disease-causing mutations in *RNU7-1* and *LSM11* impair complex formation, resulting in DNA leakage into the cytoplasm and chronic type I interferon production through the cGAS–STING pathway⁴⁸. In addition

to triggering interferon production, nucleic acid accumulation can trigger neuronal cell death, as observed in human induced pluripotent stem cells-derived neurons lacking *TREX1* or *ADAR*. In the latter case, cell death was IFN β and PKR-dependent⁶⁹ and did not require reverse transcription to DNA.

Other potential oligonucleotidopathies with broad immune activation

Three syndromes caused by specific mutations in *TREX1*, *SAMHD1*, *STING1* (also known as *TMEM173*), *IFIH1* or *RIG1* present later in life. Heterozygous, autosomal-dominant, deleterious frameshift or nonsense mutations in the C-terminus of *TREX1* give rise to retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCLS). Patients typically present with progressive visual field defects caused by small vessel vasculopathy affecting the retina and brain in early adulthood⁸⁰. Neurological features include stroke, motor impairment and cognitive decline. Other features include Raynaud phenomenon, micronodular cirrhosis and glomerular disease, which can progress to end-stage renal disease; the rate of mortality within 5 years of disease presentation is high^{80,94–96}. Mutant *TREX1* in patients with RVCLS lacks a C-terminal transmembrane anchor and translocates into the nucleus⁸⁰. This translocation leads to impaired assembly of an oligosaccharyltransferase complex⁹⁷, which triggers downstream TBK1-dependent, but cGAS–STING-independent, immune activation and autoantibody production⁹⁷. The effects of interferon and interferon-independent mechanisms on the causes of specific features of RVCLS remains under study.

Chilblain lupus (including sporadic and inherited forms) can be caused by heterozygous autosomal-dominant *TREX1* mutations (particularly p.D18N⁹⁸), a single mutation (p.I201N) in *SAMHD1* (ref. 99) and a mutation (p.G166E) in the cGAMP-binding domain of *STING1* (ref. 100). Individuals with chilblain lupus present with painful bluish-red papular or nodular skin lesions in acral areas, often triggered by exposure to cold and moisture⁹⁸. The *TREX1* mutation can lead to the development of leukoencephalitis and basal ganglia calcifications, often later in life. Selected mutations in the dsRNA sensors MDA5 (encoded by *IFIH1*) and RIG-I (encoded by *RIG1*, also known as *DDX58*), cause type 1 and type 2 Singleton–Merten syndrome (SGMRT1 and SGMRT2)¹⁰¹. Individuals with Singleton–Merten syndrome often present with aortic, valvular and tendon calcifications, dental dysplasia and early loss of dentition¹⁰². Neurological features tend to be mild; intracranial calcifications have been observed^{103,104}. Pathomechanisms causing acro-osteolysis, osteoporosis, ocular manifestations (such as glaucoma) and psoriasis-like skin disease in Singleton–Merten syndrome remain unresolved¹⁰⁵. Eleven (mostly missense) mutations in *TREX1* (ref. 106) and several (mostly heterozygous missense) variants in *RNASE2HA*, *RNASE2HB* and *RNASE2HC*¹⁰⁷ are enriched in polygenic, non-syndromic SLE cohorts, with a relative risk of 25.3 for patients with SLE carrying a heterozygous *TREX1* variant¹⁰⁶. As the parents of individuals with AGS with heterozygous variants typically do not develop SLE, other genetic or environmental factors are likely present in individuals who develop SLE.

STINGopathies

SIVI is caused by autosomal-dominant, often de novo, GOF mutations in *STING1*. *STING1* encodes the intracellular sensor and adaptor protein STING involved in dinucleotide sensing^{5,6}. Since the initial identification of mutations in *STING1*, additional disease-causing missense mutations have been found in exons 3, 5, 6 and 7 (Fig. 4), with both

de novo and inherited variants and an autosomal-recessive form of SAVI reported^{5,108–110}. COPA syndrome arises from autosomal-dominant missense mutations in *COPA*, located on chromosome 1 (1q23.2). These mutations occur within the WD40 domain of the N-terminal portion of *COPA*, which encodes the COP α subunit of the coatomer complex 1 (ref. 111). This complex regulates vesicular retrograde transport between the Golgi apparatus and the endoplasmic reticulum (ER)¹¹¹. Phenotypic similarities, a robust ISG signature and a pathogenic link between STING and *COPA*⁷ justify the inclusion of these diseases as STINGopathies.

Clinical presentation

Individuals with SAVI typically develop symptoms within the first months of life, including rash, fever and systemic inflammation marked by elevated acute phase reactants, but later presentations have been discovered^{5,6,112–114}. Vasculopathic lesions, which are most prominent in cold-sensitive acral areas, manifest variably as violaceous plaques or nodules on the hands, feet, face, nose or ears, leading to autoamputations or surgical amputations. Additional features of SAVI include nail dystrophy, acro-osteolysis (phalangeal osteolysis), and nasal septal perforation^{5,6,112–114}. Approximately 70% of patients with SAVI present with lung fibrosis, which can worsen over time and predispose individuals to infections and respiratory failure^{6,100,108,113–118}. The *STING1* p.V155M mutation is associated with more severe lung disease than seen with other *STING1* mutations^{5,6,112–114}. Non-genetic factors such as chronic aspiration and gastroesophageal reflux disease, infection and smoking contribute to the severity of the lung disease. Early mortality in SAVI is often a result of progressive lung disease, which is frequently exacerbated by infections^{5,6,113,114}.

Individuals with COPA syndrome typically present with variable severity of interstitial lung disease¹¹³. Articular manifestations appear in the early teen years, and most individuals with COPA have elevated acute phase reactants, indicating systemic inflammation^{111,119–122}. Auto-immune features are prevalent in both SAVI and COPA syndrome. Up to 62.5% of individuals with SAVI¹²³ and up to 80% of those with COPA have ANAs¹¹¹. Rheumatoid factor and anti-cyclic citrullinated peptide antibodies positivity are reported in 43% of patients with COPA with erosive arthritis¹¹¹. Low titres of antineutrophil cytoplasmic antibodies (ANCAs), specifically those that target proteinase-3 (PR3-ANCAs), which leads to a cytoplasmic (C-ANCA) staining pattern, and myeloperoxidase (MPO-ANCAs), which leads to a perinuclear (P-ANCA) staining pattern^{111,124}, are seen in 21–44% of individuals with COPA and are linked to kidney disease^{121,124}. Kidney biopsies reveal variable pathology that includes necrotizing glomerular lesions with and without immune complex deposition, IgA nephropathy with necrotizing lesions, mesangial hypercellularity¹²⁵ and endomembranous immune complex deposits⁵³. C-ANCAs and P-ANCAs are described in rare patients with SAVI who also develop kidney disease, presenting as pauci-immune, extra-capillary necrotizing and/or crescent glomerulonephritis¹²⁶. Progressive lung or glomerular disease is a major contributor to early mortality in SAVI and COPA syndrome¹²⁷.

Pathophysiology

The cGAS–STING pathway is critical in coordinating viral immunity and maintaining self-nucleic acid homeostasis^{128,129}. STING is a dimeric, ER transmembrane adaptor molecule, which undergoes conformational changes upon activation, leading to its oligomerization in the Golgi network. TBK1 is recruited to the STING oligomer and autophosphorylates on the amino acid Ser172, which is a necessary step for subsequent STING phosphorylation at amino acid

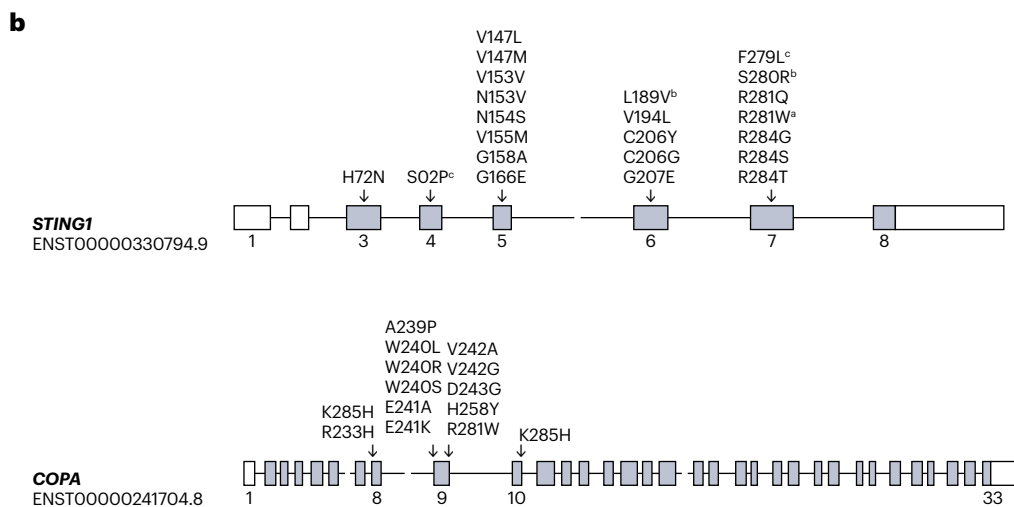
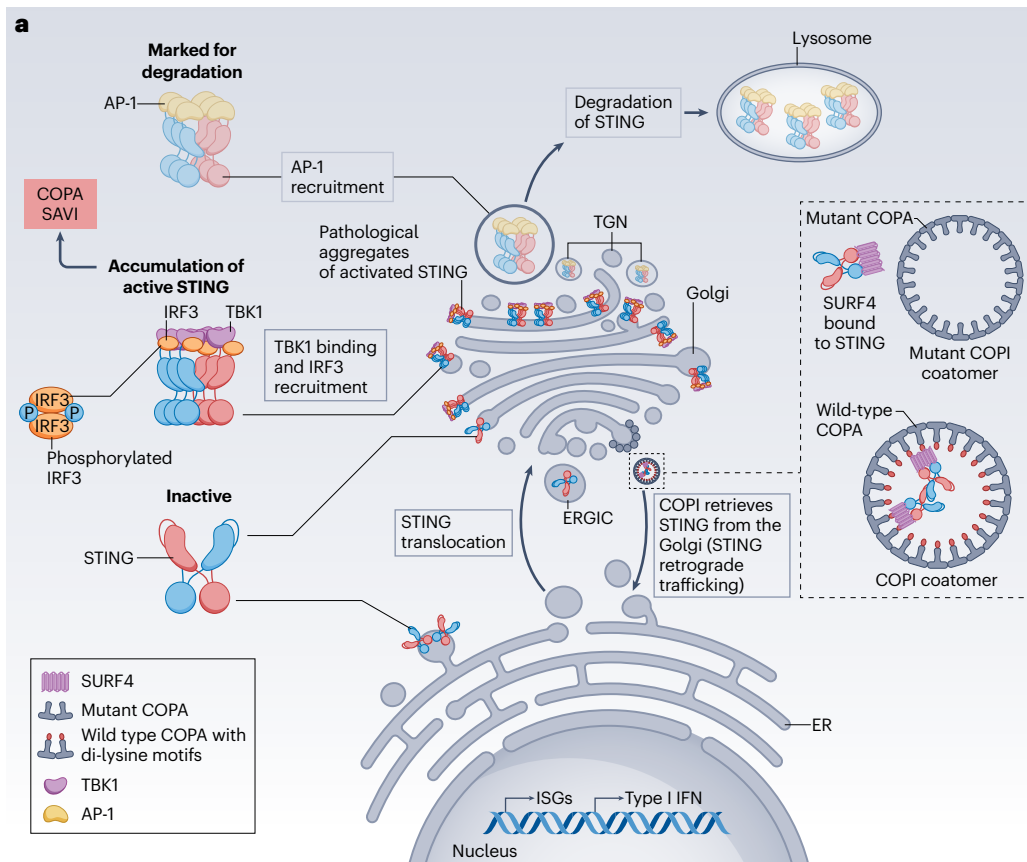
position Ser366 (ref. 130). This latter phosphorylation enables IRF3 recruitment to the now phosphorylated pLxIS motif of STING, followed by IRF3 phosphorylation, dimerization¹³¹ and nuclear translocation with transcriptional upregulation of *IFNBI* and of selected ISGs. TBK1 also facilitates dsDNA-mediated activation of NF- κ B²⁴, promoting pro-inflammatory gene transcription through mechanisms that are not fully understood^{132,133}. In AGS, aberrant accumulation of cytoplasmic self-DNA leads to cGAS activation and the generation of cGAMP, which activates STING and downstream responses. By contrast, disease-causing *STING1* mutations do not require cGAMP binding for *IFNBI* transcription in vitro^{5,6}. STING traffics in subcellular compartments that tightly regulate its activation¹³⁴.

During homeostasis, STING shuttles between the ER and Golgi¹³⁴, translocating to the ER–Golgi intermediate compartment (ERGIC) and/or Golgi, facilitated by STING ER exit protein 1 (STEEP-1) and coat protein complex II (COPII)-mediated trafficking¹³⁵. Stromal interaction molecule 1 (STIM-1), an ER retention factor, retains STING in the ER¹³⁶ a function disrupted in patients with STIM-1 deficiency¹³⁷. During homeostasis STING maintains low levels of tonic IFN β production, predominantly residing in the ER and undergoing retrograde trafficking from Golgi to ER mediated by the coat protein complex I (COPI) cargo complex. Following activation, *COPA*, which encodes COP α , a component of the COPI complex, retrieves STING from the Golgi facilitated by the cargo receptor surfet 4 (SURF4). SURF4 binds to STING and *COPA* via two C-terminal di-lysine motifs⁷ (such as KKXX and KKKXX)^{111,138} (Fig. 4). Disease-causing *COPA* mutations lead to the loss of these C-terminal di-lysine motifs¹³⁸, resulting in the inability to retrieve STING from the ER^{7–9}. In 2023, two independent groups reported individuals with a de novo mutation at position p.R99 in *ARF1*, which encodes a small GTPase that recruits and binds cytoplasmic COPI to the Golgi membrane. Individuals with this mutation present with an inflammatory phenotype, including variable chilblain-like lesions and cognitive impairment^{139,140}. These symptoms were linked to aberrant release of mitochondrial DNA (mtDNA) and the accumulation of active STING at the Golgi and/or ERGIC caused by defective retrograde transport.

Activated STING is degraded through lysosomal degradation; oligomerized STING can recruit the adaptor protein complex-1 (AP-1), which competes with TBK1 for similar STING-binding sites and sorts STING into lysosomes¹⁴¹ (Fig. 4). By contrast, nascent non-activated STING is subject to polyubiquitylation by the SEL1L–HRD1 ER-associated degradation (ERAD) protein complex that mediates proteasomal degradation¹⁴². Activated STING dimers can form a pore that functions as a proton channel, sufficient for IRF3-independent LC3B lipidation (a type of post-translation modification), autophagosomal degradation and NLRP3 activation¹⁴³; however, a role of this process in disease pathogenesis is still unclear.

Tissue-specific mechanisms

Peripheral vasculitis and interstitial lung disease are hallmarks of SAVI. Chilblain-like lesions, and Raynaud-like peripheral vasculopathy are variably seen in individuals with SAVI, chilblain lupus, AGS and were described in COVID-19 but are rarely seen in SLE. Lesional skin biopsies from individuals with COVID-19 showed type I interferon-positive macrophages in the skin adjacent to areas of endothelial cell damage¹⁴⁴ possibly owing to macrophage-dependent endothelial damage and cGAS–STING-dependent interferon production. Although not yet experimentally confirmed, the hypothesis that tissue-specific dsDNA accumulation in endothelial cells and macrophages in the skin might



trigger cGAS–STING-mediated chilblain-like lesions (that differ from the interferogenic nucleic acids that trigger CNS disease) is intriguing. This idea is supported by functional studies of two heterodimeric dominant negative *TREX1* mutations (D200N/WT or D18N/WT), which predominantly cause chilblain lesions¹⁴⁵. These *TREX1* mutations disrupt the proper positioning of two Mg²⁺ ions, preserving DNA binding but impairing dsDNA degradation, which leads to dsDNA accumulation, dsDNA–cGAS–STING activation and interferon production. The role of the cGAS–STING pathway in endothelial cell damage and senescence has been attributed to mitochondrial dysfunction leading to aberrant

mtDNA release¹⁴⁶, and mitochondrial adaptations to cold-induced stress are associated with mtDNA release into the cytoplasm¹⁴⁷, potentially linking cold exposure to endothelial damage.

The development of interstitial lung disease in SAVI and COPA syndrome, but not in AGS and PRAAS, prompts questions about cell-intrinsic pathomechanisms of STING¹⁴⁴. STING is expressed in various cell types in the lung, including endothelial cells and macrophages⁵. Immunohistochemistry of lung autopsies from patients with COVID-19 showed STING activation and phosphorylation in endothelial cells and macrophages, correlating with progressive and lethal disease, and was associated with

Fig. 4 | Pathogenesis of STINGopathies. This figure depicts stimulator of interferon genes (STING) function and pathomechanisms that drive pathogenesis in STING-associated vasculopathy with onset in infancy (SAVI) and COPA syndrome. **a**, STING-activating mutations and mutations that destroy dilysine motifs on COPA (which prevent STING retrieval from the Golgi) cause increased STING oligomerization and sublocalization in the Golgi with activation of downstream pathways. During homeostasis, STING traffics between the endoplasmic reticulum (ER) and ER Golgi intermediate compartment (ERGIC). STING can bind to the adapter SURF4, which binds to COP-I-coated vesicles, mediating the retrograde transport of STING back to the ER. cGAMP binding activates STING and induces its exit from the ER. STING translocates to the ERGIC and the Golgi, where it recruits TANK-binding kinase 1 (TBK1), which phosphorylates STING itself and interferon regulatory factor 3 (IRF3). Phosphorylated IRF3 translocates to the nucleus and induces transcription

of type I interferon genes and interferon-stimulated genes (ISGs). Activated STING can move to the trans-Golgi network (TGN) where it is marked for degradation and delivered to the lysosomes. In SAVI and COPA syndrome, STING gain-of-function mutations and the inability of SURF4-bound STING to bind mutant COPA, respectively, increase STING activation in the Golgi. **b**, Disease-causing *STING1* and *COPA* variants are depicted. First, last and affected exons are numbered. Disease-causing *STING1* variants are located in exons 3–7. The *STING1* variant p.R284W causes disease in homozygosity (marked ^a). *STING1* variants L189V and S280R (denoted ^b) and *STING1* variants S102P and F279L (marked ^c) occurred in *cis* in two patients with SAVI. Disease-causing *COPA* mutations affect a di-lysine motif that binds to SURF4. Disease-causing mutations localize mostly to exon 9. Two mutations are located on exon 8, and one mutation is located on exon 10. The process used to curate the genes depicted is detailed in Supplementary Box 1. AP-1, adaptor protein complex-1.

diffuse alveolar damage and extensive hyaline membrane formation¹⁴⁴. A role for non-haematopoietic cells in SAVI-associated lung disease is supported by experiments in mice that have a mutation in mouse *Sting* p.N153S; these mice develop lung fibrosis, the lethality and severity of which are not improved by bone marrow transplantation¹⁴⁸. STING activation in endothelial cells leads to endothelial cell layer damage with decreased expression of the endothelial cell marker CD31. CD31 (also known as PECAM-1) is highly expressed at endothelial cell–cell junctions and functions as an adhesive stress-response protein to maintain both endothelial cell junctional integrity and speed restoration of the vascular permeability barrier following inflammatory or thrombotic challenge¹⁴⁹, and increased expression of markers of endothelial inflammation (inducible nitric oxide synthase), adhesion and activation (E-selectin and ICAM-1) and coagulation (tissue factor)⁵. Furthermore, type I interferon induced endothelial cell apoptosis and impaired endothelial progenitor maturation¹⁵⁰, suggesting endothelial cell-specific interferon-dependent and interferon-independent pathologies¹⁵¹.

20S proteasomopathies

PRAAS syndromes are caused by autosomal-recessive LOF mutations in immunoproteasome genes such as *PSMB8*, *PSMB9* and *PSMB10* or the assembly molecule *PSMG2*. In addition, biallelic LOF mutations in *PSMB4*, or digenic LOF mutations in *PSMA3*, *PSMA5* or *PSMB4* in combination with one of three catalytic subunits, *PSMB8* encoding β5i, *PSMB9* encoding β1i and *PSMB10* encoding β2i, which are constitutively expressed at high levels in immunoproteasomes in immune cells and induced by oxidative stress and pro-inflammatory cytokines in most other cells¹⁵², or dominant negative LOF mutations in *PSMB8*, *PSMB9*, *PSMB10* and *POMP*, can also cause PRAAS syndromes^{10,12,13,153–159}. Biallelic variants in *PSMB1*, which encodes a component of the 20S core proteasome, are associated with neurodevelopmental delay (NDD) and systemic inflammation¹⁶⁰. Since 2017, a novel group of neurodevelopmental proteasomopathies caused by heterozygous LOF mutations in genes encoding components or assembly units of the 19S regulatory cap including *PSMD12* (refs. 161–164), *PSMD11* (ref. 165) and the proteasomal ATPases, *PSMC1* (ref. 166), *PSMC3* (ref. 167) and *PSMC5* (ref. 168) have been associated with NDD and the autism spectrum disorders with mild or no systemic features of inflammation, suggesting a distinct role of the 19S regulatory unit in these conditions.

Clinical presentations

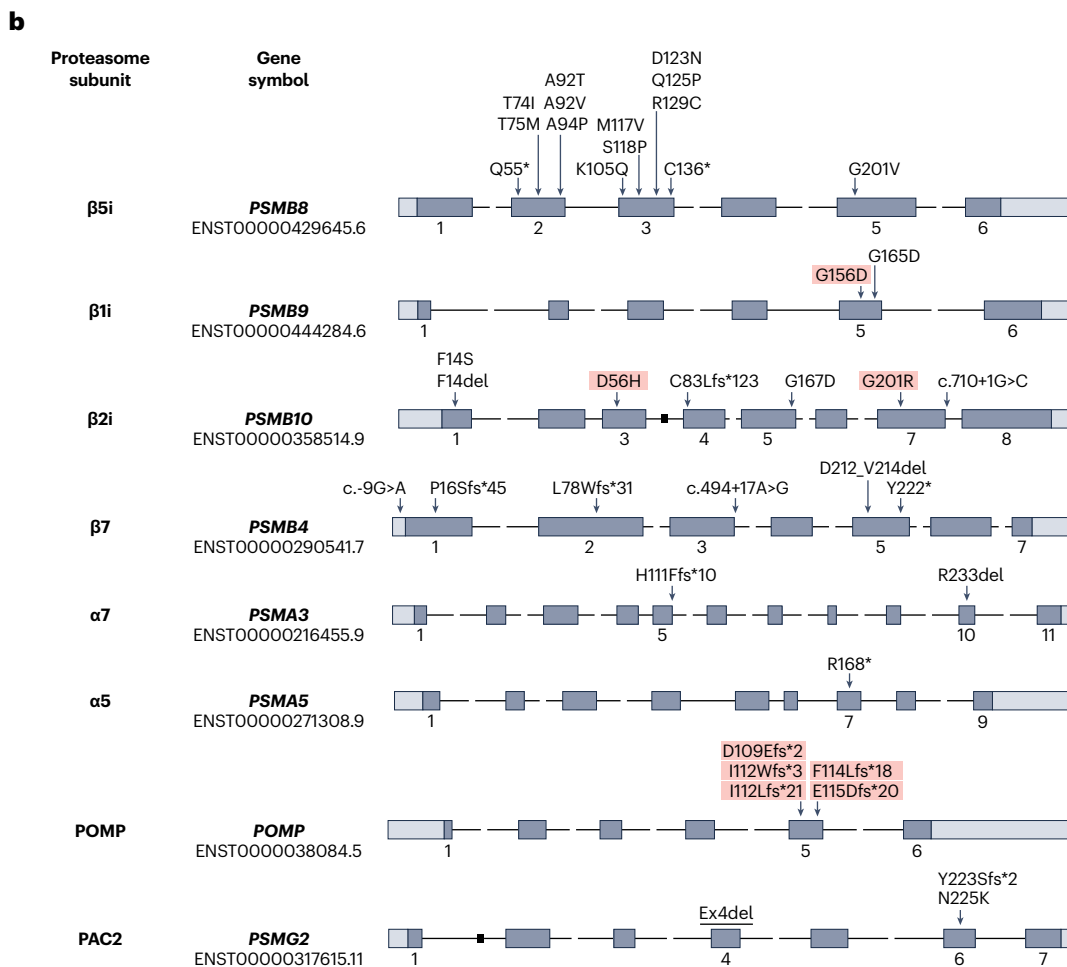
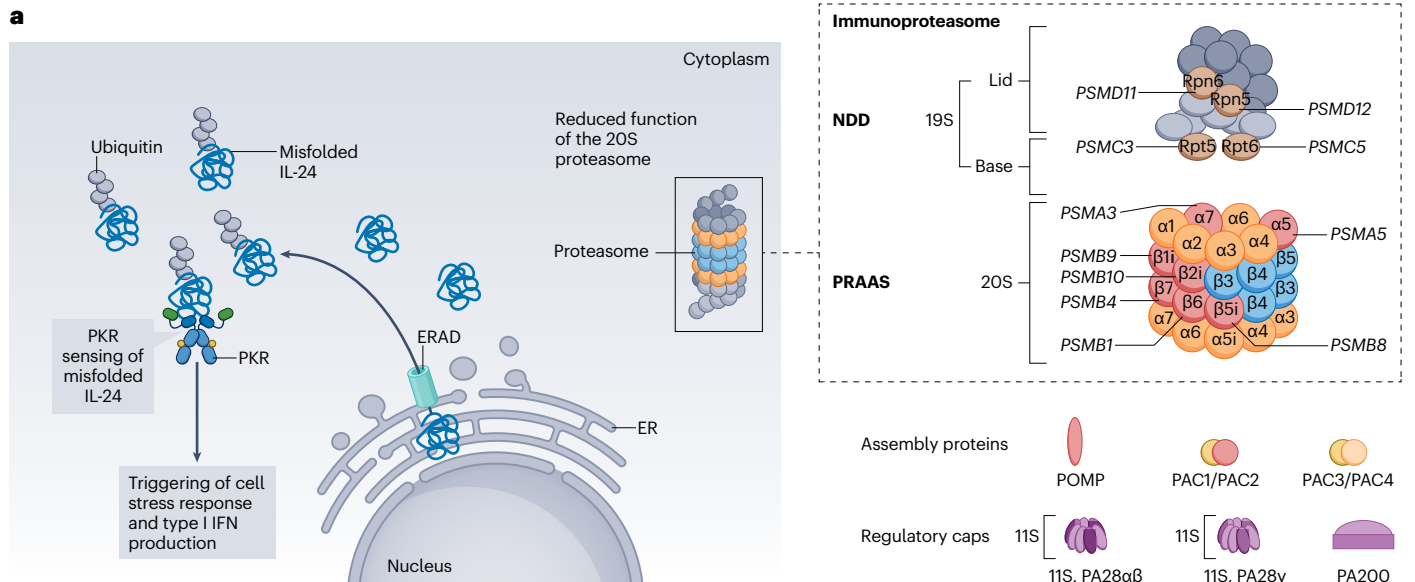
Most individuals with PRAAS present between the perinatal period and early infancy. Common clinical features include recurrent fever,

nodular skin lesions, violaceous plaques on the trunk and extremities and periorbital, heliotrope-like erythema with eyelid inflammation. Other features include joint contractures, myositis, cytopenias, basal ganglia calcifications and elevated acute phase reactants. Metabolic syndrome, including dyslipidaemia, hypertension, insulin resistance, acanthosis nigricans and hepatic steatosis can develop in up to 50% of patients^{11,153,169}. Individuals with PRAAS are also at risk of developing primary pulmonary hypertension^{170,171}. Skin biopsies typically reveal neutrophilic dermatosis and panniculitis as likely causes of progressive lipodystrophy. CNS manifestations can include basal ganglia calcifications and aseptic lymphocytic meningitis^{12,13,153}.

Transient autoantibody positivity, including ANAs and anti-phospholipid antibodies, are seen in up to 42% of individuals with PRAAS^{11,153,155,172,173}. Autoimmune manifestations rarely include autoimmune haemolytic anaemia¹⁵⁴ and immune thrombocytopenic purpura¹⁷⁴. Other rare features include alopecia areata, conjunctivitis, episcleritis, parotitis and epididymitis^{12,13}. Neutrophilic panniculitis, a typical feature of PRAAS, is not specific to these syndromes and is found in patients with other inborn errors of immunity, including LRBA deficiency, SAMD9L-associated autoinflammatory disease and NEMO-deleted exon 5 autoinflammatory syndrome¹⁷⁵. Patients with mutations in constitutive proteasome components or dominant negative mutations in *PSMB9*, *PSMB8*, *PSMB10* and *POMP* can present with more severe cytopenias and immune dysregulation resembling severe combined immunodeficiency Omenn syndrome^{156,157,159,176}. Additionally, biallelic variants in *PSMB1* have been associated with microcephaly, intellectual disability, developmental delay and short stature¹⁶⁰, but some patients can also exhibit systemic features of PRAAS. Individuals with haploinsufficiency caused by mutations in *PSMD12*, *PSMD11*, *PSMC1*, *PSMC3* and *PSMC5* predominantly present with neurodevelopmental disorders, intellectual disability and features of autism spectrum disorders, but do not present with systemic inflammation¹⁷⁷.

Pathophysiology

The ubiquitin proteasome system and lysosomal degradation are critical for maintaining protein homeostasis^{178,179}. Proteasomes are multisubunit complexes that degrade cellular proteins, whether self or foreign, and control the intracellular amino acid pool¹⁷⁸ enabling de novo protein synthesis¹⁸⁰. Proteasomes have a pivotal role in regulating various biological processes, including the cell cycle, apoptosis, cell development and MHC class I antigen presentation^{181,182}. In PRAAS, disease-causing mutations affect genes encoding the 20S



proteasome core and three alternative catalytic subunits, designated β5i/LMP7/*PSMB8*, β1i/LMP2/*PSMB9* and β2i/Mecl1/*PSMB10*, which can replace their respective constitutive proteasome counterparts,

β5/*PSMB5*, β1/*PSMB6* and β2/*PSMB7*, to form the 20S subunit of the immunoproteasome. Components of the immunoproteasome are constitutively expressed at high levels in circulating immune cells;

Fig. 5 | Pathogenesis of 20S proteasomopathies. In the autoinflammatory 20S proteasomopathies, impaired proteostasis causes cell stress and protein kinase R (PKR)-mediated type I interferon responses. **a**, Left: loss-of-function mutations in the 20S proteasome lead to the generation of misfolded IL-24. PKR serves as a sensor by binding to misfolded IL-24 that is entering the cytoplasm through ER-associated degradation (ERAD). During homeostasis and cell stress, wild-type proteasomes can upregulate their function and process ubiquitylated proteins, including misfolded IL-24. However, in individuals with PRAAS, the accumulation of misfolded IL-24 triggers interferon signalling and can activate the integrated stress response. **a**, Right: mutations in the 20S proteasome core

and assembly proteins cause the PRAAS phenotype. Subunits in the 20S core that are associated with a PRAAS phenotype are shown in red. Mutations in components of the 19S regulatory cap that cause neurodevelopmental delay (NDD) and autism spectrum disorders are shown in brown. **b**, PRAAS-causing variants in proteasome genes are depicted. Variants highlighted in red cause disease in heterozygosity through a presumed dominant-negative mechanism. First, last and affected exons are numbered. The process used to curate the genes depicted is detailed in the Supplementary Box 1. POMP, proteasome maturation protein, PAC, proteasome assembly chaperone.

however, in non-haematopoietic cells, inflammatory cytokines (including type I interferons, IFN γ , TNF and LPS) can induce immunoproteasome component expression and thus enhance the proteolytic capacity of these cells^{183,184}.

Deleterious null mutations in immunoproteasome subunits can be partially compensated for by their respective constitutive subunits, leading to a milder disease phenotype than mutations affecting constitutive proteasome subunits¹⁸⁵. The 20S proteasome is capped by several regulatory proteins. The 19S regulatory protein cap (also referred to as PA700 regulator) recruits and processes most intracellular proteins in an ubiquitin-dependent and ATP-dependent manner¹⁸⁶ (Fig. 5), whereas the 11S regulatory protein cap (also known as PA28 α and PA28 β , encoded by *PSME1* and *PSME2*) is primarily found in the immunoproteasome and mediates protein degradation independently of ubiquitin and ATP^{187,188}. The 26S proteasomes (20S subunit capped with 19S subunit) account for only 30% of total proteasomes; however, ubiquitin-independent proteolysis and proteolysis of native disordered proteins is mostly mediated by the uncapped 20S proteasome core (which can reside in the cytosol or nucleus) or by 20S proteasomes with other regulatory caps (such as the 11S subunit)¹⁸⁹. The use of advanced mass spectrometry allowed for the characterization of substrates of 20S proteasome-mediated degradation and identified nuclear and stress granule proteins containing high levels of intrinsically disordered regions, including RNA-binding and DNA-binding proteins. Other findings included processed, partially degraded substrates that were cleaved at their N termini or C termini, suggesting a fine-tuning activity of the 20S proteasome. The same study also revealed that exposure to reactive oxygen species led to oxidation of the 20S proteasomes, which had reduced proteolytic activity compared with naive proteasomes, and to oxidized protein substrates that displayed higher structural disorder than naive substrates¹⁸⁹. As the 20S proteasome cleaves the translation initiation factors, eIF4F and eIF3, which regulate assembly of the pre-initiation complex¹⁹⁰, understanding the effect of the PRAAS-causing mutations on ubiquitin-independent proteolysis and protein processing is important to understanding of the pathomechanisms that lead to the observed phenotypes.

Increased interferon signatures¹⁵³ and increased levels of CXCL10 (also known as IP-10) occur in PRAAS. The remarkable clinical response of patients with PRAAS to treatment with JAK inhibitors underscores the prominent role of type I interferon in these syndromes¹⁷¹ and has spurred investigation into the link between cellular sensing of proteotoxic stress to interferon responses. During protein starvation, fibroblasts from patients with PRAAS show features of impaired proteostasis with cytoplasmic accumulation of abnormally spliced and misfolded IL-24. IL-24 translocates from the ER to the cytosol by ERAD and triggers a PKR-mediated type I interferon response. This response was

not suppressed by a GADD34 inhibitor, which protects from unfolded protein stress, or with an inhibitor of the inositol-requiring enzyme 1 α or β (IRE1), a well-known ER transmembrane sensor that activates the unfolded protein response²⁵. This lack of suppression suggests a pivotal role for the IL-24–PKR axis in triggering interferon responses. Studies investigating fibroblasts from patients with PRAAS reveal a ‘severity-dependent’ progressive activation of the ER stress response in two patients with disease-causing *POMP* mutations, characterized by increased expression of *ATF6* and *HSPA5* (encoding BiP and GRP78, respectively), enhanced XBP1 splicing, elevated phosphorylation of eIF2 α and upregulation of TCF11 and/or NRF1 (the long and short isoforms of the transcription factor NRF-1, encoded by *NFE2L1*, which serves as regulator of the proteotoxic stress response¹⁹¹) with varying degrees of magnitude¹⁵⁶. Additionally, proteasome defects lead to a reduction of the intracellular pool of free amino acids, downregulation of mammalian target of rapamycin complex 1 (mTORC1), and phosphorylation of another serine/threonine-protein kinase, general control non-derepressible 2 (GCN2), which senses amino acid deficiency through binding to uncharged transfer RNA. GCN2 and PKR converge on the phosphorylation of eIF2 α , which executes the integrated stress response resulting in translational arrest¹⁹². These findings raise the intriguing possibility that phosphorylated PKR, could have a critical role in integrating cell stress responses with other stress-sensing serine-threonine kinases such as PERK and GCN2 (ref. 192). These pathways could establish a threshold that triggers eIF2 α phosphorylation and the subsequent arrest of the production of short-lived proteins as a late adaptation to increasing levels of proteotoxic stress¹⁹³.

Tissue-specific mechanisms

Despite the emergence of some pathomechanisms that link proteasomopathies to disease-specific characteristic clinical features (including neutrophilic panniculitis, diffuse myositis and hepatic steatosis), understanding of the 20S proteasomopathies is incomplete. Differences in the psychoneurological manifestations in the 20S autoinflammatory proteasomopathies compared with the 19S proteasomopathies, which present with behavioural disorders, reveal different organ functions of the 20S core proteasome and the 19S regulatory complex.

Panniculitis and hepatic steatosis. Neutrophilic panniculitis (neutrophil-driven inflammation of the subcutaneous fat) and fever are hallmark features of PRAAS, suggesting a pivotal role of the proteasome in adipocytes and immune cell function. In one study, loss of *PSMB4* disrupted proteostasis and adipogenesis, which was partly recovered by the activation of NRF1 (ref. 191). NRF1 is inactivated in response to cholesterol excess, oxidative stress or proteasome inhibition. In adipocytes, NRF1 translocation also upregulates activating transcription

factor-3 (ATF3), a master regulator of metabolic homeostasis¹⁹⁴, which activates inflammatory pathways and impairs adipogenesis. Interestingly, ubiquitinated intermediate filament body inclusions (also known as Mallory–Denk bodies) form in proteasome-depleted hepatocytes and are suggested as biomarkers in steatohepatitis¹⁹⁵. Further research is needed to understand how these pathways contribute to hepatic steatosis in PRAAS¹⁷¹.

Central nervous system disease. PRAAS often presents with intellectual disability in a substantial number of patients, accompanied by basal ganglia calcifications that variably develop and have become hallmark features of interferon-mediated CNS disease. In contrast to the 20S proteasomopathies in PRAAS, the 19S proteasomopathies are caused by mutations in the 19S regulatory protein subunit and present with NDD and autism spectrum disorder. Affected patients do not have a chronically elevated systemic blood interferon signature, and basal ganglia calcifications have not yet been reported. In-depth assessment of proteasome dysfunction in T cells from patients with de novo *PSMC3* mutations (who develop NDD) found increased PKR phosphorylation, an increase in an interferon response genes signature and compensatory upregulation of autophagy and mitophagy¹⁹⁶; however, a compartmentalized role of type I interferon in modifying clinical features of NDD and autism spectrum disorder in the CNS has not been established. One study suggests that the 19S regulatory protein subunit complex can function independently of the 20S proteasome¹⁹⁷. The 19S regulatory protein subunit has an interferon-independent role in regulating synaptic transmission in the CNS by mediating Lys63-ubiquitylation of synaptic proteins, essential for fast excitatory glutamatergic neurotransmission. These processes control synaptic plasticity, axonal calibre and dendritic branching¹⁹⁷ and have been implicated in the pathogenesis of autism spectrum disorder and other social behaviour disorders¹⁹⁸. It remains unclear if type I interferon aggravates this dysfunction and whether blocking interferon signalling in these disorders would be beneficial.

The IFNAR1 signalopathies

Diseases caused by selected LOF mutations in negative regulators of IFNAR signalling and GOF mutations in genes enhancing IFNAR signalling cause another group of interferonopathies. Autosomal-recessive mutations affecting AA residues in *STAT2* (refs. 15,17) (at positions p.R148 (refs. 15,17) or p.A219 (ref. 199)) or deleterious mutations in *USP18* (refs. 14,200) or in *ISG15* (refs. 16,201) manifest as pseudo-TORCH syndromes. A GOF mutation in *IFNAR* results in features resembling Degos disease¹⁸. These syndromes form a new group of interferonopathies characterized by persistently elevated interferon signatures in the blood.

Functional studies have revealed that USP18–ISG15 complexes function as negative regulators by binding to STAT2, including the critical amino acid positions p.R148 and p.A219, thereby terminating IFNAR signalling^{15,17}. Mutations preventing the formation of a functioning inhibitory dimer, including deleterious *USP18* or *ISG15* mutations^{14–17,200,201}, result in prolonged STAT2 phosphorylation, as observed in cell line knockdown experiments and in affected patients^{14,200,201} (Supplementary Fig. 1). Reported clinical features include developmental delay, elevated liver enzymes and thrombotic microangiopathy with or without proteinuria¹⁶, with a high rate of mortality in early childhood. A pathognomonic feature present in a subset of patients includes self-limiting, necrotizing and scarring skin lesions^{15–17,200}. Decreased IFN γ signalling is linked to infectious

complications such as infectious diarrhoea, *Klebsiella*, *Pseudomonas* and pneumococcal sepsis^{15,17}, as well as the development of BCGitis²⁰². It is thought that prolonged STAT2 phosphorylation is caused by the sequestration of STAT1 to form STAT1 and STAT2 heterodimers required for IFNAR1 and IFNAR2 (encoded by *IFNAR1* and *IFNAR2*, respectively) signalling, consequently depleting the STAT1 pool necessary for STAT1–STAT1 homodimerization, which is essential for IFN γ signalling^{202,203}.

A single de novo heterozygous GOF mutation in *IFNAR1* is associated with the development of Degos-like disease¹⁸. In one case, a 9-year-old girl with a heterozygous de novo variant in *IFNAR1* (p.Trp277*), presented with malignant atrophic papulosis and CNS involvement, along with an elevated interferon signature in the blood. Treatment with anifrolumab, an antibody targeting the extracellular part of IFNAR1, normalized the dysregulated interferon signature¹⁸. Mutations in signalling molecules that regulate a large group of cytokine and growth factor receptors including IFNAR (such as *STAT1* and *AK1*) are discussed later in this Review.

Monogenic SLE

Previously, individuals with autoinflammatory interferonopathies were considered to have subtypes of SLE; however, insights from studies of monogenic childhood-onset SLE highlight distinct pathways between SLE and the autoinflammatory interferonopathies. In contrast to the autoinflammatory interferonopathies, monogenic causes of paediatric-onset SLE emphasize the dysregulation of homeostatic extracellular nucleic acid elimination, the accumulation of which triggers B cell activation and autoimmunity. Nucleic acid reactive autoantibodies that can form large immune complexes or interfere with immune cells and tissue targets directly drive SLE pathology and confirm a critical role for autoreactive B cells in monogenic SLE. Immune complexes of nucleic acid-bound microparticles stimulate type I interferon production primarily by pDCs and provide distinct pathomechanisms that lead to SLE-specific clinical features that are absent in the autoinflammatory interferonopathies.

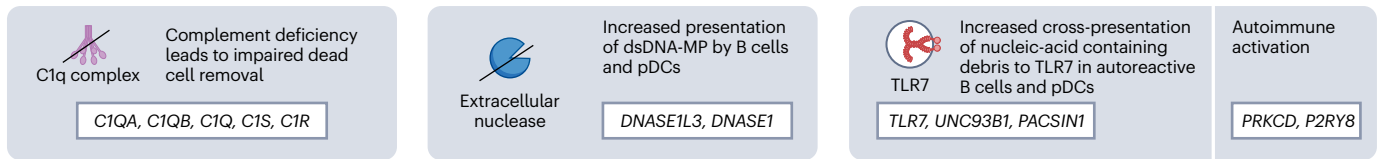
Clinical presentation

The ‘classical’ features of SLE, such as photosensitive cutaneous lupus (observed in over 50% of individuals with SLE) or immune complex-mediated glomerulonephritis (which develop in one-third of patients with SLE), are not typical features of the autoinflammatory interferonopathies²⁰⁴. Other hallmark features of SLE include mucocutaneous symptoms (such as oral and nasal ulcers), alopecia, musculoskeletal features (including arthralgia, arthritis and pleuritis) and neuropsychiatric SLE (manifesting with aseptic meningitis, demyelinating syndromes, sensorimotor and axonal neuropathies, cognitive dysfunction and psychiatric manifestations²⁰⁵).

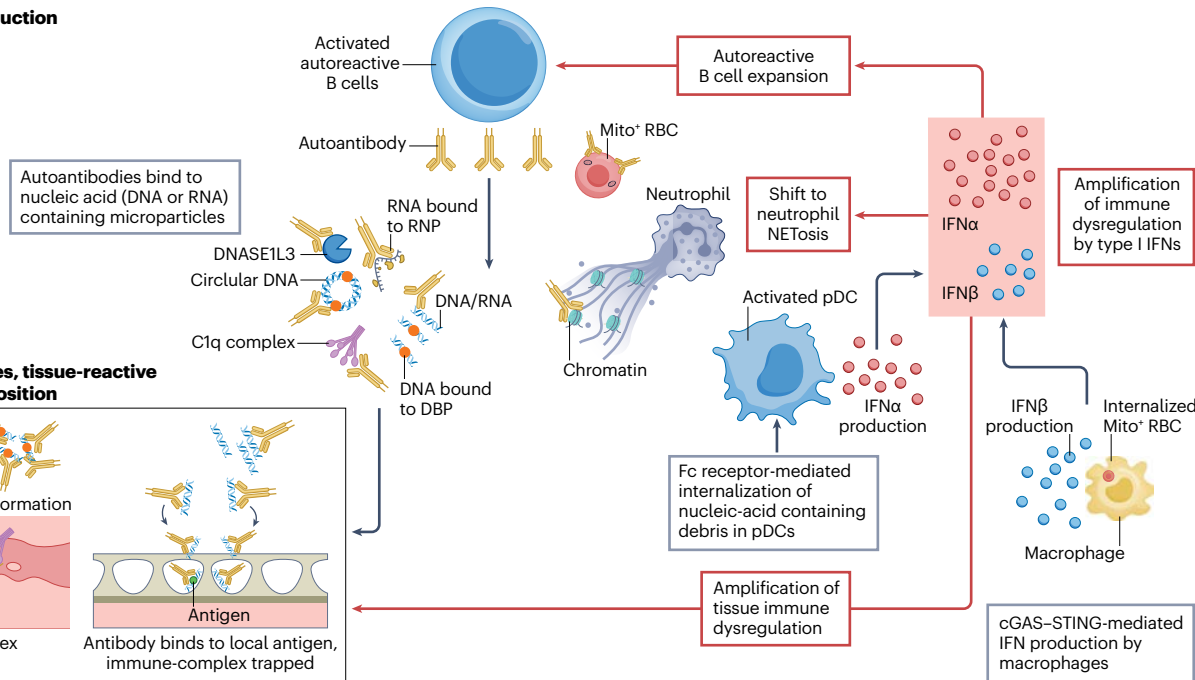
Pathophysiology

Currently around 7% of cases of paediatric-onset SLE are thought to be monogenic²⁰⁶. This number will likely grow with increased genetic sequencing of paediatric patients. Pathways identified by the SLE-causing mutations converge on the generation of autoantibodies targeting extracellular nucleic acid-containing microparticles, made up mostly of DNA-containing chromatin or RNA-containing ribosomal components (Fig. 6), and of tissue-specific autoantibodies as the cause of characteristic clinical SLE manifestations. The pathogenesis of SLE has been reviewed elsewhere^{207,208}, so we only cover relevant highlights that distinguish the pathomechanisms and

a Genetic causes



b Autoantibody production



c Immune complexes, tissue-reactive autoantibody deposition

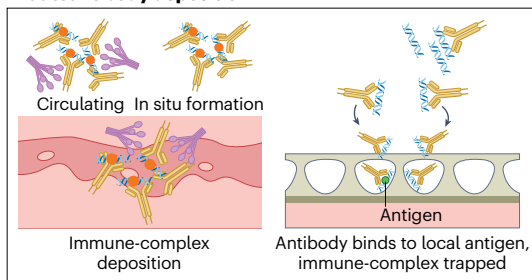


Fig. 6 | Pathogenesis of monogenic SLE. Monogenic causes of paediatric-onset systemic lupus erythematosus (SLE) that lead to extracellular nucleic acid-mediated B cell activation and autoimmunity drive SLE pathology, which can be augmented by various sources of type I interferon production, shown in this figure. **a**, Genetic causes leading to the generation of extracellular nucleic acids bound to microparticles and chromatin. *C1Q* deficiency (such as *C1QA*, *C1QB*, *C1QC*, *C1S* and *C1R*) leads to impaired removal of dead cells and immune complexes, (disease penetrance with other complement deficiencies is variable). *DNASE1* and *DNASE1L3* deficiency leads to impaired removal of nucleic acid-containing microparticles, resulting in the presentation of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) that activates autoreactive B cells and plasmacytoid dendritic cells (pDCs). Gain-of-function mutations in *TLR7* or loss-of-function mutations in Toll-like receptor (TLR) regulatory molecules (such as *UNC93B1* and *PACSIN1*), and mutations in *PRKCD* or *P2RY8* facilitate activation of autoreactive B cells and pDCs. Supplementary Fig. 4 shows curated disease-causing mutations. **b**, Mitochondrial RNA (mtRNA) and DNA-containing

microparticles trigger autoantibody formation in autoreactive B cells. Immune complexes that contain extracellular nucleic acid-bound microparticles shift neutrophils towards NETosis. Extracellular nucleic acid-containing debris are internalized by pDCs in an Fc receptor-dependent fashion, then cross-presented in the endosome, where they trigger TLR7 and/or TLR9-mediated IFN α production. In macrophages, cytoplasmic release of mtDNA or mtRNA from phagocytosed, internalized red blood cells (RBCs) or cargo containing damaged mitochondria (Mito⁺) leaks into the cytoplasm and activates IFN β in a cGAS-STING-dependent manner. Both IFN α and IFN β augment the expansion and activity of autoreactive B cells and can amplify tissue-specific damage. **c**, Immune complexes and autoantibody-mediated tissue damage occur as a result of autoreactive B cells with reactivity to nucleic acid-bound microparticles generating autoantibodies that can form large circulating immune complexes in the blood or in tissues in situ. Autoantibodies can also mediate direct tissue damage. RNP, ribonucleoprotein; DBP, DNA binding protein.

clinical manifestations of monogenic SLE from the autoinflammatory interferonopathies.

LOF mutations in the C1q complex components (encoded by *C1QA*, *C1QB* or *C1QC*) or in the two serine proteases (*C1S* and *C1R*) that activate the C1q complex and homozygous deleterious LOF mutations in extracellular deoxyribonucleases, *DNASE1L3* (refs. 209–211) plus autosomal-dominant LOF mutations in *DNASE1* (p.K5*, p.L16_K24del, A193V)²¹², cause monogenic SLE with high penetrance. The latter forms of monogenic SLE often present with

hypocomplementaemic urticarial vasculitis syndrome²¹³ and all highlight critical mechanisms of maintaining immune tolerance to extracellular nucleic acids. C1q functions as an opsonin that facilitates the removal of apoptotic cells and neutrophil extracellular traps (NETs) by macrophages, which controls serum levels of extracellular nucleic acids^{214–216} (Fig. 6). C1q also regulates homeostatic clearance of immune complexes through their capture by the C1 receptor on erythrocytes, which are subsequently phagocytosed in the liver²¹⁷. In addition, C1q facilitates intercalation of C3b and

C1q, which disrupts the formation of large insoluble immunogenic immune complexes^{218,219}. C1q maintains homeostasis by inhibiting the differentiation of antigen-presenting DCs. In the presence of damage-associated and pathogen-associated molecular patterns, C1q inhibits pro-inflammatory cytokine production and promotes anti-inflammatory macrophage polarization and in the presence of immune complexes, C1q suppresses DNA-containing immune complex-mediated pDC activation^{220,221}. All of these mechanisms are necessary to contain and resolve inflammation. At the tissue level, the C1q complex mediates clearance of apoptotic tissue cells; in *C1q*-knockout mice²²² uncleared apoptotic kidney cells expose chromatin and other self-antigen, triggering local immune complex formation, activation of the terminal membrane attack complex and direct kidney damage^{222,223}. *DNASE1L3* (ref. 209) encodes an extracellular enzyme uniquely capable of digesting chromatin and microparticle DNA released from apoptotic cells^{224,225}. Chromatin is released from necrotic and late apoptotic cells, is extruded from neutrophils during NETosis and is actively secreted by pro-inflammatory cells. Accumulation of extracellular DNA-containing microparticles can stimulate B cells to produce autoantibodies that then generate large nucleosome-containing and chromatin-containing immune complexes that are taken up by pDCs and subsequently stimulate endosomal TLR-mediated IFN α production²²⁶. Autoantibodies against C1q (found in 30–48% of patients with SLE^{227,228}) or against DNASE1L3 (over 50% of individuals with sporadic SLE and those with nephritis^{222,229}) reproduce the pathogenesis and cause phenocopies of their genetic counterparts^{229–232}. Since 2022, findings of monogenic GOF mutations in TLR7 (refs. 27–29) or in molecules that regulate TLR7 function, including autosomal-dominant GOF mutations in *UNC93B1* (ref. 233) and autosomal-dominant GOF mutations in *PACSIN1* (ref. 234), highlight a pivotal role of TLR7 in increasing B cell autoreactivity, autoantibody production and pDC activation in monogenic SLE²². TLR7 bound to UNC93B1 senses guanosine-rich and uridine-rich ssRNA oligonucleotides mostly from RNA viruses in the endosome^{235,236}. GOF mutations in *UNC93B1* cause paediatric-onset SLE by prolonging TLR7 signalling^{237–240}. Similarly, disease-causing *PACSIN1* mutations impair negative regulation of TLR7 by disrupting interactions with TRAF4 (ref. 234). Increased TLR7 signalling in B cells enhances self-reactivity²⁷ in germinal centres and extrafollicular B cells^{241,242}, activates pDCs and follicular DCs and TLR7 and TLR8 stimulation results in expansion of perivascular DCs in the bone marrow²⁴³. Activation of neutrophils by TLR7 and TLR8 triggers shedding of Fc γ RIIa (CD32), which diverts neutrophils from the phagocytosis of immune complexes to NETosis²⁴⁴. Activated neutrophils proteolytically cleave Fc γ RIIa from pDCs and monocytes, which impairs immune complex clearance and enhances C5a complement activation²⁴⁴ (Fig. 6).

Other monogenic causes of paediatric-onset SLE involve autosomal-recessive LOF mutations in *PRKCD*²⁴⁵, encoding protein kinase C δ (PKC δ), and dominant negative variants (inherited in an autosomal-dominant manner) in *P2RY8*, encoding a G α 13 protein-coupled transmembrane receptor²⁴⁶. *Prkcd*-knockout mice accumulate autoreactive B cells owing to defective negative selection in germinal centres. Autonomous B cell hyperproliferation is associated with systemic autoimmunity and presents with autoantibodies, immune complex-mediated glomerulonephritis, lymphadenopathy and splenomegaly²⁴⁷. *P2RY8* LOF mutations lead to increased activity of the signalling molecules AKT and ERK that promotes plasma cell differentiation and disrupts germinal centre tolerance,

increasing age-associated B cells and plasma cells in the periphery, which was associated with lupus nephritis²⁴⁶. Both mutations illustrate TLR7-independent functions in promoting B cell autoimmunity.

Our knowledge of cellular sources of type I interferon production in SLE is expanding²⁴⁸. It is well established that large uncleared interferogenic immune complexes undergo Fc γ R-mediated endocytosis in pDCs and trigger IRF7-mediated IFN α production through cross-presentation to endosomal TLRs²² and that type I interferon amplifies B cell-mediated pathology, accelerates the maturation of monocytes to antigen-presenting cells and has a role in DC differentiation²⁴⁹. In addition, SLE antibodies that target mitochondria and neutrophils impair both mitochondrial homeostasis and neutrophil clearance and promote NETosis (with subsequent release of chromatin, nuclear and mitochondrial material). Data from studies on SLE antibodies highlight that NETs are pivotal sources of nucleic acid triggers for ANAs and contribute to type I interferon production by pDCs and macrophages. Examples of SLE antibodies include anti-LL37 antibodies (present in some patients with SLE), which impair LL37 peptide-facilitated clearance of neutrophils during infection²⁵⁰ and, in the presence of IFN α , activate neutrophils to extrude double-stranded mtDNA²⁵¹. Similarly, anti-RNP or anti-Smith antibodies, which, when in co-culture with IFN α , stimulate the release of oxidized circular mtDNA–TFAM complexes that are internalized by pDCs in a RAGE-dependent fashion^{252,253}. In both instances double-stranded mtDNA or mtDNA–TFAM complexes stimulate endosomal TLR9-mediated type I interferon production^{252,254}. Mitochondrial circular or hypomethylated double-stranded mtDNA triggers autoantibody production and generates immune complexes that can activate pDCs^{251–253}. These examples highlight a vicious circle of mitochondrial dysfunction and type I interferon production in SLE. However, in vitro depletion of pDCs from peripheral blood mononuclear cells reduces type I interferon production by only around 40%, suggesting the involvement of other cell types in type I interferon production²⁴⁹. In SLE, anti-red blood cell (RBC) antibodies can lead to abnormal retention of mitochondria in mature red blood cells (Mito⁺ RBCs). Macrophage phagocytosis of these Mito⁺ RBCs results in leakage of mtDNA from the ingested Mito⁺ RBCs into the cytoplasm of macrophages and subsequent IFN β production in a cGAS–STING-dependent manner²⁵⁵. Mitochondrial damage during cell stress and impaired homeostasis can lead to extrusion of whole mitochondria (which are found in healthy blood) and mitochondrial fragments and/or leakage of mtRNA and mtDNA into the cytoplasm or into the extracellular space either directly or as cargo in extracellular vesicles. Although whole mitochondria are likely not immunogenic, various fragments can stimulate type I interferon production in a MDA5–RIG-I–MAVS or cGAS–STING-dependent fashion, mainly in macrophages²⁵⁶. Defective mitophagy can lead to extrusion of interferogenic oxidized mtDNA–TFAM complexes in monocytes²⁵³ and other cells but the cell sources and physiological and pathological role of mtDNA remain incompletely understood. Mitochondrial dysfunction caused by inhibition of the tricarboxylic acid cycle enzyme fumarate hydratase (encoded by *FHI*) directly drives type I interferon production^{257,258}. The relative contribution of these pathways to the unaccounted type I interferon production in SLE remains elusive. Furthermore, the antibody-independent stimulation of type I interferon production by mtDNA is not specific to patients with SLE but can amplify interferon production in other diseases including the autoinflammatory interferonopathies, infections and metabolic and degenerative diseases and could contribute to overlapping clinical features such as chilblain-like skin lesions.

Diseases with overlapping features of autoinflammatory interferonopathies and monogenic SLE

Two interferonopathies, spondyloenchondrodysplasia (SPENCD)²⁵⁹ and DNase2 deficiency²⁶⁰, caused by autosomal-recessive mutations in *ACPS5* (which encodes tartrate-resistant acid phosphatase) and autosomal-recessive LOF mutations in *DNASE2*, respectively, commonly present with autoimmune features seen in SLE. Tartrate-resistant acid phosphatase (TRAP) is an enzyme that is critical for bone resorption. Impairment of TRAP causes flattened vertebral bodies (platyspondyly) throughout the axial skeleton that are pathognomonic features in patients with SPENCD²⁵⁹. LOF autosomal-recessive mutations in *DNASE2*, encoding a deoxyribonuclease that is located in the lysosome and hydrolyses DNA under acidic conditions, cause DNase2 deficiency. Patients present with severe anaemia and thrombocytopenia, hepatosplenomegaly and recurrent fevers²⁶⁰. Individuals with SPENCD and DNase2 deficiency can develop classic features of SLE together with their respective pathognomonic disease features. Most patients have anti-dsDNA antibodies and develop SLE glomerulonephritis, underscoring a critical role of antibodies directed to DNA-containing microparticles as important drivers of the SLE-like disease pathogenesis^{261,262}.

Complex diseases with variable interferon signatures

Complex immunodysregulatory diseases with interferon signatures in the blood include ubiquitylation disorders (haploinsufficiency of A20 and OTULIN-related autoinflammatory syndrome) and diseases caused by mutations in genes involved in the JAK–STAT pathway, including *JAK1* (refs. 263,264), *STAT1* (ref. 265), *STAT2* (ref. 15) and *SOC31* (ref. 266). These mutations affect IFNAR signalling but also regulate signalling of multiple cytokine or endocrine receptors²⁶⁷. Other diseases with documented variable interferon signatures include deficiency of adenosine deaminase 2 (*DADA2*)²⁶⁸ (thought to be caused by cytoplasmic accumulation of deoxyinosine as a trigger of cytoplasmic nucleic acid sensing²⁶⁹) and Trisomy 21 (owing to a dose effect of triplication of the *IFNAR* gene cluster (*IFNAR1*, *IFNAR2*, *IFNGR2* and *IL10RB*) on chromosome 21)^{269,270}. The mechanisms of interferon production and the role of type I interferon in contributing to broader immune dysregulation seen in these diseases need to be established in treatment settings and can vary from patient to patient, even within the same disease group. Furthermore, monogenic defects in genes regulating DNA repair and mitochondrial function reveal that both the nucleus and the mitochondria are sources of interferogenic nucleic acids, which can be released in a tissue-specific manner and trigger an interferon response through cytoplasmic nucleic acid sensors (Fig. 7).

Artemis deficiency and *PRKDC* mutations encoding DNA-dependent protein kinases (which inhibit Artemis function²⁷¹) are both examples of DNA repair defects that cause cutaneous DNA damage syndromes associated with UV-induced skin cancers. Individuals with these DNA repair defects can present with increased dsDNA and cGAS–STING-mediated type I interferon production and with increased cell senescence phenotypes in the affected tissues^{272,273}. DNA damage-induced interferon production is also established in men with X-linked reticulate pigmentary disorder caused by a hypomorphic X-linked mutation (c.1375-354 A>G, in *POLAI* (refs. 274,275)) that leads to stalling of the replication-fork, DNA damage response and interferon signalling²⁷⁴. Patients often present with poikiloderma with hypopigmentation and hyperpigmentation that are characteristic of DNA repair defects²⁷⁶ (Fig. 7).

Mitochondrial damage and dysfunction are reported in individuals with Harel–Yoon syndrome caused by mutations in *ATAD3A*, a mitochondrial membrane protein. Patients can present with lactic acidosis²⁷⁷ (consistent with mitochondrial disorders) and release of mtDNA with cGAS–STING-dependent interferon signalling that is reversed upon depletion of mtDNA in THP-1 cells²⁷⁸. Other diseases, including trichohepatoenteric syndromes 1 and 2, which present with syndromic features (including intractable chronic diarrhoea²⁷⁹), can also develop interferon signatures (Fig. 7).

A heterogeneous group of inborn errors of immunity with immunodysregulatory features are caused by monogenic defects that alter B cell receptor signalling or regulatory T cell function, leading to defects in central or peripheral B cell tolerance and B cell autoimmunity independent of type I interferon. Increased T cell autoimmunity can be caused by monogenic defects that alter post-thymic selection processes and lead to increased survival of self-reactive T cells²⁸⁰ (Fig. 7 and Supplementary Table 1). In these conditions, the role of type I interferon in amplifying immune dysregulation has not been established but would not be necessary in driving autoimmunity features²⁸¹. Grouping diseases by the presence of systemic or compartmentalized interferon production, and by other pathomechanisms driving autoimmunity (for example, T cell selection and B cell tolerance) might assist in stratifying patients who could benefit from the emerging novel therapies that block type I interferons.

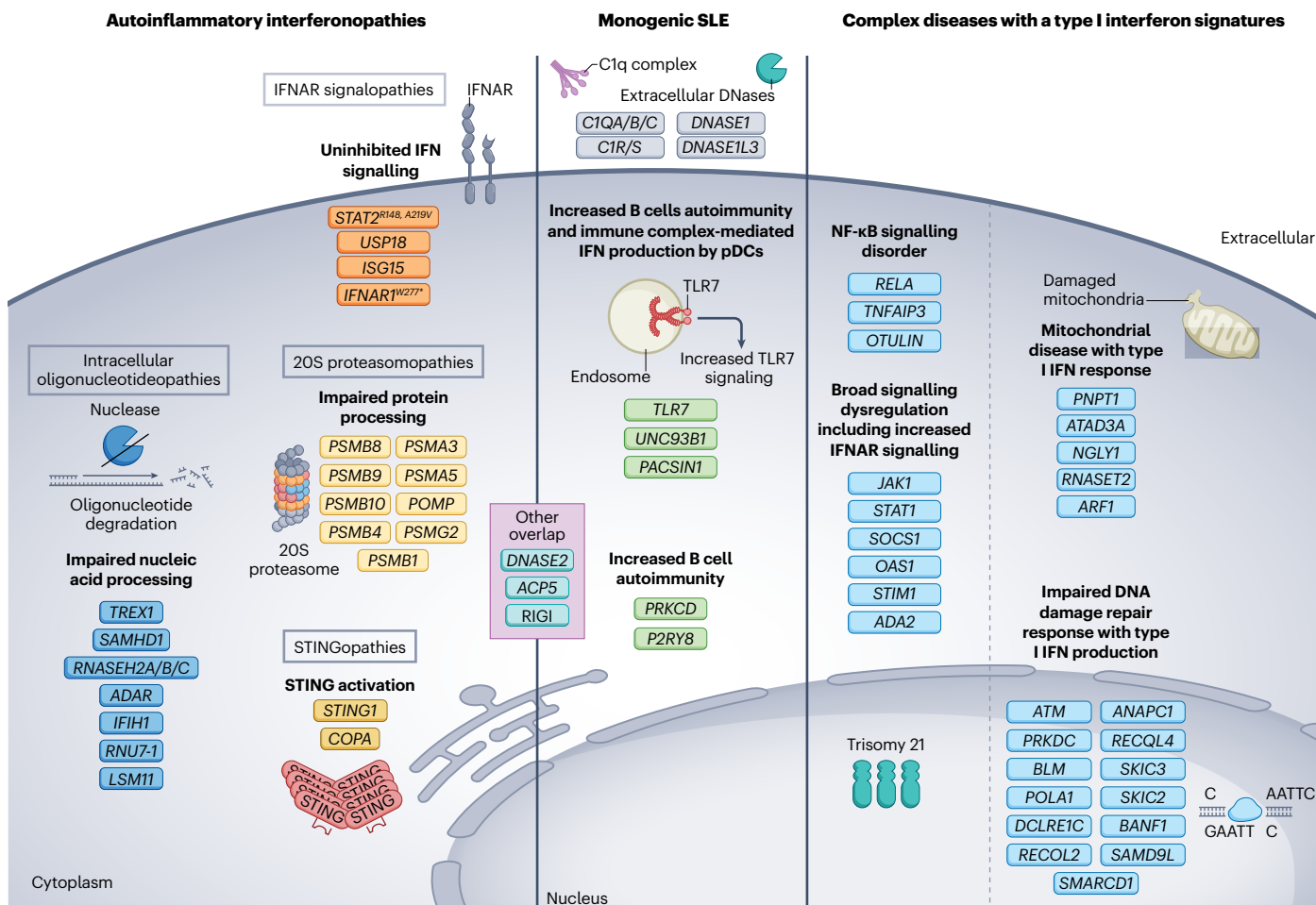
Autoimmunity in interferonopathies and monogenic SLE

The high prevalence of ANA positivity in the autoinflammatory interferonopathies (30–80%) compared with the lower prevalence in the healthy population (12–20%)²⁸⁰ and in other autoinflammatory diseases has raised questions about a role for autoantibodies in causing some of the disease manifestations of autoinflammatory interferonopathies²⁸². Despite the high prevalence of ANAs, pathogenic anti-dsDNA antibodies, which are found in 70–98% of individuals with SLE²⁸³ and carry high sensitivity and specificity for the diagnosis of SLE, are rarely found in patients with monogenic interferonopathies. Furthermore, in SLE, ANA-producing B cells undergo affinity maturation that results in specificities to dsDNA and RNP²⁸⁴, ANA antibodies can also acquire multiple reactivities; for example, ANA antibodies that target dsDNA can also develop neutralizing activity to the endonuclease DNASE1L3, which can mimic the monogenic form of SLE caused by pathogenic *DNASE1L3* mutations²³².

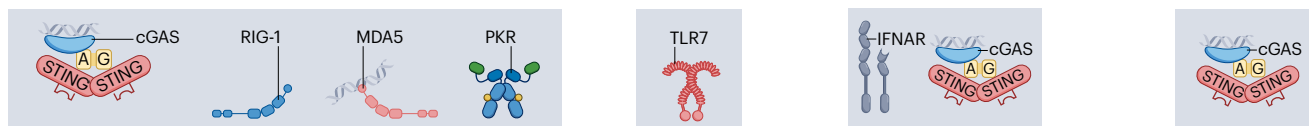
Many clinical features of SLE can be linked to specific, often affinity-matured, autoantibodies; however, ANAs in autoinflammatory interferonopathies are mostly bystanders and do not cause AGS, SAVI or PRAAS-specific disease manifestations. In this section we thus explore and compare pathogenic mechanisms of selected shared and divergent clinical features between SLE and the autoinflammatory interferonopathies.

First, cytopenias commonly occur in SLE, autoinflammatory interferonopathies and many immunodysregulatory diseases. Causes of cytopenias include both autoimmune and non-immune pathomechanisms. Autoimmune cytopenias, including autoimmune haemolytic anaemia, which is mostly caused by warm antibodies, are described in around 10% of individuals with SLE²⁸⁵. Immune thrombocytopenia, found in 20–40% of patients with SLE, is mostly caused by anti-glycoprotein antibodies and is further aggravated by the presence of antiphospholipid antibodies in SLE²⁸⁵; however, immune thrombocytopenia is rarely seen in the interferonopathies. Lymphopenia is common in individuals with various interferonopathies and occurs in

a Disease-causing genes



b Dominant sensors



c The role of type I IFN

Role of type I IFN on phenotype = impact of IFN-blocking therapy

Fig. 7 | Proposed grouping of IELs with reported interferon signatures based on pathomechanisms and the presumed contribution of type I interferon.

a, The disease-causing genes and pathomechanisms of inborn errors of immunity (IEIs) with reported interferon (IFN) signatures in blood and/or cell models with the autoinflammatory interferonopathies (left), monogenic systemic lupus erythematosus (SLE) (middle) and other complex diseases with type I interferon signatures (right). Left: Aicardi–Goutières syndrome (AGS), STING-associated vasculopathy with onset in infancy (SAVI), 20S proteasome-associated autoinflammatory syndromes (PRAAS), and by similarity also COPA, along with unopposed IFNAR signalling in the autoinflammatory interferon- α/β receptor (IFNAR) signalopathies. Emerging data from type I IFN-blocking treatments, underscore the eminent role of type I IFN in the disease pathogenesis of the autoinflammatory interferonopathies. Centre: in monogenic SLE, B cell autoreactivity drives autoantibody and immune complex formation that cause

tissue-specific pathology and can stimulate IFN α production by plasmacytoid dendritic cells. IFN is not necessary to produce the SLE phenotype but amplifies antibody-mediated pathology. Right: complex immunodysregulatory IEIs with interferon signatures in peripheral blood have been confirmed in ubiquitylation disorders, in individuals with selective JAK–STAT signalling defects, and in individuals with deficiency of adenosine deaminase 2 (DADA2) and trisomy 21, suggesting an amplifying role of type I IFN in the context of more complex underlying immune dysregulation. There are an increasing number of diseases with mitochondrial dysfunction and/or DNA repair or DNA processing defects that can present with tissue production of IFN β and increased tissue IFN-stimulated genes (ISGs) with variable but often little systemic inflammation. **b**, The dominant sensors involved in each disease group are indicated. **c**, The relative role of type I IFN in each disease group is indicated, based on the effectiveness of type I IFN-blocking therapy.

20–70% of individuals with SLE. In individuals with SLE, a drop in complement associated with cytopenias suggests a complement-mediated process²⁸⁵. However, low complement-accompanying cytopenia is rarely seen in the autoinflammatory interferonopathies, where the direct action of type I interferon on the suppression of haematopoietic stem cells and megakaryocytes is thought to cause cytopenias. Other non-immune causes of cytopenias include clonal haematopoiesis, but the relative contribution of clonal haematopoiesis to the specific cytopenias is currently unknown²⁸⁶. Future studies applying refined tools such as single-cell sequencing that profile autoimmune and non-autoimmune cells in patients with cytopenias will better discern divergent and shared pathogenic mechanisms underlying cytopenias in SLE and autoinflammatory interferonopathies.

Second, SLE-defining, autoantibody-mediated organ manifestations that are commonly seen in monogenic SLE are not seen in the interferonopathies, including kidney manifestations caused by immune complexes trapped in the mesangial and subendothelial areas of the glomerulus²⁸⁷. Kidney injury can also be caused by pathogenic anti-DNA antibodies with dual or multiple reactivity that can bind to dsDNA and additional antigens such as chromatin, mitochondria and/or glomerular membrane antigens and α -actinin.^{288–291} These antibodies can sometimes activate the terminal membrane attack complex (C5b-9)^{208,292,293} (Fig. 6). In SAVI and COPA reports of ANCA-mediated kidney disease suggest a possible role for neutrophilic endothelial damage and/or vasculopathy in causing autoantibody formation and kidney damage¹²⁶; however, kidney disease is rare in SAVI and more variable in COPA. A role for autoantibodies in the pathogenesis of neuropsychiatric manifestations of SLE was reported in over one-third of individuals with SLE²⁹⁴. The impressive specificity of selected antibodies for neuronal structures was carefully evaluated in the case of anti-dsDNA antibodies with dual reactivity or cross-reactivity to a peptide of the N-methyl-D-aspartate receptor, which causes neuronal death in the CA1 hippocampal region, presenting clinically as spatial memory defects²⁹⁵. By contrast, CNS disease in AGS presents as leukoencephalopathy; intracellular nucleic acid accumulation and high local IFN α production can cause neuronal death *in vitro*⁶⁴ but pathogenic autoantibodies have not been implicated in CNS pathogenesis of AGS²⁹⁶.

Last, individuals with SLE ($\leq 12\%$) can present with a non-erosive, deforming, but often painless Jaccoud-type arthropathy²⁹⁷, involving the hands and feet, which is also described in other autoimmune diseases, inflammatory bowel disease and acquired immunodeficiency syndromes but is rarely found in patients with autoinflammatory interferonopathies. Small vessel vasculitis, immune complex deposition and antibodies against type II collagen are implicated in the periarthritic fibrosis that causes Jaccoud-type arthropathy, but shared and divergent pathomechanisms in the various diseases remain poorly understood^{297,298}. Other manifestations of SLE that are rare or absent in the interferonopathies include photosensitivity, butterfly-distribution rash and pleuritis²⁹⁹. Emerging single-cell omics technologies will enable better characterization of pathologies that cause tissue damage in autoinflammatory interferonopathies and SLE and this information will undoubtedly influence the choice of targeted treatments that will be considered for each disease.

The role of type I interferons: cause, amplification or bystander?

The persistent type I interferon signature observed in the blood in all individuals with autoinflammatory interferonopathies and in 60–80% of patients with SLE³⁰⁰ suggests that type I interferon could have a

pathogenic role in both contexts and contribute to organ damage and morbidity. Advances in ultra-sensitive immunoassay technologies, which can measure femtomolar concentrations of interferons in serum, enabled the correlation between IFN $\alpha 2$ serum levels and interferon scores in the autoinflammatory interferonopathies³⁰¹, confirming the value of interferon response signature genes in representing interferon signalling in these diseases.

The interferon signature has also been validated in clinical trials for autoinflammatory interferonopathies^{171,301–304} and in studies of several systemic autoimmune diseases, including SLE, primary Sjögren syndrome and systemic sclerosis³⁰⁵ and is used as a diagnostic and/or therapeutic biomarker in interferonopathies. Although pathogenesis and treatment data confirm a pivotal role of type I interferon in disease pathology in some interferonopathies, classic immune complex-mediated and antibody-mediated features of SLE and other autoimmune diseases can occur in the absence of a blood interferon signature, suggesting an amplifying (or secondary) role of type I interferon in SLE^{306,307} and other autoimmune diseases, compared with its primary role in the interferonopathies (Fig. 7). Typically, various subsets of ISGs, including a subset of 6 to 28 ISGs (mostly measured using a NanoString assay), are used to quantify the interferon response in AGS^{45,303}, PRAAS, SAVI and other presumed interferon-mediated diseases³⁰⁸. Interferon signatures arise in the context of viral infections and can coexist with broader immune system dysregulation. The interferon signature is not specific to type I interferon; IFN γ can also induce some ISGs, and TNF can further amplify responses of some genes in the interferon score, particularly *CXCL10*. To quantify interferon and broader immune system dysregulation, a ratio of ISGs and NF- κ B-driven genes has been proposed³⁰⁹. Whether such stratification can aid in predicting therapeutic response to interferon-blocking treatments is under investigation. The interpretation of the interferon signatures in diseases beyond the interferonopathies, SLE and selected autoimmune diseases is challenging. Deep immune profiling studies report differential expression of type I interferon and specific ISGs in the blood and tissues of patients with COVID-19 and healthy individuals³¹⁰. The presence of localized type I interferon production in the CNS of individuals with amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease and in older individuals^{311,312} in the absence of systemic inflammation, and the presence of a tonic interferon signature in healthy people³⁰⁷, further underscore the importance of establishing blood-specific and tissue-specific normalized values of type I interferon signalling.

Treatment challenges

The goal of treating autoinflammatory diseases is to control systemic and organ-specific inflammation, halt the progression of organ damage, reduce dependency on glucocorticoids and restore normal growth and development. However, controlling the inflammatory manifestations of autoinflammatory interferonopathies with non-selective immunosuppressive agents, anti-cytokine treatments (targeting TNF, IL-1 and IL-6) and B cell-depleting therapies has proven challenging. Although high doses of steroids (1–2 mg/kg per day) can provide partial relief, long-term treatment with glucocorticoids is associated with adverse events that limit their use^{5,6,112–114,171}. JAK inhibitors reduce IFN α -mediated STAT1 phosphorylation and are recommended for the treatment of PRAAS, SAVI and AGS³¹³. Although improvement is observed in patients with these diseases after treatment with JAK inhibitors^{171,302,303,314,315}, inflammatory remission with normalization of the interferon signature and discontinuation of glucocorticoid therapy

Glossary

Autosomal-dominant mutation

A mutation that is present on one allele (copy) of a given gene.

Autosomal-recessive mutation

A mutation that is present on both alleles (copies) of a given gene.

Biallelic variants

Variants that occur in both copies of a gene; these variants can be homozygous or compound heterozygous.

Chilblain-like lesions

Skin lesions that mimic chilblains or pernio lesions, which are erythematous violaceous lesions, typically macules, papules, or nodules that develop in response to exposure to cold environments and affect mainly the acral areas, particularly toes and fingers.

Deleterious frameshift mutation

A damaging or pathogenic mutation caused by the insertion or deletion of nucleotide bases in numbers that are not multiples of three that disrupt the expected translated amino acid sequence.

Deleterious null mutation

A damaging or pathogenic mutation that leads to the gene not being transcribed into RNA and/or translated into a functional protein product; null mutation is also known as amorphic mutation.

Digenic mutations

Mutations that occur in two genes to manifest a particular phenotype or disease.

Dominant negative mutation

A heterozygous mutation that results in a protein that interferes with the normal function of the wild type protein.

Heterozygous missense mutation

A single nucleotide change in one allele that results in a different amino acid being encoded at a particular position in the resulting protein.

Hypomorphic X-linked mutation

A mutation on a gene on the X chromosome that results in a partial loss of gene function because of a reduction in the expression of RNA or protein, or by a reduction in the functional performance of the gene product; a hypomorphic mutation is also known as a leaky mutation.

Inborn errors of immunity

A group of rare genetically defined immunological disorders with increased susceptibility to immunodeficiency and infections, autoinflammation, autoimmunity, allergy and/or predisposition to lymphomas and other malignancies, mostly because of damaging germline variants in single genes.

Interferogenic

An element or molecule, such as nucleic acids, which triggers an interferon response or induces the expression of interferon stimulated genes.

Interferon score

The quantification of an interferon signature by the sum of z-scores of specific interferon-stimulated gene expression measured by RT-qPCR, NanoString or RNA-sequencing.

Nonsense mutations

Single-nucleotide changes that result in a stop codon rather than in a codon specifying an amino acid, thus leading to the production of a shortened protein.

Pseudo-TORCH syndromes

An inherited neurological disorder with clinical and neuroradiological features that mimic intrauterine toxoplasmosis, others (syphilis, hepatitis B), rubella, cytomegalovirus, herpes simplex (TORCH) infections in the absence of evidence of infection.

Warm antibodies

Autoantibodies that are active only at body temperatures of 37°C or higher and attach to and prematurely destroy red blood cells.

is only achieved in around half of patients with PRAAS¹⁷¹ (Supplementary Fig. 2). In other patients treated with JAK inhibitors, interferon scores do not normalize and clinical improvements can wane over time. Upper respiratory infections and viral reactivation (including BK viraemia) require monitoring^{154,171,313,316,317}. In AGS, the limited benefit of reverse transcriptase inhibitors³¹⁸ suggests the involvement of interferogenic triggers other than reverse-transcribed retroelements, as discussed earlier. Allogeneic HSC transplantation is beneficial in some patients with PRAAS^{157,319–322}, although no benefits of this type of treatment were reported in murine models of SAVI¹⁴⁸. Anifrolumab, a recombinant anti-IFNAR antibody approved for the treatment of SLE³²³, has shown promise in a patient with a GOF *IFNARI* mutation¹⁸, a patient with LOF *DNASE2* mutation³²⁴, a patient with cutaneous manifestations of SAVI³²⁵ and in a cohort of individuals with SAVI, PRAAS, COPA or undifferentiated interferonopathies refractory to treatment with JAK inhibitors³²⁶. Anifrolumab effectively suppressed the interferon signature³²⁶, providing proof of mechanism and a tool to probe other presumed interferon-mediated diseases. However, the long-term effect of this treatment on preventing the progression of organ damage requires further evaluation. Although current treatment strategies are limited to blocking interferon signalling, treatments that target intracellular nucleic acid and protein aggregate sensing pathways are emerging. These drugs promise a shift in the treatment paradigm to reduce the production of type I interferon by blocking the cGAS–STING, RIG-I–MDA5–MAVS, PRK and/or TLR7–MYD88 sensing pathways (Supplementary Fig. 2) that could enable the targeting of mutated pathways in the autoinflammatory interferonopathies and possibly improve safety.

Conclusion

Over the past two decades, advances in both basic and translational research have enhanced the understanding of disease-specific pathways that drive type I interferon production in the genetically defined autoinflammatory interferonopathies and monogenic SLE. This research has improved pathogenic characterization, disease monitoring and identified novel treatment targets. The discovery of the predominant role of intracellular nucleic acid sensor pathways in AGS, alongside the characterization of sensors of proteotoxic stress, and endosomal TLR7 in monogenic SLE represent substantial progress. The development and validation of the type I interferon signature as a diagnostic and therapeutic biomarker are promising for monitoring inflammation in autoinflammatory interferonopathies, and its use in a broader spectrum of diseases is being evaluated.

Use of advanced models of tissue and organ inflammation involving multi-cellular platforms (tissue, cell lines and organoids derived from induced pluripotent stem cells) and profiling of lesional tissue from patients using spatial transcriptomics, proteomics and metabolomics have started to unravel the effect of disease-causing mutations on critical cells and tissues such as the CNS, endothelial cells, adipocytes, liver and lungs. With the application of treatments that effectively block interferon signalling, the role of interferon in disease pathogenesis can be evaluated. Interferon-independent effects of mutations on target tissues might be blocked by targeting sensors such as cGAS, STING, PKR and TLR7 and provide novel strategies for the treatment of rare autoinflammatory interferonopathies and the broader spectrum of diseases with increased sterile interferon signalling. The ability to characterize inflammatory pathways by molecular profiling and the availability of targeted biomarkers herald a new era of precision immunology that could enable upstream targets to

be pinpointed to improve efficacy and safety profiles in the treatment of interferon-mediated diseases beyond the genetically defined autoinflammatory interferonopathies and monogenic SLE.

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




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An update on autoantibodies in the idiopathic inflammatory myopathies

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Abstract

Myositis-specific autoantibodies (MSAs) have become pivotal biomarkers for idiopathic inflammatory myopathies and have revolutionized understanding of the heterogeneous disease spectrum that affects both adults and children. The discovery and characterization of MSAs have substantially enhanced patient stratification based on clinical phenotype, thereby facilitating more precise diagnosis and ultimately improving management strategies. Advances in immunoassay technologies in the past 20 years have further propelled the field forward, enabling the detection of a growing repertoire of autoantibodies with high specificity and sensitivity; however, evolving research over the past decade has revealed that even within antibody-defined subsets, considerable clinical diversity exists, suggesting a broader spectrum of disease manifestations than previously acknowledged. Challenges persist, particularly among patients who are seronegative, where the failure to identify certain rare MSAs stems from the use of diverse detection methodologies and inadequate consensus-guided standardization and validation protocols. Bridging these diagnostic gaps is crucial for optimizing patient care and refining prognostic stratification in idiopathic inflammatory myopathies.

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Key points

- Autoantibodies, found in over half of adult and paediatric patients with idiopathic inflammatory myopathies (IIMs), correlate with specific clinical phenotypes, aiding in the classification, diagnosis and prognostic assessment of these diseases.
- The emergence of new myositis autoantibodies, along with a deeper understanding of serological categories in the past 10 years, enriches the diagnostic and prognostic repertoire for IIMs.
- A more concerted global exploration of ethnic, environmental and genetic diversities is crucial to improve the characterization of subsets within the serological categories already known in IIMs.
- Numerous new immunoassays for detecting myositis-specific autoantibodies, including line blot techniques, have variable performance and lack standardized protocols, requiring further validation for reliable myositis autoantibody detection.

Introduction

Idiopathic inflammatory myopathies (IIMs), also known as myositis, encompass a complex and diverse array of diseases characterized by muscle inflammation, often accompanied by extramuscular manifestations affecting the skin, lungs, heart and joints. The historical classification of IIMs initially included dermatomyositis and polymyositis^{1,2}, and was further refined with the identification of two additional subgroups: inclusion body myositis (IBM)³ and immune-mediated necrotizing myopathy (IMNM)⁴, which are defined by distinct clinical and histopathological features. However, these classifications have been challenged owing to the emergence of autoimmunity as a key factor in the pathogenesis of IIM, prompting reclassification efforts as early as 1991 (ref. 5). Autoantibodies are detected in 50–70%^{6–10} of adults and children with IIMs and the identification of these autoantibodies has enhanced the understanding of IIMs overall. These autoantibodies target various cellular components, including both nuclear and cytoplasmic elements, and are traditionally classified into two groups: myositis-associated autoantibodies (MAAs) and myositis-specific autoantibodies (MSAs). MAAs can occur in conjunction with other autoantibodies and are associated with various autoimmune rheumatic diseases, not just limited to IIM subtypes. Conversely, MSAs are exclusively found in patients with IIM, with only a small percentage of patients with IIM showing multiple MSAs^{6–8,11,12} and infrequently showing overlap with other MSAs and MAAs¹³. Despite not being specific to IIM, seropositivity for MAAs can help to differentiate between inflammatory and non-autoimmune myopathies¹⁴.

MSAs and MAAs are increasingly recognized as diagnostic and prognostic biomarkers for IIMs, which can contribute to the diagnosis and classification of patients into more homogenous groups, such as juvenile IIM, dermatomyositis, IMNM, antisynthetase syndrome (ASyS), IBM and subsets with debated nomenclature, such as polymyositis and overlap myositis. The current understanding of IIM classification is summarized in Box 1; however, challenges in disease management have highlighted that heterogeneity often exists even within these autoantibody-defined subsets, suggesting an even broader clinical spectrum than previously recognized. In this Review,

we provide an updated perspective on the expanding repertoire of MSAs and MAAs identified over the past 5 years, highlighting how MSAs relate to the various subtypes of IIMs and the clinical associations of MSAs and MAAs in both adults and children with IIM. In addition, we outline and discuss the increasing array of autoantibody detection methods and the challenges they present (Box 2). This Review will not focus on the pathogenic mechanisms and management of IIM.

Autoantibodies in dermatomyositis

Dermatomyositis is marked by distinctive skin manifestations and often accompanied by muscle inflammation. This section explores the various MSAs linked with dermatomyositis, highlighting their clinical significance and correlation with disease manifestations and prognosis.

The majority of individuals with dermatomyositis are positive for at least one MSA, including anti-Mi2 (ref. 15), anti-melanoma differentiation-associated gene 5 (anti-MDA5)^{16,17}, anti-transcriptional intermediary factor 1 (anti-TIF1)^{18,19}, anti-nuclear matrix protein 2 (anti-NXP2)²⁰, or anti-small ubiquitin-like modifier activating enzyme (anti-SAE)²¹ autoantibodies. In the EuroMyositis registry, MSAs were identified in 42.9% of patients across four centres⁶. Patients with dermatomyositis typically exhibit distinctive cutaneous features that can include a periorbital heliotrope (blue–purple) rash with oedema, erythematous rash on the face, anterior chest ('V sign'), back and shoulders ('Shawl sign') or the lateral aspect of the thighs ('Holster sign'), and violaceous papules or plaques or erythema located on the dorsal part of the metacarpophalangeal or interphalangeal joints ('Gottron papules' or 'Gottron's sign'). However, not all patients will present with all skin signs. Aside from these characteristic skin manifestations, patients with dermatomyositis can present with specific clinical phenotypes that vary depending on the presence of specific autoantibodies (Table 1 and Fig. 1). The clinical phenotype associated with some MSAs also differs between cases of juvenile-onset and adult-onset dermatomyositis¹⁰.

Anti-melanoma differentiation-associated gene 5 autoantibodies

Anti-MDA5 antibody-positive dermatomyositis (MDA5-DM) is a distinct subtype of IIM. Anti-MDA5 autoantibodies bind to the helicase domains of the MDA5 protein²². Type I interferon transcription is induced by activated MDA5 (MDA5 is activated by anti-MDA5 autoantibodies binding to double-stranded RNA)²³. MDA5-DM represents less than 2% of adult-onset IIMs in Europe and exhibits a prevalence ranging from 7% to 60% in Asian populations²⁴, whereas the reported prevalence of juvenile-onset dermatomyositis is 6–13% in European cohorts^{7,25} and 28% in Asian cohorts²⁶.

Although MDA5-DM shares the classic cutaneous features of dermatomyositis associated with other autoantibodies, this subtype is characterized by a distinct phenotype that includes palmar papules and skin ulcerations²⁴. These manifestations, which reflect vasculopathy linked to elevated type I interferon levels, are less common in other forms of dermatomyositis and might not always be recognized by general physicians. Lack of detection of MDA5-DM-associated manifestations can complicate early diagnosis and management. Notably, skin ulceration, which is particularly prevalent in MDA5-DM, is considered a strong predictor of interstitial lung disease (ILD) development in affected patients²⁷. Muscle involvement can be minimal, and potentially even amyopathic, in children and adults with MDA5-DM compared with other forms of dermatomyositis.

The manifestations of MDA5-DM are reviewed extensively elsewhere²⁸. One of the most common clinical features of MDA5-DM

Box 1 | Overview of the current classification of IIM

The classification of idiopathic inflammatory myopathies (IIMs) has evolved since the initial criteria were published in 1975 (refs. 1,2). Autoantibodies have become essential for diagnosing and classifying IIMs, allowing a more precise categorization of patients into specific subtypes. Polymyositis is now recognized as part of a broader spectrum of IIM entities, which include: dermatomyositis, immune-mediated necrotizing myopathy (IMNM), antisynthetase syndrome (ASyS), inclusion body myositis and overlap myositis syndromes.

Although advances have been made in the definition of these subgroups of IIMs, which are defined by the current classification criteria for IIMs, as published by the EULAR and the ACR in 2017 (ref. 243), heterogeneity remains an issue. The field of IIMs continues to evolve, with ongoing research in transcriptomics, proteomics and machine learning, which provide deeper insights into the disease.

is ILD^{24,29}; rapidly progressive ILD (RP-ILD) is the most severe manifestation and is associated with a high mortality rate^{30,31}. Disparities between the association of anti-MDA5 antibodies and RP-ILD have been reported in different cohorts. For example, in populations in East Asia, 82–100% of patients with MDA5-DM have ILD, with RP-ILD reported in 39–100% of patients²⁴. In populations from Brazil, North America and Europe the incidence of RP-ILD is lower, with 38–73% of patients with MDA5-DM having ILD and 20–57% having RP-ILD²⁴; however, many of the studies on the association between ILD with anti-MDA5 antibodies are contradictory and therefore this area requires further research. Radiographic patterns of ILD described in MDA5-DM include organizing pneumonia³², non-specific interstitial pneumonia and usual interstitial pneumonia, with organizing pneumonia being the most predominant radiographic pattern³³. The efficacy of intensive first-line combination therapy has been shown in two studies of patients with MDA5-DM-associated RP-ILD; this intensive therapy involves either a combination of glucocorticoids, tacrolimus and intravenous cyclophosphamide or a combination of glucocorticoids, rituximab, tofacitinib and/or plasma exchange^{34,35}. Despite these treatment options, RP-ILD associated with MDA5-DM remains potentially fatal in both children and adults¹⁰. RP-ILD has a high mortality rate (approximately 50%) and the majority of deaths occur during the very early stages of disease³⁶, but data from some studies suggest that disease progression tends to slow down 6 months after onset and relapse seems to be uncommon³⁷. However, there are reports of disease recurrence in the form of cutaneous and/or severe respiratory relapse that can occur many years after onset and after months of clinical remission^{38,39}. The strategic timing of administration of this aggressive first-line combination therapy and the assessment of clinical presentations merits further investigation. Notably, anti-Ro52 antibodies are highly prevalent in patients with MDA5-DM (up to 75%), and the presence of these autoantibodies in patients with MDA5-DM correlates with an elevated rate of RP-ILD and mortality^{40,41}.

The heterogeneity of clinical presentations has led to the subclassification of MDA5-DM into three main phenotypes on the basis of predominant symptoms: RP-ILD with mechanic's hands (which has the

highest mortality compared with other MDA5-DM phenotypes), a pure dermatological pattern with cutaneous manifestations and inflammatory arthritis and/or arthralgia (which has a better prognosis than other MDA5-DM phenotypes), and a vasculopathic dermatomyositis associated with cutaneous findings of digital necrosis, calcinosis and Raynaud phenomenon, as well as an increased incidence of muscle inflammation (which has an intermediate prognosis)^{24,42}. Numerous cases of MDA5 autoimmunity were reported during the COVID-19 pandemic; a retrospective analysis of a large cohort of patients in the UK who developed MIP-C (MDA5-autoimmunity and MIP-Interstitial Pneumonitis Contemporaneous with the COVID-19 Pandemic) revealed a shared immunopathology that was similar to the established mechanisms seen in autoimmune lung diseases⁴³. Interestingly, the rate of ILD was much lower in the MIP-C cohort than in historical cohorts of individuals with MDA5-DM⁴³. This observation was particularly intriguing given that MDA5 functions as a retinoic acid-inducible gene 1 (RIG-I) helicase, which can sense RNA and function as a crucial pattern recognition receptor for the SARS-CoV-2 virus^{16,44}.

Distinctive laboratory features of MDA5-DM include elevated ferritin levels, which correlate with disease severity, particularly that of ILD^{32,45–47}, and liver enzyme abnormalities without a concurrent increase in muscle enzyme levels. Markers such as the neutrophil-to-lymphocyte ratio⁴⁸, peripheral lymphocyte count⁴⁹ and serum KL-6 concentration³² are indicative of prognosis in MDA5-DM, with higher ratios and lower lymphocyte counts associated with worse outcomes and elevated KL-6 levels correlating with severe ILD and increased mortality. New biomarkers, including plasma keratin 19 (KRT19)⁵⁰, IFN β and eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2)⁵¹, show promise in aiding diagnosis and prognosis prediction of MDA5-DM-associated ILD. The potential association between anti-MDA5 antibodies and malignancy has been assessed in numerous different cohorts, but clear answers have not yet emerged. In East Asian cohort studies, no association has been found between anti-MDA5 antibody positivity and malignancy^{37,52}. Conversely, a French cohort study reported that 7.6% of patients with MDA5-DM were diagnosed with a malignancy, the prevalence of which was much lower than paraneoplastic cases in MDA5-autoantibody-negative dermatomyositis⁴².

Further insights are necessary to refine predictive factors and underlying mechanisms of MDA5-DM across different ethnicities, especially owing to the lower prevalence of MDA5-DM-associated RP-ILD in non-Asian populations. A better and urgent understanding of biomarkers and treatment guidelines that are tailored to the different clinical phenotypes of MDA5-DM are equally crucial to improve patient outcomes.

Anti-transcriptional intermediary factor 1 autoantibodies

Anti-TIF1 autoantibodies (also known as anti-155/140 or anti-p155 antibodies) specifically recognize a 155 kDa nuclear protein, sometimes in combination with a 140 kDa protein. Initially identified in both juvenile and adult patients with dermatomyositis^{19,53}, these antibodies were further characterized as targeting TIF1 γ (p155), TIF1 α (p140) and TIF1 γ/α (p155/140) proteins¹⁸. Anti-TIF1 autoantibodies are the most prevalent MSA found in juvenile-onset IIM, with reported frequencies ranging from 17% to 35% (refs. 7,10). These autoantibodies are most frequently observed in white children and in those diagnosed at a younger age (median age -6–7 years)^{10,54}.

Patients with anti-TIF1 autoantibodies often present with mild muscular symptoms⁵⁵, yet dysphagia can be frequent and severe⁵⁶. In addition, patients commonly experience severe skin manifestations,

including a heightened risk of ulceration, calcinosis and lipodystrophy, which is particularly notable in paediatric populations^{10,57}, along with non-classical features, such as hyperkeratotic papules and psoriasis-like lesions⁵⁸. Intriguingly, in adults, anti-TIF1 autoantibodies are thought to be protective against calcinosis, and these patients rarely develop ILD^{59–61}.

An important feature of anti-TIF1 antibodies is their strong correlation with cancer in adult patients, particularly the anti-TIF1γ IgG2 subtype^{53,62–64}; approximately 50–84% of adults with dermatomyositis who test positive for anti-TIF1 autoantibodies also develop malignancies^{55,66}. TIF1γ is crucial for the regulation of transcription and post-translational modification and has been shown to suppress various tumours (including non-small-cell lung cancer, breast cancer, glioma and clear-cell renal cell carcinoma); tumour suppression by TIF1γ is mediated through the TGFβ–SMAD and the Wnt–β-catenin signalling pathways⁶⁷. It is hypothesized that in anti-TIF1γ antibody-positive cancer-associated dermatomyositis, cancer triggers the development of dermatomyositis; TIF1γ is implicated as a possible tumour autoantigen. The expression of tumour-specific neoantigens that subsequently stimulate autoantibody production could be caused by mutations, such as loss of heterozygosity, in the gene that encodes TIF1γ in malignant

tissues. The resulting autoantibodies could potentially cross-react with antigens found in the muscle and skin and therefore promote the development of dermatomyositis^{66–68}. The types of cancer seen in patients with IIM can vary depending on their geographical and ethnic backgrounds.

Interestingly, although anti-TIF1 autoantibodies are more prevalent in juvenile-onset IIM than in adult-onset IIM, the association with cancer has not been observed in children^{7,69}. Similarly, this association has not been seen in children with anti-NXP2 autoantibodies¹⁰, which will be discussed in more detail in the next section. Mechanisms other than cancer could contribute to the breakdown of tolerance to the TIF1γ or NXP2 antigens in juvenile-onset IIM. The development of anti-TIF1γ antibodies and anti-NXP2 antibodies in children could be a result of an anti-cancer immune response targeting abnormal cells (which might or might not be cancerous). The vigorous immune response targeting neoantigens might be strong enough to eliminate any subclinical malignancy before it is detected⁷⁰. In addition, associations between HLA haplotypes and the presence of anti-TIF1γ autoantibodies are different between adults and children, suggesting that there might be differences in the precise antigen targets between these groups. Adults with anti-TIF1γ antibody-positive IIM

Box 2 | Autoantibodies in idiopathic inflammatory myopathies

Classification and clinical relevance

- **Dermatomyositis subtypes:** Include autoantibodies such as anti-Mi2, anti-MDA5, anti-TIF1 and anti-NXP2 autoantibodies, each of which is linked to specific clinical features.
- **Inclusion body myositis (IBM):** Often detected with anti-cN1A autoantibodies, especially in men 45 years of age and older.
- **Immune-mediated necrotizing myopathy (IMNM):** Associated with substantial muscle weakness and necrosis and characterized by the presence of anti-SRP and anti-HMGCR autoantibodies.

Autoantibody detection methods

- **Immunoprecipitation:** The gold standard for detecting autoantibodies, particularly for identifying protein and RNA components.
- **Enzyme-linked immunosorbent assay (ELISA):** A highly sensitive method for detecting specific myositis-specific autoantibodies (MSAs), which is used for monitoring antibody levels and disease progression.
- **Line immunoassay (LIA) and dot blot assay:** Multiplex methods that enable the simultaneous detection of various autoantibodies, although the performance of these methods can vary.
- **Particle-based multianalyte technology (PMAT):** Shows promise for detecting specific myositis autoantibodies and results align well with immunoprecipitation results.
- **Immunoprecipitation in combination with mass spectrometry (IP-MS):** Shows potential for uncovering autoantibodies, particularly those directed against complex autoantigens.

Diagnostic and prognostic utility

- **Myositis-specific autoantibodies:** Exclusively found in patients with IIMs; the detection of these antibodies aids in disease classification and predicting clinical outcomes.

- **Myositis-associated autoantibodies (MAAs):** Found in IIM and other autoimmune diseases; could be helpful in distinguishing inflammatory myopathies from non-autoimmune myopathies.

Clinical challenges

- **Heterogeneity in autoantibody subsets:** Clinical presentation varies among patients, even within antibody-defined groups, which complicates management strategies.
- **Reliability of detection methods:** Variability and lack of standardized protocols across different immunoassays.

Emerging insights

- **Novel autoantibodies:** New autoantibody discoveries, such as anti-specificity protein 4, anti-cell division cycle and apoptosis regulator 1, anti-cortactin antibody, anti-eukaryotic initiation factor 3, expand the understanding of IIM subtypes.
- **Pathogenic role:** The role of autoantibodies in disease mechanisms remains an ongoing research area, with some studies showing correlation of autoantibodies with disease activity.

Clinical implications

- **Cancer associations:** Anti-TIF1 and anti-NXP2 autoantibodies are linked with an increased risk of malignancy, necessitating thorough cancer screening.
- **Extramuscular manifestations:** Specific autoantibodies are associated with conditions such as interstitial lung disease and cardiac involvement, which influence management strategies.
- **Paediatric considerations:** Unique clinical courses and manifestations in juvenile patients, with some autoantibodies, such as anti-NXP2, showing a higher incidence of calcinosis, and no association of cancer with anti-TIF1γ and anti-NXP2 autoantibodies.

Table 1 | Main clinical associations of myositis-specific, myositis-associated and novel autoantibodies in adult and juvenile IIM

Autoantibody	Target antigen	Main clinical associations and associated phenotype	Autoantibody	Target antigen	Main clinical associations and associated phenotype
Myositis-specific autoantibodies			Novel autoantibodies		
Anti-Jo1	Histidyl-tRNA synthetase	ASyS ILD	Anti-U1-RNP	U1 ribonucleoprotein	SLE-myositis overlap MCTD
Anti-Ha/YRS	Tyrosyl-tRNA synthetase	ASyS ILD	Anti-Ro52 and Anti-Ro60	Ro-52 (also known as TRIM21) Ro-60	ASyS ILD
Anti-Zo	Phenylalanyl-tRNA synthetase	ASyS ILD	Anti-Ku	DNA-binding protein	SSc-myositis overlap
Anti-EJ	Glycyl-tRNA synthetase	ASyS ILD	Anti-cN1A	Cytosolic 5' nucleotidase 1A	IBM
Anti-PL7	Threonyl-tRNA synthetase	ASyS ILD	Anti-RuvBL1 and RuvBL2	RuvBL1 and RuvBL2	SSc-myositis overlap
Anti-PL12	Alanyl-tRNA synthetase	ASyS ILD	Anti-SMN complex	Survival of motor neuron complex	SSc-myositis overlap MCTD
Anti-OJ	Components of the multi-enzyme synthetase complex	ASyS ILD	Anti-FHL1	Four-and-a-half LIM protein 1	Severe muscle involvement including dysphagia
Anti-KS	Asparaginyl-tRNA synthetase	ASyS ILD Arthritis Sicca syndrome	Novel autoantibodies		
Anti-Ly	Cysteinyl-tRNA synthetase	Unknown	Anti-CCAR1	Cell division cycle and apoptosis regulator 1	Decreased risk of cancer development when present alongside anti-TIF1γ antibodies
Anti-MDA5	Melanoma differentiation-associated protein 5	Severe skin manifestations (ulcerations) RP-ILD Inflammatory arthritis and arthralgia	Anti-Sp4	Specificity protein 4	Cutaneous dermatomyositis Raynaud phenomenon Decreased risk of cancer development when present alongside anti-TIF1γ antibodies
Anti-TIF1	Transcriptional intermediary factor 1	Severe skin manifestations Mild muscular symptoms Dysphagia Malignancy	Anti-CTTN	Cortactin	ILD
Anti-NXP2	Nuclear matrix protein 2	Calcinosis Muscle involvement Gastrointestinal symptoms Malignancy	AMA	Non-organ-specific mitochondrial antigens ^a	Severe muscle involvement, including paraspinal muscle involvement, respiratory dysfunction and cardiomyopathy
Anti-Mi2	Nucleosome remodelling deacetylase complex	Classical cutaneous dermatomyositis Severe muscle involvement	Anti-eIF3	Eukaryotic translation initiation factor 3 subunit	Undefined
Anti-SAE	Small ubiquitin-like modifier-activating enzyme	Classical cutaneous dermatomyositis Mild muscular symptoms Dysphagia ILD Malignancy	<p>^aNumerically classified as M1–M9. M2 is the most commonly recognized antigen complex, consisting of the 2-oxo-acid dehydrogenase complex (2-OADHC) family located in the inner mitochondrial membrane. AMA, anti-mitochondrial antibody; ASyS, antisynthetase syndrome; CCAR1, cell division cycle and apoptosis regulator 1; cN1A, cytosolic 5'-nucleotidase 1A; CTTN, cortactin; eIF3, eukaryotic initiation factor 3; FHL1, four and a half LIM domains 1; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IBM, inclusion body myositis; ILD, interstitial lung disease; MCTD, mixed connective tissue disease; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; SAE, small ubiquitin-like modifier activating enzyme; SLE, systemic lupus erythematosus; SMN, survival of motor neuron; Sp4, specificity protein 4; SRP, signal recognition particle; SSc, systemic sclerosis; TIF1, transcriptional intermediary factor 1; TRIM21, tripartite motif-containing protein 21; U1RNP, U1-ribonucleoprotein. Table adapted from Lundberg et al.⁷⁹, Springer Nature Limited.</p>		
Anti-SRP	Signal recognition particle	Severe muscle involvement, including dysphagia and cardiac involvement	<p>exhibit a strong association with HLA-DQB1*02:02 (OR 3.31, 95% CI 1.89 to 5.84)⁷¹. Conversely, patients with juvenile-onset IIM exhibited a strong association with HLA-DQB1*02:01, although the strongest association was with HLA-DRB1*03:01 (OR 2.69, 95% CI 1.75 to 4.15), which is on the same haplotype as HLA-DQB1*02:01 (ref. 71). These differences in haplotype association between anti-TIF1γ autoantibodies in adult-onset versus juvenile-onset IIM might provide an explanation</p>		
Anti-HMGCR	3-hydroxy-3-methylglutaryl coenzyme A reductase	Severe muscle involvement			
Myositis-associated autoantibodies					
Anti-PM/Scl	Ribonuclease (PM/Scl-100, PM/Scl-75 subunits)	SSc-myositis overlap			

for the lack of association with cancer in anti-TIF1γ antibody-positive juvenile-onset IIM.

Given the strong association between anti-TIF1γ antibodies and cancer-associated dermatomyositis, adult patients, 40 years of age and older at the time of anti-TIF1γ antibody-positive IIM onset should undergo thorough country-specific or region-specific, age-appropriate and sex-appropriate surveillance and screening for malignancies. International guidelines and summary of recommendations for IIM-associated cancer risk stratification, cancer screening modalities and screening frequency were developed by the International Myositis Assessment and Clinical Studies (IMACS) group in 2023 (ref. 72).

Anti-Nuclear Matrix Protein 2 autoantibodies

Anti-NXP2 antibodies (formerly known as anti-MJ antibodies⁷³) target a nuclear protein called NXP2 (also known as MORC3 (ref. 74)), which is crucial for transcriptional regulation, RNA binding, cellular architecture maintenance and tumour suppression through p53 activation and induction of cellular senescence^{59,71}. Anti-NXP2 antibodies were originally

described in a subset of patients with juvenile-onset IIM and linked to severe, treatment-refractory dermatomyositis with clinical manifestations such as polyarthritis, joint contractures, severe calcinosis and intestinal vasculitis⁷⁵.

Patients with anti-NXP2 dermatomyositis commonly experience severe muscle disease, including dysphagia and dysphonia, whereas skin symptoms are typically less prominent and can lack a pathognomonic rash in some patients⁷⁶. The highest incidence of calcinosis when comparing various juvenile-onset dermatomyositis subtypes is in patients with juvenile-onset dermatomyositis who are positive for anti-NXP2 autoantibodies, with the age of an individual at disease-onset correlating linearly with risk of calcinosis^{10,77}. Adults with anti-NXP2 antibody-positive dermatomyositis often present with calcinosis and subcutaneous oedema^{59,60,78,79}. Gastrointestinal involvement has also been documented in both children and adults with anti-NXP2 antibody-positive dermatomyositis^{80–82}. In a Chinese cohort, gastrointestinal symptoms, such as abdominal pain and melena, typically appeared approximately 10 months after diagnosis, with gastrointestinal perforation occurring in 80% of cases within 2 weeks to

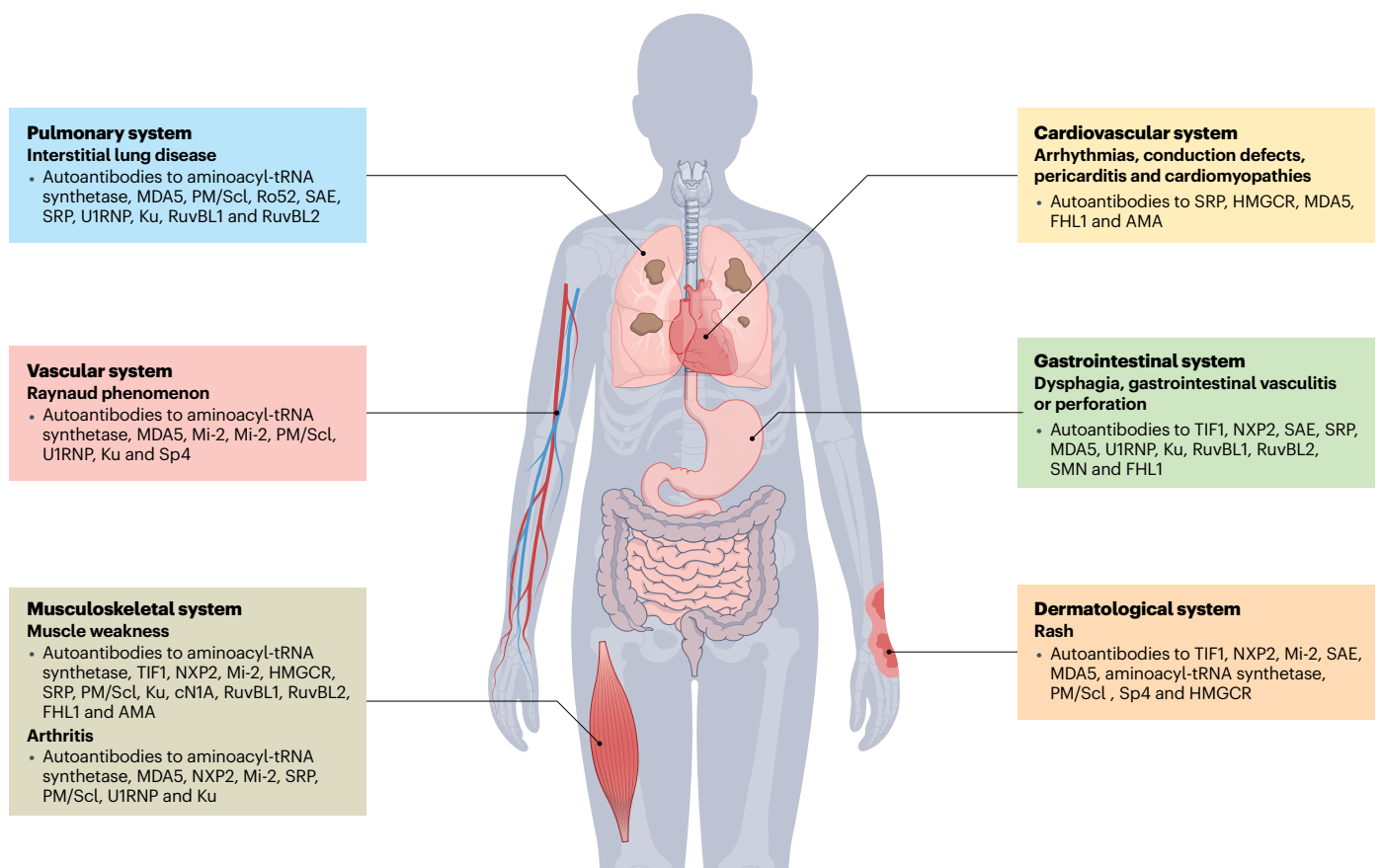


Fig. 1 | Autoantibodies associated with different IIM manifestations. Individuals with idiopathic inflammatory myopathies (IIMs) present with various muscular complications (myositis) and extramuscular complications, such as in the lungs (interstitial lung disease), vasculature (Raynaud phenomenon), heart (arrhythmias, conduction defects, pericarditis and cardiomyopathies), skin (rash), musculoskeletal system (muscle weakness and arthritis) and gastrointestinal tract (dysphagia, gastrointestinal vasculitis and perforation). Some of these manifestations have a strong association with the presence of

specific circulating autoantibodies, which are depicted in this figure. AMA, anti-mitochondrial antibodies; cN1A, cytosolic 5'-nucleotidase 1A; FHL1, four and a half LIM domains 1; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; MDA5, melanoma differentiation-associated protein 5; NXP2, nuclear matrix protein 2; SAE, small ubiquitin-like modifier activating enzyme; SMN, survival of motor neuron complex; Sp4, specificity protein 4; SRP, signal recognition particle; TIF1, transcriptional intermediary factor 1; tRNA, transfer RNA; U1RNP, U1-ribonucleoprotein. Adapted from ref. 79, Springer Nature Limited.

3 months after symptom onset⁸¹. Common histopathological features of anti-NXP2 antibody-positive dermatomyositis include vasculitis, vasculopathy, intestinal smooth muscle necrosis and serosal calcinosis⁸¹; these severe gastrointestinal involvements indicate an underlying role for type I interferon signalling. Various immunosuppressive agents, including vedolizumab⁸¹, infliximab⁸³, rituximab⁸³ and Janus kinase inhibitors⁸³, have shown some efficacy in managing severe gastrointestinal involvement in patients with anti-NXP2 antibody-positive dermatomyositis. Akin to those positive for anti-TIF1 antibodies, adult patients with anti-NXP2 antibody-positive dermatomyositis are at an increased risk of malignancy^{72,78,84} compared with the general population and require screening for malignancy.

Anti-Mi-2 autoantibodies

Anti-Mi2 antibodies target a nuclear helicase protein that is part of the nucleosome remodelling deacetylase complex and is crucial for gene transcription regulation. Patients with anti-Mi-2 antibody-positive dermatomyositis (2–38% of adult-onset dermatomyositis and 4–10% of juvenile-onset dermatomyositis) typically exhibit classic skin manifestations of dermatomyositis^{10,85,86}, along with symptoms such as joint pain, arthritis and Raynaud phenomenon⁸⁵. Both adults and children with anti-Mi2 antibody-positive dermatomyositis often experience severe muscle involvement, characterized by muscle weakness and elevated creatine kinase levels. Biopsy-obtained muscle samples from these patients commonly show substantial necrosis and high severity scores, with a tendency for complement deposition along the muscle membrane^{85,87–89}. Conversely, anti-Mi2 antibody positivity is associated with lower incidences of calcinosis⁸⁸, ILD^{85,88} and malignancy^{85,90} than anti-Mi2 antibody-negative dermatomyositis. Patients generally respond well to conventional treatment, leading to favourable outcomes⁹⁰. Levels of anti-Mi2 autoantibodies have been shown to correlate with creatine kinase levels and muscle strength, which reflects disease severity⁹⁰.

Anti-small ubiquitin-like modifier activating enzyme autoantibodies

Anti-SAE antibodies are a rare MSA found in both adult and paediatric patients, with prevalence of this antibody ranging from <1% to 8% (refs. 21,91). This autoantibody is frequently associated with dysphagia and sometimes linked with ILD⁹². The classic dermatomyositis rash, often extensive^{58,93}, and preceding muscle weakness^{10,91,94}, are prominent manifestations. Muscle weakness tends to be mild⁹³, yet dysphagia^{93,95,96} is notably prevalent in anti-SAE antibody-positive dermatomyositis.

In Asian populations, the incidence of anti-SAE antibody-associated-ILD is higher than in white populations⁹¹, often presenting with an organizing pneumonia pattern^{92,94,97}; however, the development of ILD does not typically worsen the overall prognosis of anti-SAE antibody-positive dermatomyositis. Anti-SAE antibody-positive dermatomyositis has been linked with an increased risk of malignancy^{93,95,98} and is consequently categorized as an intermediate risk factor for cancer among IIMs⁷², necessitating careful screening for malignancy. The prognosis of dermatomyositis associated with anti-SAE autoantibodies remains uncertain, especially as skin disease can be challenging to manage effectively⁹⁴.

Autoantibodies in immune-mediated necrotizing myopathy

Anti-signal recognition particle (anti-SRP) and anti-3-hydroxy-3-methylglutaryl CoA reductase (anti-HMGCR) autoantibodies are both

associated with IMNM. Myofibre necrosis and minimal inflammatory infiltrates are reported in histological biopsy-obtained muscle samples from patients with IMNM⁹⁹. Clinical manifestations often observed in individuals with IMNM typically include severe muscle weakness, high levels of creatine kinase and a poor response to traditional immunosuppressive treatments.

Anti-signal recognition particle autoantibodies

Anti-SRP antibodies target a cytoplasmic ribonuclear protein that transports synthesized proteins to the endoplasmic reticulum. Anti-SRP antibody-positive IIM is more prevalent among Asian populations than in European cohorts^{100–102}. Extramuscular manifestations involving the skin, lungs and heart can occur in patients with SRP antibody-positive IMNM^{103–106}, whereas in patients with HMGCR antibody-positive IMNM it is mainly the skeletal muscles that are clinically affected¹⁰⁷. Although anti-SRP antibodies are uncommon in juvenile-onset IIM (1.6–4% of juvenile onset IIM), the clinical presentation of individuals positive for these antibodies resembles that of adults with rapid disease onset and severe weakness^{108,109}. IIM patients with anti-SRP autoantibodies experience more severe disease than those with anti-HMGCR autoantibodies⁹⁹. Notably, younger age at onset correlates with more severe weakness in anti-SRP antibody-positive IIM than anti-HMGCR antibody-positive IIM¹¹⁰. The presence of anti-SRP autoantibodies has been associated with cardiac involvement, including rhythm or conduction abnormalities, as well as cardiac insufficiency^{105,107}, and a more severe degree of muscle inflammation than that in patients who are positive for anti-HMGCR autoantibodies¹¹⁰.

Anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase autoantibodies

HMGCR is a glycoprotein that catalyses the conversion of HMG-CoA to mevalonic acid, a crucial step in cholesterol biosynthesis. Anti-HMGCR antibodies specifically target the intracellular C-terminal region of the enzyme¹¹¹. Statin (which inhibits HMGCR) is a known, though rare, risk factor for developing IIM associated with anti-HMGCR antibodies^{112,113}. A strong association has been demonstrated between the presence of the HLA DRB1*11:01 haplotype and anti-HMGCR antibody-related myopathy, suggesting a mechanistic link involving statin-induced upregulation of HMGCR expression and potential presentation of HMGCR-derived peptides by DRB1*11:01 (ref. 71). It is hypothesized that statin exposure leads to increased expression and/or structural changes in HMGCR, potentially disrupting immune tolerance in genetically susceptible individuals who develop IMNM. Interestingly, a study found that adults in Asia with anti-HMGCR antibody-positive IMNM generally have lower rates of statin exposure than patients in North America, Europe and Oceania¹¹⁴. It is unclear whether this difference is because of varying rates of statin prescriptions or an increased awareness of anti-HMGCR antibody-positive IMNM. These findings suggest that other factors, such as genetic predispositions, environmental influences or dietary elements (including the presence of statins in the diet) might also have a role in anti-HMGCR antibody-positive IMNM^{111,114}; studies have identified other genetic factors, including DRB1*07:01, that could influence susceptibility to disease¹¹³.

Anti-HMGCR antibody-positive IMNM is less common in juvenile-onset IIM but shares a similar phenotype with adult-onset disease¹⁰. Adults 53 years of age or younger, without prior or lower statin exposure, exhibited a distinctive clinical course with a poorer prognosis compared to the older, statin-exposed group 61 years of age or older¹¹⁵. Despite aggressive immunosuppressive therapy, patients

with anti-HMGCR antibodies often experience relapses, with younger patients typically exhibiting more treatment-resistant disease^{10,113,115}. Although extramuscular manifestations are rare in adult-onset IMNM, skin involvement is frequently reported in childhood-onset disease and more rarely in adult-onset disease. In patients with HMGCR antibody-associated IMNM, rashes can include skin eruptions (similar to those seen in classic dermatomyositis), pruritic erythematous papules with photosensitivity, as well as dermatological manifestations resembling Jessner–Kanoff disease or cutaneous lymphoma¹¹¹. The association of anti-HMGCR antibodies with malignancy risk remains controversial^{98,114,116} and requires further evaluation through larger cohort studies.

Autoantibodies in antisynthetase syndrome

MSAs that target aminoacyl-transfer RNA synthetases (ARS)⁸ are associated with a distinct clinical phenotype known as ASyS that is characterized by the presence of one of the anti-ARS autoantibodies and one or more of the following clinical features¹¹⁷: ILD, muscle inflammation, inflammatory arthritis, Raynaud phenomenon, persistent and unexplained fever or mechanic's hands. Anti-ARS antibodies include anti-Jo1 (anti-histidyl-tRNA synthetase), anti-PL7 (anti-threonyl-tRNA synthetase), anti-PL12 (anti-alanyl-tRNA synthetase), anti-EJ (anti-glycyl-tRNA synthetase), anti-OJ, (anti-isoleucyl-tRNA synthetase), anti-KS (anti-asparaginyl-tRNA synthetase), anti-Zo (anti-phenylalanyl-tRNA synthetase) and anti-Ha/Yrs (anti-tyrosyl-tRNA synthetase) antibodies. Emerging data have identified novel autoantibodies that target other ARS, including autoantibodies against cysteinyl-tRNA synthetase, anti-Ly and valyl-tRNA synthetase^{118–120}. However, the clinical importance of these new autoantibodies currently remains undefined. ASyS is rare in children¹⁰. Patients with ASyS can exhibit the aforementioned clinical manifestations over different time periods. This variability can make diagnosis challenging. In some patients, ILD might be the first symptom to appear, occurring before muscle, joint and skin manifestations¹²¹.

Anti-Jo1 antibodies are the most common anti-ARS antibodies. Raynaud phenomenon is an early feature of anti-Jo1 antibody-positive ASyS and can predate the onset of muscle inflammation⁵⁸. Antibodies targeting non-Jo1 proteins, especially PL7 and PL12, are linked to earlier onset and more severe ILD, along with a poorer prognosis than patients who are positive for anti-Jo1 antibodies^{122,123}. In a study comparing 75 patients who were positive for anti-Jo1 antibodies with 20 patients who were positive for anti-PL7 and/or PL12 antibodies, it was found that the latter group exhibited less severe muscle involvement and lower rates of muscle disease recurrence^{124,125}. Moreover, those with non-anti-Jo1 antibodies have been shown to be more prone to developing ILD without concurrent muscle disease¹²². Research conducted in the past 5 years investigating why non-anti-Jo1 autoantibodies are uncommon has provided intriguing insights into anti-OJ autoantibodies. Unlike single autoantigen targets, anti-OJ antibodies target a multi-enzyme synthetase complex, which could explain its infrequent detection in routine commercial immunoassays¹²⁶. In cohorts in which anti-OJ antibodies have been identified, it is associated with ASyS, often presenting with ILD as the primary manifestation¹²⁷. Some reports suggest that anti-OJ antibody positivity might also be linked to severe muscle involvement^{128,129}. Most patients with anti-OJ autoantibodies show a favourable response to glucocorticoid therapy¹²⁶. However, larger studies are needed to better characterize the precise clinical phenotype associated with anti-OJ autoantibodies.

In an intriguing initiative to enhance stratification of patients with ASyS into more homogeneous subgroups, a study evaluated the

transcriptional signatures associated with distinct clinical phenotypes in a retrospective cohort of Chinese patients¹³⁰. The researchers used unsupervised clustering analysis, which was based on comprehensive clinical features and routine laboratory markers; the study identified three new endotypes among patients with ASyS, which were RP-ILD, dermatomyositis-like and arthritis clusters. These groups showed variations in both clinical outcomes and gene signatures. Although this categorization certainly offers new perspectives, external validation in multicentre cohorts is required.

Myositis-associated autoantibodies

MAAs are not specific to IIM and can be detected in other connective tissue diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren syndrome, rheumatoid arthritis and particularly in overlap myositis – a heterogeneous subtype of IIM characterized by the presence of IIM alongside clinical features from one of the aforementioned autoimmune rheumatic diseases^{131,132}.

Anti-PM/Scl autoantibodies

Anti-PM/Scl-75 antibodies, along with anti-Ro52 antibodies, are one of the most prevalent MAAs in adult IIMs but are less common in juvenile-onset disease¹⁰. Anti-PM/Scl-75 and anti-PM/Scl-100 antibodies are associated with a very diverse group of clinical manifestations, encompassing features of SSc and IIM. These include, in addition to skeletal muscle involvement, inflammatory arthritis, Raynaud phenomenon, dysphagia, gastroesophageal reflux, ILD, puffy hands, sclerodactyly and skin lesions that are characteristic of both SSc and IIM, such as telangiectasia, calcinosis, heliotrope rash, mechanic's hands and Gottron papules¹³³. Renal involvement is possible but is seen in less than 25% of cases and is highly variable across different case series^{134,135}. Anti-PM/Scl antibodies have also been associated with sicca symptoms, pointing to a potential link with Sjögren syndrome^{133,136,137}.

It remains unclear if there are distinct clinical profiles in individuals who are positive for a single PM/Scl antibody or positive for both anti-PM/Scl-75 and anti-PM/Scl-100 antibodies¹³³. In both children and adults, there is a strong correlation between these antibodies and connective tissue disease overlap myositis, although they can also be found in isolation in adult-onset IIM and in juvenile-onset IIM. Anti-PM/Scl autoantibodies do not correlate with clinical activity and are generally observed in individuals with a relatively good long-term prognosis^{138,139}.

Anti-UI-ribonucleoprotein autoantibodies

In both adults and children, anti-UIRNP antibodies were initially regarded as a key diagnostic marker for mixed connective tissue disease (MCTD)¹⁴⁰; however, subsequent research has raised questions about whether MCTD should be considered a distinct disease entity. The substantial overlap in clinical features and disease progression with other autoimmune rheumatic diseases, such as SLE and SSc, has fuelled the ongoing debate on the classification of MCTD¹⁴¹. Additionally, anti-UIRNP antibody positivity is not exclusive to MCTD and can be present in other connective tissue diseases, complicating its role as a definitive diagnostic marker. Despite these challenges, patients with anti-UIRNP antibodies are notable for their prominent extramuscular manifestations. Anti-UIRNP antibody positivity has a strong correlation with ILD, with up to 60% of patients with IIM who test positive for this autoantibody also exhibiting symptoms of ILD, which generally tends to be mild^{142,143}. There are also reported associations of anti-UIRNP antibody positivity with inflammatory arthritis, dysphagia, Raynaud phenomenon, cutaneous features of dermatomyositis, necrotizing

myopathy, pulmonary hypertension, glomerulonephritis, pericarditis, sclerodactyly, calcinosis, subcutaneous oedema and fever^{6,143–146}.

Anti-Ro52 and anti-Ro60 autoantibodies

Anti-Ro antibodies are found in a large number of autoimmune rheumatic diseases, including Sjögren syndrome, SLE, SSc and IIM. Anti-Ro52 autoantibodies are present in nearly one-third of individuals with IIM, and have a strong association with the ASyS subtype (40–60%)^{147,148}, ILD and dysphagia¹⁴⁹. Anti-Ro52 antibodies are found in 6–14% of patients with juvenile-onset IIM¹⁰. Although anti-PM/Scl-75 antibodies are common MAAs in adult-onset IIM, the prevalence of this antibody is generally lower than that of anti-Ro52 antibodies, particularly in ASyS, in which anti-Ro52 antibodies are found more frequently than anti-PM/Scl-75 antibodies. In ASyS, anti-Ro52 antibodies present in isolation, without concomitant positivity for anti-Ro60 antibodies, unlike other connective tissue diseases. Anti-Ro52 antibodies frequently occur with anti-ARS antibodies, most commonly with anti-Jo-1 antibodies, in predominantly white cohorts¹⁵⁰ and with anti-PL7, anti-PL12 and anti-EJ antibodies in Asian cohorts^{150–154}. Anti-Ro52 antibodies have been associated with ILD and a more aggressive disease course, both owing to symptomatic ILD and severe muscle inflammation compared with patients with ASyS without anti-Ro52 antibodies^{155,156}. One prospective observational study found that patients with isolated anti-Ro52 antibodies and non-rapidly progressive ILD typically exhibited a non-specific interstitial pneumonia pattern on imaging. In contrast, those who developed RP-ILD more frequently showed an organizing pneumonia pattern¹⁵⁷. This aggressive disease course has been observed in both adults and children with IIM. Specifically, in juvenile-onset IIMs, the prevalence of ILD is 46% in those who are anti-Ro52 positive compared with 4% in those who are negative for anti-Ro52 antibodies, with a more chronic disease course and less frequent achievement of remission^{158,159}. The association of anti-Ro52 with cancer in adults has been proposed, but there are many more studies opposing this hypothesis than supporting it¹⁵⁸.

Anti-Ku autoantibodies

Anti-Ku antibodies occur in 9–19% of cases of overlap syndromes, particularly those that involve a combination of IIM and SSc¹⁶⁰. Common clinical features in patients with IIM that have anti-Ku antibodies include myalgia, proximal muscle weakness, dysphagia, arthralgia, Raynaud phenomenon and ILD, typically with a poor response to glucocorticoids¹⁶⁰. Some studies have also reported a rare association between anti-Ku antibodies and camptocormia, also referred to as ‘bent spine syndrome’^{160,161}.

The phenotypic characteristics of patients who are anti-Ku antibody-positive described in the literature indicate two distinct clusters. One cluster is characterized by substantial distal muscle weakness with elevated creatine kinase, along with scleroderma-associated features, such as Raynaud phenomenon, sclerodactyly, ILD and oesophageal dysmotility. In contrast, the second cluster exhibits features associated with SLE, including cytopenia, renal involvement, lupus rash and anti-double-stranded-DNA antibody positivity^{162–165}.

Reports of children with IIMs and positive anti-Ku antibodies are rare; hence, a clear phenotype has not yet been described. In a systematic literature review that included 11 paediatric patients positive for anti-Ku antibodies, the authors observed that cutaneous manifestations and joint symptoms were much more common in paediatric patients than in adult patients¹⁶⁶. Serum creatine kinase levels were also more frequently elevated in children than in adults. Conversely,

ILD and oesophageal involvement were less common in children than in adults¹⁶⁶.

Anti-cytosolic 5′-nucleotidase 1 A autoantibodies

The identification of autoantibodies that target cytosolic 5′-nucleotidase 1 A (cN1A; encoded by *NT5C1A*) in 2013 represented a pivotal advancement in the understanding of IBM^{167,168}. IBM is a disease that stands out from other IIMs owing to its slower progression, resistance to treatment and unique clinical and pathological features, such as asymmetric muscle involvement. IBM primarily affects men who are 45 years of age or older¹⁶⁹, and is characterized by a gradual onset of muscle weakness and atrophy affecting both proximal and distal limb muscles, notably the deep finger flexors, knee extensors, ankle dorsiflexors and facial and pharyngeal muscles¹⁷⁰. IBM does not feature specific MSAs. Anti-cN1A antibodies have been detected in some patients with IBM; however, detection of this antibody lacks both sensitivity and specificity.

IBM was previously believed to be primarily driven by T cell-specific autoimmune responses but the discovery of anti-cN1A autoantibodies indicated that IBM might now also encompass elements of humoral immunity. The sensitivity of anti-cN1A antibodies in diagnostic assays for IBM ranges from 33% to 89% (ref. 171). In one study anti-cN1A antibodies were detected in 27% of patients with juvenile-onset IIM¹⁷²; however, the presence of anti-cN1A antibodies in paediatric patients remains controversial, as further studies using several other detection methods have not detected anti-cN1A antibodies in juvenile-onset IIM^{173,174}. Patients diagnosed with systemic autoimmune rheumatic diseases such as Sjögren syndrome and SLE frequently exhibit high rates of anti-cN1A antibody positivity (up to 36.4% in Sjögren syndrome and 20.5% in SLE¹⁷⁵). The detection of anti-cN1A antibodies in diseases such as Sjögren syndrome and SLE can pose challenges to the specificity of IBM diagnosis.

It would be reasonable to conclude that anti-cN1A antibodies primarily serve as a supplementary tool in supporting IBM diagnosis. Diagnosis of IBM remains largely based on the distinctive clinical presentation, electromyography results and characteristic histopathological findings of this disease, rather than autoantibody profiles. Attempts to establish the predictive value of anti-cN1A antibodies in defining specific clinical phenotypes, correlating with histopathological findings, or predicting survival outcomes, have yielded limited conclusive results¹⁷¹.

Anti-RuvBL1 and anti-RuvBL2 autoantibodies

Anti-RuvBL1 and anti-RuvBL2 antibodies were first identified in 2014 and are associated with a unique combination of clinical features that include rapidly progressive diffuse cutaneous scleroderma, skeletal muscle involvement and ILD^{176,177}. In Japanese cohorts, anti-RuvBL1 and anti-RuvBL2 antibody positivity was more frequent in older patients and also in men¹⁷⁶. These antibodies are less frequently associated with conditions such as morphea and necrotizing muscle disease. Thus far, anti-RuvBL1 and anti-RuvBL2 antibodies have been described in 52 patients with SSc and scleromyositis overlap syndromes¹⁷⁷, but the true prevalence of anti-RuvBL1 and anti-RuvBL2-positive IIM and SSc overlap is likely to be underestimated, as these autoantibodies are not included in current commercially available assays. To our knowledge, no cases of anti-RuvBL1 and anti-RuvBL2 positivity have been reported in children, underscoring the need for further research to clarify their full clinical implications and to develop more comprehensive diagnostic tools.

Anti-survival of motor neuron complex autoantibodies

A novel subset of scleromyositis associated with anti-survival of motor neuron (SMN) autoantibodies has been described¹⁷⁸.

Anti-SMN antibodies have been detected in patients with anti-U1-RNP antibody-positive IIM, indicating a potential overlap with other connective tissue diseases. In a 2023 cohort study that included 66 patients with MCTD, anti-SMN antibodies were present in 59% of patients¹⁷⁹. The presence of these antibodies was associated with IIM and severe SSc with myocarditis and lower gastrointestinal tract involvement¹⁷⁹. Notably, patients with high-titre anti-SMN antibodies exhibited an increased risk of developing myocarditis, fingertip pitting scars and small intestinal bacterial overgrowth¹⁷⁹. These findings underscore the potential of anti-SMN antibodies as biomarkers for the identification of patients at risk of a more aggressive disease course and emphasize the need for further research to fully elucidate the role of these antibodies in diagnosis and clinical management.

Anti-four and a half LIM domains 1 autoantibodies

Anti-four-and-a-half-LIM-domain 1 (FHL1) autoantibodies were identified in a Swedish cohort of adult patients with IIM in 2015 (ref. 180). Anti-FHL1 antibodies target a muscle-specific protein that is prevalent in skeletal and heart muscles¹⁸¹. These autoantibodies are present in approximately 14–25% of adults with IIM and around 10.9% of patients with juvenile-onset IIM^{180,182,183}. Initially, anti-FHL1 antibodies were associated with severe muscle involvement and dysphagia¹⁸⁰; however, these clinical features were not consistently replicated in other cohorts^{182,183}. In one study of patients with juvenile-onset IIM, in comparison with those without anti-FHL1 autoantibodies, those with anti-FHL1 autoantibodies had a higher frequency of MAAs, particularly anti-Ro52 autoantibodies (35% versus 13%)¹⁸³. Separately, in a study conducted in an adult cohort in Australia, anti-FHL1 autoantibodies were detected in a subset of patients with SSc and IBM¹⁸². Arguably, therefore, anti-FHL1 antibodies should be considered as MAAs rather than MSAs given the lack of distinctive clinical features of IIM associated with these antibodies. Further research is needed to gain a more comprehensive understanding of the clinical implications and pathogenic mechanisms of anti-FHL1 autoantibodies.

Novel autoantibodies to close the seronegative gap

The serological landscape of overlap myositis has been broadened by the discovery of new autoantibodies, with the majority of discoveries occurring in the past 5 years. The following section offers detailed insights into these findings.

Anti-cell division cycle and apoptosis regulator 1 autoantibodies

The presence of anti-TIF1 γ antibodies in patients with dermatomyositis is linked to an increased risk of cancer, yet a substantial number of these patients do not develop cancer or are diagnosed more than 3 years after disease onset. Research has revealed a broad range of autoantibodies in anti-TIF1 γ antibody-positive patients with dermatomyositis who remain cancer free, with cell division cycle and apoptosis regulator protein 1 (CCAR1) identified as a key antigen in this context¹⁸⁴. Data from a 2023 study in the USA found that antibodies that target CCAR1 were present in at least one-third of the cohort of patients with dermatomyositis who were positive for anti-TIF1 γ antibodies¹⁸⁵. This study, examining adults with dermatomyositis who were positive for both anti-TIF1 γ and anti-CCAR1 antibodies, suggests that the additional presence of anti-CCAR1 antibodies can lower the incidence of cancer to levels that are comparable with those of the general population¹⁸⁵. The possible identification of a subset of patients with dermatomyositis who are at

a decreased risk of developing cancer could mean that these patients might not require intensive cancer screening beyond age-appropriate measures. The study also found that anti-CCAR1 antibodies were absent in healthy individuals and infrequently present in other disease control groups, such as those with anti-HMGCR antibody-positive IMNM, IBM and SLE; anti-CCAR1 antibodies were primarily detected in patients with dermatomyositis with anti-TIF1 γ antibodies. The observation that anti-CCAR1 antibodies are predominantly found in patients with dermatomyositis who are also positive for anti-TIF1 γ antibodies indicates that intermolecular epitope spreading could be caused by these antigens interacting in a molecular complex. Future studies are needed to determine if there is an association between anti-CCAR1 autoantibodies and malignancy in children with IIM.

Anti-specificity protein 4 autoantibodies

Anti-specificity protein 4 (anti-Sp4) autoantibodies have been reported in adults and children with IIM and almost exclusively occur in those also possessing anti-TIF1 γ autoantibodies¹⁸⁶. Sp4 regulates genes that are involved in neurotransmission, metabolism and oncogenesis and are overexpressed in various types of cancer¹⁸⁷. Similar clinical features are observed in both adults and children with IIM who test positive for anti-Sp4 autoantibodies. Notably, these patients exhibit substantial cutaneous manifestations, including Raynaud phenomenon, V-sign rash, malar rash, photosensitivity and linear extensor erythema¹⁸⁸. Adult patients who are positive for both anti-TIF1 γ and anti-Sp4 autoantibodies have a distinctive phenotype characterized by a notably higher prevalence of Raynaud phenomenon and less severe muscle involvement than those positive for anti-TIF1 γ autoantibodies alone. Furthermore, adult patients who are positive for both anti-TIF1 γ and anti-Sp4 autoantibodies show a comparatively lower risk of developing malignancies¹⁸⁶.

Anti-cortactin autoantibodies

Anti-cortactin (anti-CTTN) antibodies were first identified in 2014 as a 68-kD band on confirmatory immunoblotting in patients who tested positive for anti-MDA5 antibodies or anti-HMGCR antibodies via an enzyme-linked immunosorbent assay (ELISA), but were negative for MDA5 and HMGCR antigens on confirmatory immunoblotting¹⁸⁹; these antibodies have since been found to co-occur in patients with anti-Mi2 and anti-NXP2 autoantibodies¹⁹⁰. Cortactin, initially recognized as a key substrate of the oncogene Src tyrosine kinase, is linked to cancer progression¹⁹¹. A single longitudinal cohort study assessed the prevalence of anti-CTTN antibodies in patients with known IIM. This study demonstrated that patients with IIM with coincident anti-CTTN antibodies had a higher prevalence of dysphagia than those who were anti-CTTN antibody negative. The study additionally showed that the titres of anti-CTTN antibodies were higher in patients with ILD than in those without ILD¹⁹⁰; no additional clinical or biological characteristics have been identified thus far. Anti-CTTN antibodies can also be present in some patients with SSc and SLE¹⁸⁹. No differences in the prevalence of anti-CTTN autoantibodies were observed in individuals with juvenile-onset IIM (2%) compared with healthy children (4%)¹⁹².

Anti-mitochondrial autoantibodies

There is growing evidence indicating that mitochondria have crucial roles in the onset and progression of autoimmune and degenerative disorders, particularly those associated with impaired cell metabolism. Beyond the primary role of mitochondria in energy production, mitochondrial involvement is now recognized in inflammatory processes,

potentially contributing to autoimmune conditions, as evidenced by the presence of anti-mitochondrial antibodies (AMAs) in 2%–20% of IIM cases, particularly in East Asia^{193,194}. AMAs target non-organ-specific mitochondrial antigens categorized numerically from M1 to M9. The M2 antigen complex, which is the most widely recognized, consists of the 2-oxo-acid dehydrogenase complex family found in the inner mitochondrial membrane. Notably, AMA-M2 is a serological hallmark for primary biliary cholangitis¹⁹³. The association of IIM with AMAs was first documented in 1974 in a patient with muscle inflammation that was complicated by primary biliary cholangitis¹⁹⁵. Studies over the past 6 years have also highlighted atypical and severe manifestations beyond myopathy, including respiratory dysfunction and cardiomyopathy in patients with IIM associated with AMAs^{196,197}. In addition to proximal muscle weakness, involvement of muscles such as the paraspinal muscles (which results in a lordotic posture), adductor magnus and semimembranosus has been noted^{193,198,199}. This phenomenon is hypothesized to stem from the predominance of type I muscle fibres and the abundance of mitochondria in these muscle groups¹⁹⁸.

Anti-eukaryotic initiation factor 3 autoantibodies

Anti-eukaryotic initiation factor 3 (eIF3) autoantibody is a novel, and rare autoantibody (0.44%) that has been identified in a UK cohort of patients with IIM²⁰⁰. Three patients exhibiting clinical symptoms resembling polymyositis in this cohort tested positive for anti-eIF3 antibodies (which target a cytoplasmic complex of proteins known as eIF3). Interestingly, the presence of these autoantibodies seems to correlate with a positive prognosis, as these patients showed no signs of malignancy or ILD and responded well to immunosuppressive therapy²⁰⁰. However, further studies are needed to fully characterize this association.

Potential pathogenicity of myositis autoantibodies

The pathophysiology of IIM is characterized by an aberrant adaptive immune response pertaining to the presence of T cells and B cells within muscle tissue inflammatory infiltrates, which is often accompanied by the presence of autoantibodies and is associated with specific HLA-DR alleles¹²³. The role of type I and II interferons, and IL-6, which is a key cytokine involved in the initiation of the innate immune responses, has been implicated in the pathogenesis of IIM^{10,201}. Some studies indicate that the immune pathways involved could differ depending on the specific myositis autoantibody group, but more evidence is required to confirm this distinction²⁰².

Whether MSAs have a role in the pathogenesis of IIM or are simply an epiphenomenon remains controversial. In some diseases, the pathogenic role of autoantibodies is well established. For example, in myasthenia gravis, antibodies against the acetylcholine receptor on the postsynaptic membrane and against muscle-specific tyrosine kinase lead to the failure of nerve impulse transmission at the neuromuscular junction, resulting in the fluctuating muscle weakness and fatigue that characterize the disease²⁰³. In addition, epidemiological studies and research in mouse models have elucidated how anti-citrullinated protein antibodies trigger pathogenic mechanisms in rheumatoid arthritis, including effects on macrophage and osteoclast activation, bone loss, modulation of synovial fibroblasts, formation of neutrophil extracellular traps (NETs)-derived immune complexes and pain pathways^{204,205}.

The positive correlation between serum levels of myositis autoantibodies and disease activity is potential evidence to support the argument for myositis autoantibodies having a pathogenic role. The literature includes examples of this relationship in the case of some MSAs, such as anti-MDA5 antibodies, which have been observed in small IIM

cohorts^{206–208}. Decreased titres of anti-MDA5 antibodies are associated with disease remission, and in some patients, these antibodies can become undetectable. Furthermore, an increase in anti-MDA5 antibody titres has also been shown to predict disease relapse, particularly in anti-MDA5-associated RP-ILD^{208,209}. These findings have not, however, been consistently replicated in other studies^{210,211}. A correlation between the levels of both anti-SRP and anti-HMGCR antibodies and the activity of IMNM has also been described^{212,213}. Although the association of MSAs with certain clinical phenotypes and correlations with disease activity supports their pathogenic role, the evidence remains circumstantial.

Emerging evidence indicates the involvement of neutrophil dysregulation and NETs in the pathogenesis of IIMs, but further research is still needed. Increased levels of circulating and tissue neutrophils and NETs, which are composed of nucleic acids, histones and neutrophil-derived proteins, activate the type I and II interferon pathways in patients with IIM^{10,214,215}. This activation contributes to ILD, muscle injury and vasculopathy²¹⁴. Enhanced NET formation has been specifically observed in patients with anti-MDA5 and anti-TIF1- γ antibodies^{216–218}. Furthermore, patients with anti-Jo-1 antibodies exhibit abnormally low DNase I activity, which impairs the degradation of NETs, thereby exacerbating tissue damage²¹⁹. In vitro studies of purified anti-HMGCR and anti-SRP autoantibodies show that these antibodies can reduce the formation of myotubes and induce myotube atrophy²²⁰. Injecting mice with purified IgG from patients who are positive for anti-HMGCR and anti-SRP antibodies leads to muscle weakness and histopathological findings of necrotic myofibres, these findings indicate a complement-mediated pathogenicity of these antibodies²²¹. Findings from a 2024 study revealed that autoantibodies are internalized into muscle fibres in patients with IIM, disrupting the normal function of their autoantigens and contributing to disease pathogenesis²³. For example, anti-Mi2 autoantibodies interfere with the Mi2–NuRD complex, leading to the transcriptional derepression of over 100 genes. In addition, the study found that anti-PM/Scl autoantibodies disrupt the nuclear RNA exosome complex, resulting in impaired RNA degradation. Together, these observations lend support to the potential pathogenic role of at least some MSAs and MAAs, but further research is necessary to elucidate their mechanisms and clinical implications fully.

Methods of autoantibody detection

Historically, the clinical associations of MSAs and MAAs have predominantly been established using immunoprecipitation or double immunodiffusion assays^{222,223}, which detect direct and indirect antibody–antigen interactions²²⁴, respectively. Immunoprecipitation, which is often regarded as the gold standard for autoantibody detection²²⁵, facilitates the identification of protein and/or RNA components of autoantigens on the basis of the known molecular weights of these autoantigens in a single assay¹²². Immunoprecipitation uses S-methionine-labelled cell extracts for detection¹²². There has been a surge in the range of commercially available immunoassays aimed at streamlining the detection and characterization of MSAs. These include ELISAs, line immunoassay (LIA) and dot blot multiplex immunoassays, particle-based multianalyte technology (PMAT) and protein immunoprecipitation in combination with mass spectrometry (IP-MS). Table 2 provides a detailed comparison of the advantages and limitations of these various techniques.

Enzyme-linked immunosorbent assay

Using an ELISA (a single-plex immunoassay) for serological screening is a highly sensitive method for the detection MSAs, but necessitates

Table 2 | Advantages and limitations of the various methods of autoantibody detection

Name of method	Advantages	Limitations	Refs.
Immunoprecipitation	Considered the gold standard for autoantibody detection	Expertise needed to interpret precipitation bands, particularly in the 100 to 200 kDa range on gel electrophoresis; antigens such as MDA5, NXP2 and TIF1 γ show similar migration and immunoprecipitation patterns (for example, a 140 kDa band) Labour-intensive and time-consuming nature of this technique restricts its utility in routine diagnostics	7,10,53,225, 233,235
ELISA	Highly sensitive for MSA detection Strong concordance with immunoprecipitation in identifying anti-Mi2, anti-MDA5, anti-Jo1, anti-EJ, anti-KS, anti-PL7 and anti-PL12 autoantibodies (97.1% sensitivity, 99.8% specificity for anti-ARS antibodies and 98.2% sensitivity, 100% specificity for anti-MDA5 antibodies) Quantifies antibody titres, which is essential for monitoring levels and changes during disease progression or therapy	Factors that can compromise the diagnostic reliability include poor recombinant protein purification, variability in protein expression and stability and the presence of other autoantibodies, paraproteins or hypergammaglobulinaemia Might not be cost-effective to test a single antibody, when assessing a heterogeneous rare disease	24,58,226, 236–239
LIA and dot blot assay	Easily accessible Enable automation and higher throughput for simultaneous detection of various MSAs Enable detection of anti-MDA5, anti-NXP2 and anti-TIF1 γ , which can be challenging to identify through immunoprecipitation Able to provide a semiquantitative result when evaluated with densitometry	Variations in specificity (reactivity in controls) from the lack of standardization across commercial assays from different manufacturers (for example, myositis autoantibodies are detected in up to 22% of healthy individuals) High prevalence of multiple positive results compared with immunoprecipitation Multiplex nature complicates optimization of diagnostic accuracy for each specific myositis autoantibody	148,227,228, 230,232,233, 235,240,241
PMAT	Good agreement with immunoprecipitation (particularly for anti-NXP2, anti-TIF1 γ and anti-Mi2 antibodies) Better correlation with immunoprecipitation than LIA	Larger cohorts needed to fully assess performance	224,229,242
IP-MS	Promising method for detection of complex autoantigen (such as anti-OJ autoantibodies)	Larger cohorts needed to fully assess performance	120

anti-ARS, autoantibodies targeting aminoacyl-transfer RNA synthetases; anti-EJ, anti-glycyl tRNA synthetase; anti-Jo1, anti-histidyl-tRNA synthetase; anti-KS, anti-asparaginyl-tRNA synthetase; anti-MDA5, anti-melanoma differentiation-associated gene 5; anti-NXP2, anti-nuclear matrix protein 2; anti-OJ, anti-isoleucyl-tRNA synthetase; anti-PL7, anti-threonyl-tRNA synthetase; anti-PL12, anti-alanyl-tRNA synthetase; anti-TIF1, anti-transcriptional intermediary factor 1; ELISA, enzyme-linked immunosorbent assay; IP-MS, immunoprecipitation in combination with mass spectrometry; LIA, line immunoassay; MSA, myositis-specific autoantibody; PMAT, particle-based multianalyte technology.

the use of a purified recombinant protein²²⁶. Poor recombinant protein purification, inadequate protein expression and protein instability increase the risk of false-positive detection of autoantibodies when using ELISA²²⁶. ELISAs offer the ability to screen and quantify antibody titres, making it invaluable for monitoring changes in antibody levels over the course of disease or in response to therapy^{24,58}.

Line immunoassay and dot blot assay

The multiplex LIA and dot blot have emerged as reliable alternatives to the labour-intensive immunoprecipitation method for the simultaneous detection of various MSAs²²⁷. Utilizing recombinant and purified antigens, these multiplex immunoassays enable automation and high throughput, with the EUROLINE Autoimmune Inflammatory Myopathies Profile (manufactured by EUROIMMUN) being the most widely used product in clinical and research settings²²⁸. Membranes (often nitrocellulose) are used in LIA as the solid phase that can passively absorb proteins²²⁴. The detection of autoantibodies is based on colorimetric precipitation on the membrane that can be semi-quantified²²⁴. Although these commercial assays are easily accessible, they lack standardization between different manufacturers and can exhibit low specificity. The actual antigens used in the assays are commercial secrets, but the full-length protein is rarely used.

Particle-based multianalyte technology

Particle-based assay for autoantibody detection, also known as PMAT, has shown promising potential as an alternative to the established methods of autoantibody detection²²⁹. PMAT utilizes particles coated with specific autoantigens. When serum from patients is applied, autoantibodies present in the serum bind to these antigens. PMAT can be adapted for high-throughput screening, akin to LIA and dot blot assays, allowing the analysis of many samples simultaneously, which is particularly beneficial in clinical settings. A study conducted on a European cohort of 54 adult patients with IIM revealed a good correlation between the autoantibody results obtained from PMAT and those from immunoprecipitation²²⁹; however, additional validation in larger populations is essential.

Immunoprecipitation in combination with mass spectrometry

The discovery of anti-Ly autoantibodies, which target CARS1, was made using IP-MS, specifically protein immunoprecipitation combined with gel-free liquid chromatography-tandem mass spectrometry¹²⁰. The study that reported the discovery of anti-Ly autoantibodies also highlighted additional patients with anti-OJ autoantibodies that were overlooked by conventional LIA and dot blot immunoassays, indicating

IP-MS as a promising method for discovering and detecting autoantibodies, particularly those against complex autoantigens. As IP-MS is a new technique, described in 2023, further research is warranted to fully explore its potential and broader implications.

Challenges in comparing the different immunoassays

A 2020 survey conducted among prominent international myositis experts, who were also members of the IMACS group, highlighted the diverse techniques used globally for MSA detection. The survey revealed that ELISA is the predominant method for MSA detection globally, followed by LIA. Accordingly, ensuring the reliability of the numerous assays available for autoantibody detection is paramount as MSAs have a crucial role in clinical decision making. Current assessment of these assays, however, reveals variability in their performance, compounded by the absence of agreed standardized protocols for detecting myositis autoantibodies²²⁸.

The validation of testing methods and comparing their sensitivity, specificity and positive predictive value (PPV) poses substantial challenges. Discrepancies among laboratories persist, potentially attributable to the lack of analyte-specific quality controls and adequate calibration, subjectivity in interpretation, alongside variability across different manufacturers^{224,230,231}. Evaluation of these assays needs to be performed in well-defined clinical contexts.

Furthermore, certain assay types exhibit poor performance for specific myositis autoantibodies. For instance, detection of anti-TIF1γ autoantibodies can be limited when using blotting-based assays (which use denatured antigen for detection) as the target of these antibodies is a conformational epitope²³². Accordingly, one study reported that false negatives occurred in 40% of samples when using LIA and 76% when using dot blot assays to detect anti-TIF1γ antibodies in comparison with immunoprecipitation²³². Importantly, ELISA had a higher sensitivity for the detection of TIF1γ autoantibodies in patients with cancer compared with immunoprecipitation and line blot. In a comparison of LIA with immunoprecipitation across three studies, none of the 25 serum samples that tested positive for anti-OJ antibodies by immunoprecipitation (as confirmed by RNA and protein immunoprecipitation or protein immunoprecipitation alone) tested positive on LIA¹²⁶. This disparity emphasizes the importance of developing a more practical assay capable of capturing both direct and indirect (quaternary) interactions involving anti-OJ antibodies and the multi-enzyme synthetase complex that the autoantigen belongs to.

Differences in patient cohorts can have a substantial effect on the interpretation of autoantibody detection results. In particular, the PPV of myositis antibody testing is heavily dependent on disease prevalence within the tested population. Variability in clinical practices among different centres regarding when myositis autoantibody tests are ordered can also affect the resulting PPV outcomes²³³. A retrospective cohort study demonstrated an overall PPV of 63% for myositis antibody LIA testing in patients with suspected IIM²³⁴, contrasting with the lower PPV observed in a New Zealand cohort²³³ of 16.7% owing to lower disease prevalence in the New Zealand population that was studied; only 5% of patients with a myositis autoantibody request in New Zealand cohort had a true-positive result.

The rarity of certain autoantibodies makes validation of autoantibody detection challenging. For instance, certain antisynthetase MSAs included in commercial immunoassays are detected in only 0.3% of patients with IIM⁶. Validation has often been restricted to small cohorts, with a substantial proportion of MSA specificities being underpowered for meaningful analysis. Accurate classification of patients with IIMs

is crucial, in particular, distinguishing the true positives with clinical correlation from false-positive results, defined by the absence of IIM or disease-associated features despite autoantibody positivity, remains pivotal. Importantly, autoantibodies can precede IIM diagnosis, and the presence of autoantibodies can indicate a risk of developing IIMs in the future²³³, highlighting the need for careful clinical correlation when interpreting autoantibody findings.

Multicentre studies involving well-defined patient populations are needed to comprehensively assess the diagnostic performance of various myositis autoantibody tests. Future PPV studies should prioritize clarity in the tested populations and involve larger sample sizes. Clinical correlation integrates all available clinical and ancillary test data, such as physical examination findings, creatine kinase levels, electromyography, muscle biopsy and imaging results, and is essential for interpreting myositis autoantibody test outcomes.

Conclusions

In the past decade, substantial strides have been made in understanding both adult-onset and juvenile-onset IIM, particularly through the identification of distinct serological subgroups. However, the increasing recognition of subsets within these serological categories has also underscored that much remains unknown surrounding this complex disease. Future research should focus on characterization of IIMs through robust global collaborations that explore ethnic, environmental and genetic signatures, diversities and biological processes. The validation and standardization of commercially available autoantibody assays with large, longitudinally followed cohorts are also paramount. Perhaps a shift towards re-defining IIM into distinct endotypes could instead offer new insights into the heterogeneity of this complex disease and could inform the development of personalized treatment strategies.

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Author contributions

D.A.I., N.A.A. and A.I.R.-L. researched data for the article and contributed substantially to discussion of the content. All authors wrote the article and reviewed and/or edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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 Check for updates

Julia Lichtnekert & Hans-Joachim Anders 

In the version of the article initially published, in Fig. 2b, the label “Ilanalumab” was incorrect and has now been amended to “lanalumab”. Additionally, the target of lanalumab was incorrectly shown to be BAFF-R/BCMA/TACI, and the labels “Epratuzumab” and “Anifrolumab” originally both read “Ilanalumab”. These errors have now been corrected in the HTML and PDF versions of the article.

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