Evolving concepts in systemic lupus erythematosus damage assessment

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The Systemic Lupus International Collaborating Clinics–ACR Damage Index (SDI) has been widely used for 25 years. This index, however, has its limitations, providing a rationale for a global initiative to create a revised, updated SDI to capture organ damage across the age-spectrum.

> “no clinical trial has employed the SDI as a primary outcome measure”

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease with diverse manifestations. The assessment of patients with SLE in clinical care and research studies is challenging. Accurate evaluation requires the measurement of five essential domains including disease activity, health-related quality of life, economic impact, adverse effects of treatment and chronic damage[1]. The index currently in wide use for assessing damage does not adequately capture several important concepts. A joint initiative between the Systemic Lupus International Collaborating Clinics (SLICC), the Lupus Foundation of America (LFA) and the ACR is now underway to address this challenge.

In 1996, the SLICC–ACR Damage Index (SDI) was developed to quantify irreversible organ dysfunction that occurs in patients with SLE[2]. The SDI assesses 41 items across 12 organ systems. Most items are assigned one point if present, with two points possible for recurrent events such as avascular necrosis or myocardial infarction and three points for end-stage renal disease, for a possible total score of 45. Attribution to SLE is not necessary, because it is often impossible to discern whether damage is the consequence of disease activity, its treatment or a concomitant illness. To distinguish damage from reversible disease activity, an item generally must be present for six months to be scored.

The SDI is a robust instrument for quantifying damage and has been extensively validated[3]. This tool has prognostic value, with numerous studies showing that damage predicts morbidity and mortality[3]. Increased damage is also associated with increased economic costs[4] and reduced health-related quality of life[3].

An overarching principle of the treat-to-target strategy in SLE is the prevention of organ damage[5], and damage mitigation is a major therapeutic goal in clinical trials. Both the European Medicines Agency (EMA) and the FDA recommend damage as an outcome for SLE clinical trials[6]. To date, however, no clinical trial has employed the SDI as a primary outcome measure.

Over the past 25 years, the SDI has been used in adult and paediatric populations, in cohort studies and in clinical trials. With widespread use, a number of limitations have been described. We will consider some of the key limitations of the SDI and the rationale for a SLICC–LFA–ACR initiative to revise the index.

Timing and duration of damage

The SDI includes items that have occurred “since diagnosis of SLE”[2] and, with the exception of sudden events such as stroke or myocardial infarction, items should be present for six months to be scored[2]. Therefore, at SLE diagnosis, the SDI score is zero. However, some circumstances might merit item inclusion and scoring even if they occurred prior to a formal SLE diagnosis, which is often delayed by several years. In the SLE prodrome, many patients are diagnosed with associated disorders such as cutaneous lupus, mixed connective tissue disease (MCTD) or primary antiphospholipid antibody syndrome (APS). Organ damage might occur as part of that initial diagnosis when a patient is on the path to developing SLE, for example, APS-related stroke or interstitial lung disease in a patient with MCTD. In the current SDI, such damage is not scored even though it is assuredly a manifestation of the same disease process (FIG. 1).

The initial justification for requiring the presence of items for at least six months prior to scoring was to ensure the SDI captured irreversible organ damage, as opposed to transient inflammation. Some items might, however, reverse even after six months; for example, recovery of proteinuria in lupus nephritis is slow, with about half of patients recovering within two years, and in 22% of patients, recovery might take up to five years[7]. Duration of proteinuria and other damage items might need re-evaluation to increase the likelihood that they represent irreversible damage.

Organ domains and item inclusion

In a study of over 1,000 individuals with childhood-onset SLE, many items were infrequently scored, with almost half of all items occurring in less than one percent of individuals[8]. In the same study, the researchers also noted that only four of 41 items in the SDI were associated with a physician-scored visual analogue scale (VAS) of

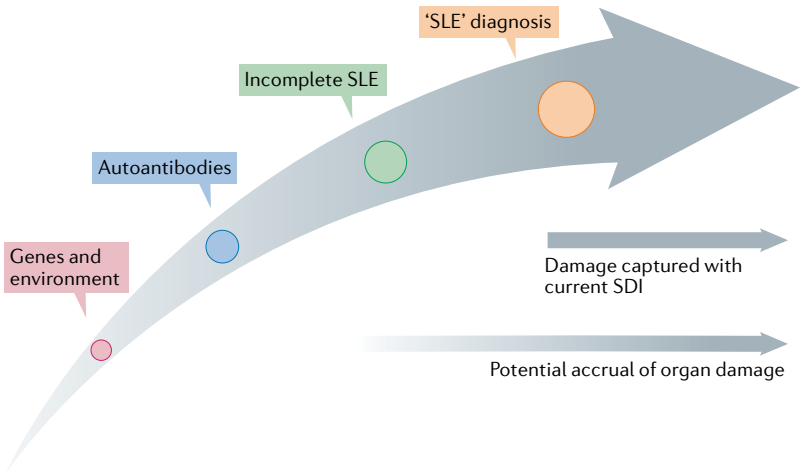

Fig. 1 | **The development of SLE and consequent accrual of organ damage.** Systemic lupus erythematosus (SLE) develops in susceptible individuals along a continuum from autoantibody formation to the development of early features of the disease (incomplete SLE) to the eventual fulfillment of SLE classification criteria. Organ damage might accrue in a prodromal period prior to a formal diagnosis of SLE being made. Such damage is not captured by the current SLICC–ACR Damage Index (SDI).

damage severity. Expert consensus was that the current SDI does not adequately capture damage severity in children[8]. It could be argued, therefore, that the current item list is over-inclusive and that some items could be removed without loss of important metric properties.

Conversely, certain items might affect patients with SLE but are not represented in the current SDI. Some forms of organ damage are specific to paediatric populations including growth failure and delayed puberty. In adults, other items might be worthy of further consideration, such as hypothyroidism, adverse pregnancy outcomes and cardiovascular risk factors.

Item redefinition

Over the past 25 years, new definitions of certain items are now in widespread use. The initial rationale for basing most item definitions on clinical assessment was that technology might not be widely available[2], yet modern diagnostics are now more accessible across health-care settings globally than ever before. For instance, in the current SDI, pulmonary arterial hypertension is defined as a loud pulmonary second sound and/or parasternal heave; however, a formal diagnosis requires objective investigations such as an echocardiogram and/or right heart catheterization. Similarly, the SDI defines pulmonary fibrosis on the basis of a chest radiograph; however, high-resolution CT is now considered as standard-of-care. More nuanced item definitions might also be needed to reflect our modern understanding of their prognostic relevance. In the renal domain, persistent proteinuria in the SDI is currently defined as >3.5 g per day for more than six months. However, in SLE, poor outcomes occur at much lower levels. Similarly, the Kidney Disease Improving Global Outcomes (KDIGO) stages or biopsy features are used to define outcomes in chronic kidney disease populations and are more precise than the SDI definition of renal impairment.

Any effort to modernize items must, however, balance the improved accuracy of item definition with the potential effect in resource-poor settings, as well as the ability in any health-care setting for a full assessment to be undertaken at regular intervals. Removing all clinical definitions could have the adverse consequence of increasing the already pronounced global disparity in SLE research and patient care.

> “An update is necessary to ensure this tool truly captures the extent of organ damage”

Scoring range and weighting

Although the SDI has a scoring range of 0–45, a notable floor effect occurs in the majority of patients who have a score between zero and three, and in half of all patients with a score of zero[9]. Potential ways to address this floor effect might be the inclusion of additional items and/or the provision of more sensitive definitions of items. A related issue is whether items should be weighted. For example, a cataract and a myocardial infarction both score as one point, but these items likely reflect different severities and effects to the patient.

Moving forwards

The SDI has been a widely used and powerful tool in SLE research for the past quarter-century. An update is necessary to ensure this tool truly captures the extent of organ damage. Accurate damage assessment is essential to determine disease prognosis. With trials and the routine use of new medicines for SLE, a new damage index must be able to accurately assess the long-term consequences of this chronic illness across the age-spectrum. An international collaboration between SLICC, the LFA and the ACR to modernize the SDI is now in progress.

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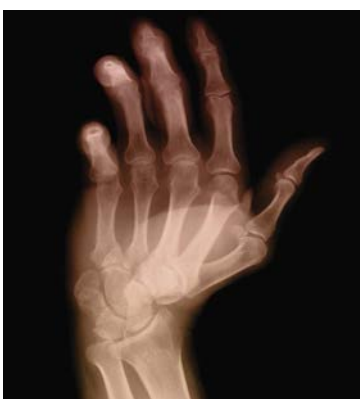
RHEUMATOID ARTHRITIS

Complement primes joints for inflammation

> metabolic changes in primed synovial fibroblasts were dependent on the complement system

Chronic inflammatory diseases, such as rheumatoid arthritis (RA), often involve disease flares, in which inflammation can recur at specific sites. Why certain sites are more prone to recurring inflammation than others has been something of a mystery, as have the mechanisms involved. Theories about the involvement of innate immune cells and so-called 'trained immunity' (a form of immunologic memory in innate immune cells) have been proposed, but fail to explain why only certain sites are affected. This issue has now been addressed in a new study published in *Immunity*, which suggests that a process described as 'inflammatory tissue priming' could be taking place.

"The key motivation for starting this research was to shed more light on the question of how inflammation becomes persistent. Related to this issue is the question of why inflammation has a tendency to recur at specific sites, and if it does, why it is then often more severe than before," says corresponding author Markus Hoffmann. "The phenomenon of inflammatory tissue priming we describe in this study turned out to be fully localized and, after excluding a role for tissue-resident macrophages, we observed that local fibroblasts mediate tissue priming."



Credit: PhotoDisc/GettyImages

Hoffmann and colleagues first established that repeated exposure to an inflammatory challenge led to a shift from an acute and resolving form of arthritis to a more chronic arthritis phenotype in experimental models. Similar results were obtained using different types of inflammatory stimuli across several rodent models of inflammatory arthritis, suggesting that a consistent inflammatory priming process occurs.

Hypothesizing that joint-resident cells might become adapted in some way following exposure to an inflammatory challenge, the researchers focused their attention on synovial fibroblasts. Adoptive transfer of synovial fibroblasts from mouse joints previously challenged twice with monosodium urate (MSU) crystals into recipient mice, followed by subsequent re-challenge with MSU crystals, caused a prolonged form of arthritis compared with transfer of synovial fibroblasts from joints previously challenged only once with MSU crystals. Further in vivo, synovial tissue organoid culture and RNA sequencing experiments confirmed that repeatedly primed synovial fibroblasts have a hypertrophic, pro-inflammatory phenotype, and suggested some mechanisms that could be responsible for this transformation.

"We observed that during priming, fibroblasts undergo metabolic reprogramming with increased aerobic glycolysis and a shift to a pro-inflammatory state, characterized by increased invasiveness, migration and NLRP3 inflammasome activation," states Hoffmann.

Remarkably, these metabolic changes in primed synovial fibroblasts were dependent on the complement system, components of which were upregulated in primed synovial fibroblasts. Complement proteins C3, C5 and their receptors are known to induce metabolic changes that can activate T cells, but a similar role in stromal cells had not previously been shown.

"In a mechanism that is reminiscent of what has been described for T cells, intracellular complement expression (C3 and C3a receptor) and activation of the kinase mTOR and the transcription regulator HIF1α are instrumental for the metabolic invigoration of fibroblasts," explains Hoffmann. "In the absence of C3, mTOR or HIF1α, fibroblasts cannot compensate for the high energy demands that the primed cell state demands and undergo cellular senescence, which is associated with faster resolution of inflammation."

Analysis of synovial sub-lining fibroblasts from patients with RA revealed that they express C3 and slowly increase its production when repeatedly stimulated with TNF in vitro. Synovial fibroblasts from patients with established RA also showed a glycolytic shift and increased invasiveness compared with synovial fibroblasts from patients with very early RA, suggesting that inflammatory tissue priming might also take place in humans.

"This work opens up new avenues for therapeutic intervention," states Hoffmann. "The aim would be to block or even revert tissue priming without immunosuppression. Currently, we are trying to follow up on this by looking into projects that interfere with epigenetic or metabolic reprogramming of primed fibroblasts," he concludes.

Joanna Clarke

ORIGINAL ARTICLE Friščić, J. et al. The complement system drives local inflammatory tissue priming by metabolic reprogramming of synovial fibroblasts. *Immunity* https://doi.org/10.1016/j.immuni.2021.03.003 (2021)
RELATED ARTICLE Nygaard, G. & Firestein, G. S. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. *Nat. Rev. Rheumatol.* **16**, 316–333 (2020)

IMMUNOMETABOLISM

IFNs disrupt T cell metabolism in SLE

Systemic lupus erythematosus (SLE) is often accompanied by increased expression of type I interferon (IFN)-stimulated genes (ISGs), commonly referred to as the type I IFN signature, that is strongly linked to disease activity. Emerging evidence also suggests that metabolic changes in immune cells, including mitochondrial abnormalities, contribute to immune dysregulation and SLE pathogenesis. New findings link chronic type I IFN signalling with mitochondrial abnormalities in $CD8^+$ T cells in patients with SLE and provide further rationale for exploring metabolic pathways as therapeutic targets in this disease.

In searching for differentially expressed genes in the T cell signatures of patients with SLE versus healthy individuals, Buang et al. discovered an enrichment in mitochondrial-associated metabolic pathways, particularly in $CD8^+$ T cells, that was linked to the expression of ISGs. They also identified various mitochondrial changes in the $CD8^+$ T cells of patients with SLE that were associated with a high IFN signature, including an increase in mitochondrial size and membrane hyperpolarization and a reduced spare respiratory capacity. By contrast, the $CD4^+$ T cells had no detectable changes in any of the mitochondrial parameters assessed.

In cultures of peripheral blood mononuclear cells from healthy individuals, exposure of $CD8^+$ T cells to chronic IFNα and T cell receptor (TCR) signalling phenocopied the mitochondrial abnormalities observed in those cells from patients with SLE and a high IFN signature. Notably, prolonged IFNα exposure together with TCR activation reduced cell survival upon TCR restimulation.

Further analysis suggested that chronic type I IFN signalling in $CD8^+$ T cells increases the consumption of nicotinamide adenine dinucleotide (NAD^+), decreasing the NAD^+ to $NADH^+$ ratio and leading to mitochondrial dysfunction and reduced cell viability. Indeed, treatment with a NAD^+ precursor could reverse the SLE-like metabolic changes associated with chronic IFNα and TCR stimulation and improved the viability of the cells upon TCR restimulation.

Jessica McHugh

ORIGINAL ARTICLE Buang, N. et al. Type I interferons affect the metabolic fitness of $CD8^+$ T cells from patients with systemic lupus erythematosus. *Nat. Commun.* **12**, 1980 (2021)

PSORIATIC ARTHRITIS

Upadacitinib improves PsA in phase III trial

In the SELECT-PsA 1 trial, the Janus kinase (JAK) inhibitor upadacitinib improved disease activity and inhibited radiographic progression in patients with active psoriatic arthritis (PsA) who had previously had an inadequate response to non-biologic DMARDs. Together with the results of the SELECT-PsA 2 trial, which enrolled patients who had an inadequate response to biologic DMARDs, the findings suggest that upadacitinib could be a promising treatment option for PsA.

The phase III, double-blind SELECT-PsA 1 study compared treatment with oral upadacitinib (at a daily dose of 15 mg or 30 mg) with placebo and with subcutaneous adalimumab (40 mg every other week) as an active comparator. Patients ($n = 1{,}704$) were randomly assigned to receive one of the four treatments in a 1:1:1:1 ratio.

At week 12, an ACR20 response (the primary end point) was achieved by 70.6% of patients who received the 15 mg dose of upadacitinib and 78.5% of those who received the 30 mg dose, compared with 36.2% of patients who received placebo. The 30 mg dose of upadacitinib, but not the 15 mg dose, was superior to adalimumab with respect to the ACR20 response at week 12; 65.0% of patients in the adalimumab group achieved this end point.

Results for several additional end points were better with either dose of upadacitinib than with placebo, including change from baseline to 24 weeks in the modified total Sharp–van der Heijde score, resolution of enthesitis and PASI75 response at 16 weeks.

The incidence of adverse events was similar with the 15 mg upadacitinib dose and adalimumab, but was higher with the 30 mg upadacitinib dose.

Additional trials with larger numbers of patients are needed to determine the long-term safety of upadacitinib and to compare its efficacy with that of other drugs used to treat PsA.

Sarah Onuora

ORIGINAL ARTICLE McInnes, I. B. et al. Trial of upadacitinib and adalimumab for psoriatic arthritis. *N. Engl. J. Med.* **384**, 1227–1239 (2021)
RELATED ARTICLE Mease, P. J. et al. Upadacitinib for psoriatic arthritis refractory to biologics: SELECT-PsA 2. *Ann. Rheum. Dis.* **80**, 312–320 (2021)

LUPUS NEPHRITIS

Glycosylation influences IgG effects in LN

Lupus nephritis (LN) is a serious complication of systemic lupus erythematosus (SLE), but current treatments and biomarkers for this condition are inadequate. New research published in *JCI Insight* suggests that differential glycosylation of IgG antibodies affects their pathogenicity in LN via a calcium/calmodulin kinase IV (CaMK4)–nephrin pathway, and that urinary CaMK4 could act as a biomarker for LN.

Podocyte-specific CaMK4 inhibition has previously been shown to prevent the deposition of immune complexes and kidney damage in lupus-prone MRL/*lpr* mice, suggesting that it could have potential as a therapeutic target in LN. In the new study, the authors determined that IgG from patients with LN, but not from those with SLE without LN, was able to upregulate the expression of CaMK4 in podocytes in vitro.

Interestingly, the upregulation of CaMK4 by IgG was dependent on the type of glycosylation present on the antibodies: the presence of fucose residues increased CaMK4 expression, whereas the presence of galactose residues reduced CaMK4 expression. Antibodies from patients with LN did not have increased fucosylation compared with antibodies from patients with SLE without LN, but rather had reduced galactosylation. In vitro experiments in podocytes revealed that the increased CaMK4 expression induced by LN IgG suppresses the transcription of nephrin, which is necessary for normal podocyte function, suggesting that a kidney-protective feedback loop is dysregulated in patients with LN.

In patients with LN, CaMK4 expression was high in kidney tissue samples and correlated with disease activity. Furthermore, the amount of CaMK4 mRNA in podocytes in urine samples from patients with LN also corresponded with disease activity, suggesting that urinary CaMK4 could potentially be used as a surrogate biomarker for active LN without the need for biopsies.

Joanna Clarke

ORIGINAL ARTICLE Bhargava, R. et al. Aberrantly glycosylated IgG elicits pathogenic signaling in podocytes and signifies lupus nephritis. *JCI Insight* https://doi.org/10.1172/jci.insight.147789 (2021)
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RHEUMATOID ARTHRITIS

Repurposed drugs could target PIM kinases in early RA

The PIM family of serine/threonine-protein kinases, which have previously been implicated in the pathogenesis of cancer, could be a therapeutic target in a subset of patients with early rheumatoid arthritis (RA), according to new research published in *Arthritis & Rheumatology*.

"PIM kinases can shape adaptive immune responses and even drive aberrant synovial fibroblast proliferation," reports Nicola Maney, first author of the publication. "Importantly, they are already targeted by small molecule inhibitors in development in oncology."

In the study, the researchers confirmed their earlier observation that *PIM1* expression discriminates patients with early RA, noting that *PIM1* is upregulated in $CD4^+$ T cells within peripheral blood mononuclear cells (PBMCs) from patients with early RA compared with patients in the same early arthritis cohort with a diagnosis other than RA. Notably, the study used a readily applicable flow cytometric assay that can measure *PIM1* expression at a single-cell level in frozen PBMCs, which was validated against 'gold standard' quantitative real-time PCR assays.

Analysis of synovial tissue from patients with untreated inflammatory arthritis revealed that PIM1 protein expression was similarly increased in infiltrating $CD4^+$ T cells in early RA synovium compared with non-RA synovium, although correlations between synovial tissue PIM1 protein expression and peripheral blood *PIM1* gene expression in paired samples did not reach statistical significance.

In T cell receptor-stimulated $CD4^+$ T cells isolated from patients with early, untreated RA, exposure to commercially available, small-molecule PIM inhibitors decreased the activation and proliferation of the cells without affecting their viability. PIM inhibition also reduced the production of the pro-inflammatory cytokines IFNγ and IL-17, and led to an expansion of regulatory T cells. In vivo, treatment with PIM inhibitors reduced arthritis severity and cartilage destruction in mice with collagen-induced arthritis.

> "treatment with PIM inhibitors reduced arthritis severity and cartilage destruction in mice with collagen-induced arthritis"

The researchers propose that studies should be undertaken to explore whether PIM inhibitors could be repurposed for use in RA. "We are validating target engagement assays for use in such studies," says corresponding author Arthur Pratt. "Conceivably, the aforementioned PIM1 assay readouts could have additional value as a molecular diagnostic, enabling patients in whom the signalling pathway is most active to be preferentially targeted with PIM1 blockade."

Sarah Onuora

ORIGINAL ARTICLE Maney, N. J. et al. Pim kinases are therapeutic targets in early rheumatoid arthritis. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41744 (2021)

EXPERIMENTAL ARTHRITIS

Inducing apoptosis in joint cells improves advanced arthritis

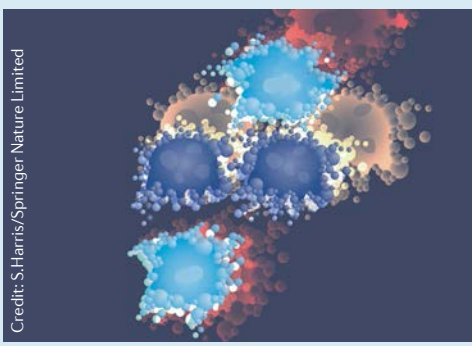
In joints affected by rheumatoid arthritis (RA), the resistance of macrophages and osteoclasts to apoptosis contributes to the progression of synovial inflammation and joint destruction. New research suggests that selectively inducing apoptosis in these cells by targeted chemotherapy with celastrol could have potential for the treatment of advanced inflammatory arthritis.

One potential disadvantage of celastrol, which is a compound isolated from the roots of the *Tripterygium wilfordii* plant (also known as thunder god vine), is that it can be severely toxic to off-target organs including the brain, heart and liver when delivered systemically. The researchers involved in the new study had previously demonstrated in a model of glomerulonephritis that targeted delivery of celastrol to the disease site increased its therapeutic efficacy and decreased its systemic toxicity. "Inspired by our previous findings, we hypothesized that selectively delivering celastrol to both macrophages and osteoclasts in RA joints could efficiently induce apoptosis in these cells, thus reducing synovial inflammation and reversing bone erosion in RA," explains corresponding author Guanghua Lei.

> "Treatment … with CEL-PRNPs alleviated synovial inflammation, reduced cartilage loss and, remarkably, reversed bone erosion"

To that end, the researchers developed RGD peptide-modified PLGA nanoparticles covered with MMP9-cleavable polyethylene glycol chains. In the presence of MMP9, celastrol-loaded modified nanoparticles (termed CEL-PRNPs) led to high rates of apoptosis in both osteoclasts and inflammatory macrophages from patients with late-stage RA.

In rats with advanced adjuvant-induced arthritis, CEL-PRNPs were selectively distributed in osteoclasts and inflammatory macrophages in the joints and decreased the numbers of these cells. Treatment of the rats with CEL-PRNPs alleviated synovial inflammation, reduced cartilage loss and, remarkably, reversed bone erosion, with negligible adverse effects; by contrast, anti-TNF therapy was less effective than CEL-PRNPs at reducing inflammation and failed to reverse bone damage in this model of advanced arthritis.


Credit: S.Harris/Springer Nature Limited

"We plan to carry out further clinical translation research, including studying the long-term toxicity of using CEL-PRNPs," says Lei.

Sarah Onuora

ORIGINAL ARTICLE Deng, C. et al. Targeted apoptosis of macrophages and osteoclasts in arthritic joints is effective against advanced inflammatory arthritis. *Nat. Commun.* **12**, 2174 (2021)

IN BRIEF

SPONDYLOARTHRITIS

Polygenic risk scores outperform other tests in AS

Polygenic risk scores (PRSs) can better discriminate patients with ankylosing spondylitis (AS) from healthy individuals and individuals with chronic back pain than other standard diagnostic tests, according to a receiver operator characteristic analysis. A PRS developed for individuals of European decent had a higher discriminatory capacity (area under the curve (AUC) = 0.924) than *HLA-B27* testing (AUC = 0.869), MRI (AUC = 0.885) or C-reactive protein (AUC = 0.700) in this population. Similarly, a PRS that had been developed specifically for East Asian populations had a better discriminatory capacity than *HLA-B27* testing in individuals of East Asian descent.

ORIGINAL ARTICLE Li, Z. et al. Polygenic risk scores have high diagnostic capacity in ankylosing spondylitis. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2020-219446 (2021)

OSTEOARTHRITIS

Osteoarthritis risk factors differ between sexes

According to an analysis of the Rotterdam Study, the prevalence and strength of various risk factors for knee osteoarthritis differs between men and women. These findings might inform the development of sex-specific risk tools. The majority of the risk factors assessed had a higher prevalence in women than in men, with the exception of alcohol intake and smoking, which were higher in men, and high BMI, which was equal for both sexes. The relative risk associated with high physical activity or a Kellgren–Lawrence score of 1 at baseline was higher for men than for women, whereas the relative risk associated with a BMI ≥27 was higher for women.

ORIGINAL ARTICLE Szilagyi, I. A. et al. Towards sex-specific osteoarthritis risk models: evaluation of risk factors for knee osteoarthritis in males and females. *Rheumatology* https://doi.org/10.1093/rheumatology/keab378 (2021)

SJÖGREN SYNDROME

Ultrasound scoring system shows promise in pSS

Findings from a cross-sectional, single-centre, observational study support the use of the OMERACT ultrasound scoring system in the diagnosis of primary Sjögren syndrome (pSS). Of 134 patients suspected of having pSS, those patients who fulfilled the ACR–EULAR pSS classification criteria more often had a score of ≥2 in ≥1 gland than those patients who did not fulfil the criteria (72% versus 13%; $P < 0.001$). At this scoring cut-off point, the scoring system had a good sensitivity (72%) and specificity (91%) for diagnosing pSS when using the 2016 ACR–EULAR criteria as a reference standard.

ORIGINAL ARTICLE Fana, V. et al. Application of the OMERACT Grey-scale Ultrasound Scoring System for salivary glands in a single-centre cohort of patients with suspected Sjögren's syndrome. *RMD Open* **7**, e001516 (2021)

PAEDIATRICS

MIS-C is a risk factor for thrombotic events

In a multicentre retrospective cohort study of children and adolescents hospitalized with COVID-19 or multisystem inflammatory syndrome in children (MIS-C), thrombotic events occurred in 9/426 patients (2.1%) with COVID-19 and 9/138 patients (6.5%) with MIS-C. In addition to MIS-C, risk factors for thrombosis in these patients included age ≥12 years, cancer and the presence of a central venous catheter. In those patients who developed thrombosis, the mortality was high (28%).

ORIGINAL ARTICLE Whitworth, H. B. et al. Rate of thrombosis in children and adolescents hospitalized with COVID-19 or MIS-C. *Blood* https://doi.org/10.1182/blood.2020010218 (2021)

AUTOINFLAMMATORY DISEASES

Pathogenic *UBA1* variants define a subset of relapsing polychondritis

Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome is a newly discovered condition caused by somatic mutations in *UBA1* and is characterized by systemic inflammation that affects multiple tissues. Because of its wide range of phenotypes, this condition often meets criteria for other rheumatic diseases, including relapsing polychondritis. Two new studies have identified *UBA1* variants in a subgroup of patients with relapsing polychondritis, supporting the concept that relapsing polychondritis is more than one disease.

Relapsing polychondritis is a rare idiopathic inflammatory disease characterized by inflammation of the cartilage in various tissues. Given the discovery of VEXAS syndrome and the heterogeneity of relapsing polychondritis, two teams of researchers independently sought to identify and characterize patients with VEXAS-associated *UBA1* mutations among cohorts of patients with relapsing polychondritis.

In a prospective observational cohort of 92 patients with relapsing polychondritis (72 female and 20 male), Ferrada et al. identified seven patients with somatic mutations in *UBA1* using exome and targeted sequencing. These patients were exclusively male and were also characterized by older age, ear and nose chondritis and haematologic abnormalities.

"We derived an evidence-based clinical algorithm that identified every patient with VEXAS syndrome within our cohort with near perfect accuracy based on male sex and two common laboratory tests," says corresponding author Marcela Ferrada. This algorithm had a 100% sensitivity and 96% specificity.

In a separate study, Tsuchida et al. identified eight patients with VEXAS-associated *UBA1* mutations among a group of 13 patients (11 male and two female) using Sanger sequencing. All of the patients were male and skin inflammation was a prominent feature. "Because low prevalence somatic mutation can be missed with Sanger sequencing, we then performed droplet digital PCR and peptide nucleic acid-clamping PCR to identify low-frequency mutations," explains Yohei Kirino, corresponding author on the Tsuchida et al. study. They identified for the first time a female patient with a somatic variant in *UBA1* (that had a low allele prevalence of 0.14%).

The clinical observations across both studies were similar, with a few differences. Some of the patients with VEXAS syndrome in the Tsuchida et al. study had airway involvement, which was not observed in the other study. "We are not sure why this difference occurred, but we believe it might be due to ethnicity differences," remarks Kirino.

"The data suggest that *UBA1* genetic screening of patients with relapsing polychondritis, especially in men with skin lesions, might be useful in the development of precision medicine for relapsing polychondritis. Further collaborations to determine the specific clinical features of this disease and the optimal strategy for *UBA1* screening, including which specimens and cut-off values to use, are needed," says Kirino.

"We are currently trying to better understand the natural history and clinical features of patients with VEXAS syndrome," explains David Beck, co-senior author on the Ferrada et al. study. "Our ultimate goal is to use these studies to find effective therapies for patients with VEXAS syndrome."

Jessica McHugh

ORIGINAL ARTICLES Ferrada, M. A. et al. Somatic mutations in *UBA1* define a distinct subset of relapsing polychondritis patients with VEXAS syndrome. *Arthritis Rheumatol.* https://doi.org/10.1002/art.41743 (2021) | Tsuchida, N. et al. Pathogenic *UBA1* variants associated with VEXAS syndrome in Japanese patients with relapsing polychondritis. *Ann. Rheum. Dis.* https://doi.org/10.1136/annrheumdis-2021-220089 (2021)

STEM CELLS

Streamlining cell fate decisions during chondrogenesis

Yeri Alice Rim and Ji Hyeon Ju

The accurate homogeneous differentiation of human induced pluripotent stem cells into chondrocytes is crucial for cartilage regenerative therapies. Discovery of the signalling pathways responsible for the differentiation of unwanted cell types during in vitro chondrogenesis could herald a breakthrough for in vitro cartilage generation.

Refers to Wu, C. L. et al. Single cell transcriptomic analysis of human pluripotent stem cell chondrogenesis. *Nat. Commun.* **12**, 362 (2021).

More than a decade has passed since human induced pluripotent stem cells (hiPSCs) were first developed and shown to be easily obtainable and remarkably regenerative, sparking interest in them as potential candidates for regenerative therapeutics[1]. Articular cartilage lacks natural healing ability[1]; thus, various attempts have been conducted to generate and characterize the most ideal articular cartilage-like tissue from stem cells. Although there have been many positive achievements in stem cell research, improved methods are still needed for the differentiation of stem cells into chondrocytes[2]. A new study by Wu et al.[3] has attempted to address this challenge by identifying the signalling pathways responsible for the differentiation of stem cells into unwanted cell types (off-target differentiation) in the hope of enhancing the yield and homogeneity of hiPSC-derived chondrocytes for cartilage repair.

Increased DNA methylation in hiPSCs has the potential to induce off-target cell generation even during directed differentiation towards a specific lineage[4]. Therefore, it is important to understand the gene regulatory networks that lead to on-target or off-target differentiation, as well as the effects of the off-target cells during differentiation at a single-cell level[4]. Such analysis can be achieved using next-generation sequencing and mass cytometry tools. In their study, Wu et al.[3] used both bulk RNA sequencing (RNA-seq) and single-cell RNA-seq to examine differentiated hiPSCs at various time points during mesodermal and chondrogenic differentiation to determine the transcriptomic differences between chondrocytes and off-target cells generated from hiPSCs. Successful chondrogenesis was confirmed by increased expression of chondrogenic markers such as *MATN4*, *ACAN*, *COL2A1*, *COL6A3*, *COL9A1*, *SOX6* and *SOX9*. Bulk RNA-seq data also revealed that hiPSC-derived chondrocytes had a similar phenotype to human embryonic limb bud chondrocytes.

Interestingly, increased expression of *NEUROD4*, the product of which activates neuronal differentiation, was revealed in day 14 chondrogenic pellets[3]. On the basis of this discovery, Wu et al. could identify an off-target neurogenic cell population in pellets. In addition, although most cells in the pellets were characterized as having chondrocyte-like phenotypes, black-pigmented regions were found on the surface, suggesting the presence of another non-chondrogenic off-target cell population. Neurogenic cells were confirmed among the hiPSC-derived chondroprogenitor cells, which were maintained until day 7 of differentiation, and cells that underwent melanogenic differentiation were also confirmed in day 7 and day 14 chondrogenic pellets. These results led to the conclusion that the black regions on the surface of the pellets were melanin pigment accumulated in melanocytes. As such, this study is the first to identify the specific off-target cell populations that occur within differentiated hiPSC-derived chondrogenic pellets. Wu and colleagues also identified the hub genes responsible for both neurogenesis and melanogenesis during in vitro chondrogenesis in hiPSCs. Ectodermal cells give rise to neurons and skin, and WNT signalling has a critical role in both lineages[5,6]; therefore, it was not surprising that *WNT4* was strongly associated with transcription factors that regulate neural differentiation, and *WNT2B* was associated with melanocyte-specific gene expression. Although Wu et al.[3] confirmed mesodermal lineage markers in the differentiated cells, it would have been interesting if they had also investigated specific markers for ectoderm at various time points to confirm the exact time point of the appearance of these off-target cells.

> “WNT inhibitors could be attractive chemical agents for improved in vitro chondrogenic differentiation”

In addition to detecting off-target differentiation-inducing genes, Wu et al. also investigated methods for blocking off-target differentiation[3]. WNT expression was mostly located in the perichondral layer consisting of heterogeneous cells, so the authors hypothesized that the inhibition of WNT signalling might improve the quality of hiPSC-derived chondrogenic pellets by increasing the homogeneity of cells (FIG. 1). As predicted, administration of the WNT inhibitor Wnt-C59 during chondrogenesis increased cell homogeneity in chondrogenic pellets, which suggests that WNT inhibitors could be attractive chemical agents for improved in vitro chondrogenic differentiation.

Notably, long-lasting chondrogenesis does not always lead to cartilage, but rather leads to osteogenic differentiation[7,8]. This process, known as endochondral ossification, occurs naturally during bone development and requires the hypertrophic differentiation of chondrocytes. Chondrocyte hypertrophy is sometimes followed by osteogenesis; however, hypertrophic and osteogenic differentiation should be avoided during cartilage regenerative therapy. Previous studies have shown that activated WNTs stimulate chondrocyte hypertrophy and endochondral ossification in the developing limbs of chicks[9,10]. In the study by Wu et al., WNT3A treatment increased off-target cell differentiation by inducing *COL1A1* and *COL10A1* expression

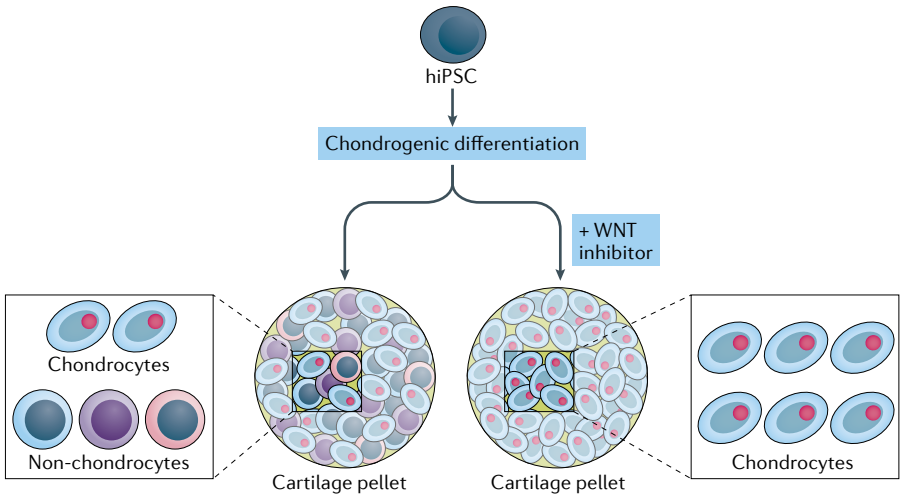


Fig. 1 | **Improving the quality of human induced pluripotent stem cell-derived chondrocytes.** One of the main hurdles to overcome during in vitro chondrogenesis using human induced pluripotent stem cells (hiPSCs) is the generation of heterogeneous populations of cells within chondrogenic pellets that include unwanted cell types. The generation of undesired melanocytes and neurons, which are induced by WNT signalling, can be reduced using the WNT inhibitor Wnt-C59, thereby increasing the homogeneity of chondrocytes within chondrogenic pellets.

in the perichondrium, which was reversed by Wnt-C59 treatment[3]. Canonical correlation analysis revealed one mesenchymal cell subpopulation and four chondrocyte subsets in Wnt-C59-treated pellets, indicating that a fairly homogeneous population occurred after WNT inhibition. Most importantly, Wu et al. discovered a unique chondrocyte subset that expressed interferon-related genes such as *ISG15*, *IFI6* and *MX1*. This population was later identified as mature, hypertrophic chondrocytes that expressed two main hypertrophic markers, *VEGFA* and *MMP13*, and was greatly increased at day 28 of differentiation. Little information has so far been reported about specific markers that can identify hypertrophic chondrocytes, so this discovery could suggest *ISG15*, *IFI6* and *MX1* as new cellular markers that can distinguish hypertrophic chondrocytes during chondrogenesis.

Although pioneering in several ways, the study by Wu and colleagues[3] leaves in abeyance several research topics. Wnt-C59 treatment during mesenchymal differentiation reduced the efficacy of in vitro chondrogenesis by reducing a population of $CD146^{+}CD166^{+}$ potential progenitor cells. Further characterization of these $CD146^{+}CD166^{+}$ mesenchymal cells needs to be carried out to confirm their potential as a candidate cell population for chondrogenic differentiation. The identification of several additional hub genes involved with chondrogenic differentiation also remains to be determined. Furthermore, it will be interesting to compare the overall results to chondrocytes in a diseased condition, such as osteoarthritis. It will be also exciting to confirm the regenerative ability of WNT inhibitor-treated chondrogenic pellets in pre-clinical trials in various cartilage defect animal models to consider whether these pellets could be a potential regenerative therapy for cartilage defects.

In summary, using single-cell-transcriptome analysis, Wu and colleagues have revealed that WNT signalling induces heterogeneity during in vitro chondrogenesis[3]. WNT inhibition had dual effects on hiPSC-based chondrogenesis by removing off-target cells and preventing hypertrophic differentiation. Most importantly, Wu et al. provide an improved chondrogenic differentiation protocol to generate chondrogenic pellets with increased homogeneity that has been validated in a variety of cell lines. This innovative solution gives hope for the development of cartilage regeneration therapeutics and provides a roadmap for the use of single-cell analysis in hiPSC-based differentiation studies.

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Competing interests
The authors declare no competing interests.

Systemic and organ-specific immune-related manifestations of COVID-19

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Abstract | Immune-related manifestations are increasingly recognized conditions in patients with COVID-19, with around 3,000 cases reported worldwide comprising more than 70 different systemic and organ-specific disorders. Although the inflammation caused by SARS-CoV-2 infection is predominantly centred on the respiratory system, some patients can develop an abnormal inflammatory reaction involving extrapulmonary tissues. The signs and symptoms associated with this excessive immune response are very diverse and can resemble some autoimmune or inflammatory diseases, with the clinical phenotype that is seemingly influenced by epidemiological factors such as age, sex or ethnicity. The severity of the manifestations is also very varied, ranging from benign and self-limiting features to life-threatening systemic syndromes. Little is known about the pathogenesis of these manifestations, and some tend to emerge within the first 2 weeks of SARS-CoV-2 infection, whereas others tend to appear in a late post-infectious stage or even in asymptomatic patients. As the body of evidence comprises predominantly case series and uncontrolled studies, diagnostic and therapeutic decision-making is unsurprisingly often based on the scarcely reported experience and expert opinion. Additional studies are required to learn about the mechanisms involved in the development of these manifestations and apply that knowledge to achieve early diagnosis and the most suitable therapy.

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In January 2020, a novel coronavirus — severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) — was identified as the aetiological agent for a cluster of pneumonia cases initially detected in Wuhan City, China[1]. The disease caused by SARS-CoV-2, COVID-19, has a very wide clinical spectrum, ranging from asymptomatic cases (accounting for a substantial proportion of infections) to development of bilateral pneumonia that can progress to respiratory failure and, in some cases, multi-organ failure and death[2]. Some patients can develop a hyperinflammatory response caused by an excessive reaction to the virus, characterized by a highly impaired interferon type I response associated with a persistent blood viral load, with inflammation being partially driven by the transcription factor NF-κB with increased TNF and IL-6 production[3]. This exacerbated immune-related inflammatory response can promote the development of extrapulmonary features[4], including immune-related manifestations that can mimic a wide variety of systemic and organ-specific inflammatory and autoimmune diseases (BOX 1), as has been reported in other viral infections[5]. To date, reports of these manifestations have been scattered among hundreds of manuscripts. This Review compiles the current knowledge of immune-related manifestations associated with the COVID-19 (both systemic and organ-specific) and focuses principally on specific epidemiological, clinical and virological aspects that could help specialists to identify and manage patients presenting with these features.

Immune-related systemic manifestations

Some patients with COVID-19 can develop a severe, acute virus-induced lung injury under the umbrella of acute respiratory distress syndrome (ARDS), a clinical syndrome characterized by acute lung inflammation and increased-permeability pulmonary oedema due to injury to the alveolar capillary barrier[6]. The hyperinflammatory phenotype of ARDS is characterized by elevated concentrations of pro-inflammatory cytokines, an increased incidence of shock and adverse clinical outcomes[7,8]. Although the mechanisms of COVID-19-induced ARDS are still being elucidated[9], the term 'cytokine storm' has become synonymous with its pathophysiology, although some authors have suggested use of this term is misleading in the context of severe COVID-19 (REF.[10]).

Key points

- COVID-19 can produce a systemic inflammatory reaction involving extra-pulmonary organs.
- Immune-related manifestations are increasingly recognized conditions in patients with COVID-19.
- ~3,000 cases involving >70 different systemic and organ-specific immune-related disorders have been reported.
- The clinical phenotype varies and seems to be influenced by age, sex and/or ethnicity.
- The severity of immune-related manifestations of COVID-19 ranges from completely benign, self-limiting manifestations to systemic, life-threatening syndromes.
- Some features tend to appear within the first 2 weeks of SARS-CoV-2 infection, and others emerge in a late post-infectious stage or even in asymptomatic patients.

Even the term cytokine storm has been frequently interchanged with the term 'cytokine release syndrome' (CRS)[10], which describes an immune-related dysregulation associated with the release of large amounts of cytokines that trigger systemic inflammation with multi-organ failure and high mortality rates. CRS is one of the most frequent serious adverse effects of chimeric antigen receptor T (CAR-T) cell therapies[11] and is characterized by fever, tachycardia, tachypnoea and hypotension, the key symptoms that define systemic inflammatory response syndrome[10]. IL-6, a pro-inflammatory cytokine, is an important mediator of the acute inflammatory response in ARDS and CRS, and seems to also factor in severe COVID-19, contributing to elevated C-reactive protein concentrations, hypercoagulation and hyperferritinaemia[12,13]. However, serum IL-6 concentrations reported in patients with COVID-19 are substantially lower than those reported in patients with CRS, ARDS or sepsis[10,14], or even influenza[15]. Why some patients with severe COVID-19 rapidly enter a state of multi-organ failure is unknown, but the pathophysiology of COVID-19-associated ARDS seems to be more complex than a simple overproduction of cytokines[9].

The systemic phenotype related to the inflammatory reaction triggered by SARS-CoV-2 infection is very broad and can be reminiscent of that of some autoimmune or inflammatory diseases. In children, systemic involvement has a substantial overlap with Kawasaki disease, whereas in adults it seems to be closer to haemophagocytic lymphohistiocytosis (HLH), anti-phospholipid syndrome (APS) or systemic vasculitis (TABLE 1, FIG. 1).

Multisystem inflammatory syndrome in children. In April 2020, a number of seriously ill children presented with systemic inflammatory response syndrome features resembling Kawasaki disease were reported in the UK, some of whom tested positive for SARS-CoV-2 infection, a condition that was termed paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 infection (PIMS-TS), also called multisystem inflammatory syndrome in children (MIS-C)[16]. Reports of more than 700 cases of MIS-C, including a large case series in the USA[17], indicate that this syndrome mainly affects children who are aged >5 years and predominantly of non-white ethnicity, with a multisystemic presentation including gastrointestinal (92%), cardiovascular (80%), mucocutaneous (74%) and respiratory (70%) symptoms. The clinical presentation is severe; around half of the reported cases fulfilled the criteria for defined or incomplete Kawasaki disease and 73% required critical care, with an overall mortality rate of 1.7% (Supplementary Table 1). MIS-C has some notable differences from classic Kawasaki disease[18–21], which predominantly affects children under the age of 5 years and most prominently in Asian populations, and has lower rates of admission to an intensive care unit (ICU) and death than does MIS-C. However, the systemic phenotype of MIS-C and the specific involvement of some organs, such as the coronary arteries, suggest a close link with Kawasaki disease[22] and, in fact, the frequency of coronary aneurysms reported in the largest series of children diagnosed with MIS-C (8–23%)[17,23–25] is quite similar to that reported in children with classic Kawasaki disease (6–17%)[18,19]. Most cases of MIS-C could be the result of a post-viral immune-related response. In non-Asian countries with large outbreaks of SARS-CoV-2 (such as France, Italy, Spain, UK and USA), most cases of MIS-C were reported in the late stages of the first wave of the pandemic[17,26] (the reason why Asian countries such as China and Japan have not reported a similar number of MIS-C cases is unknown[27]) and were diagnosed a mean of 25–45 days after onset of SARS-CoV-2 infection[17,28]. Accordingly, the rate of SARS-CoV-2 infection confirmed by serological tests in children with MIS-C is ~90%, in contrast to only ~40% positivity using PCR tests (indicating an ongoing infection) (Supplementary Table 1). The development of a Kawasaki disease-like presentation in children presenting with severe COVID-19 cannot be considered unexpected[29], in view of the important contribution of respiratory viral infections to the aetiopathogenesis of Kawasaki disease[30]. However, the question of whether MIS-C should be considered a discrete entity or could be an aetiologically driven subset of Kawasaki disease is still unresolved[31], as the inflammatory response in MIS-C shares several features with Kawasaki disease but differs with respect to the T cell subsets involved, lack of IL-17A-mediated hyperinflammation and levels of biomarkers related to arterial damage[32].

Cases of young adults with COVID-19 presenting with a classic phenotype of Kawasaki disease have also been reported[33–36]; these cases suggest that the Kawasaki disease phenotype presented by adults with COVID-19 follows the spectrum of classic Kawasaki disease, of which rare cases of adult-onset disease have been reported[37] (Supplementary Table 2).

Haemophagocytic syndromes. Primary and secondary HLH are hyperferritinaemic hyperinflammatory syndromes that have a common terminal pathway but different pathogenetic roots, including viruses as one of the main external triggering factors[38]. The features of COVID-19 and the diagnostic criteria for HLH (high fever, splenomegaly, cytopenia, hypertriglyceridaemia, hyperferritinaemia, hypofibrinogenemia, high serum concentrations of soluble IL-2 receptor (also known as sCD25), low activity of natural killer cells and haemophagocytosis) overlap substantially[39–47]. However, the

Box 1 | **Immune-related disorders reported in patients with COVID-19**

Systemic immune-related manifestations

- Multisystem inflammatory syndrome in children
- Haemophagocytic syndromes or macrophage activation syndrome
- Vasculitis
  - Kawasaki disease in children and adults
  - Retinal vasculitis
  - Cutaneous leukocytoclastic vasculitis
  - IgA vasculitis
  - Small and medium-sized vessel gastrointestinal vasculitis
  - Diffuse alveolar haemorrhage
  - Central nervous system vasculitis
- Antiphospholipid antibodies
- Myositis
  - Acute myalgia
  - Rhabdomyolysis
  - Autoimmune inflammatory myopathy
  - Necrotizing autoimmune myopathy
- Arthritis
  - Acute arthralgias
  - Symmetric polyarthritis
  - Asymmetric oligoarthritis
  - Monoarthritis
  - Psoriatic arthritis
- Other systemic autoimmune diseases
  - Systemic lupus erythematosus-related symptoms
  - Sicca symptoms and/or parotid enlargement
  - Sarcoidosis

Organ-specific immune-related manifestations

- Cutaneous
  - Chilblain lesions
  - Erythema multiforme
  - Livedo reticularis
  - Retiform purpura
  - Oral ulcers
  - Erythema nodosum
  - Periorbital erythema
  - Generalized pustular figurate erythema
  - Sweet syndrome
  - Livedo racemose
- Haematological
  - Immune thrombocytopenic purpura
  - Thrombotic thrombocytopenic purpura
  - Autoimmune haemolytic anaemia
  - Evans syndrome
- Neurological
  - Guillain–Barré syndrome
  - Miller Fisher syndrome
  - Meningoencephalitis
  - Autoimmune encephalitis
  - Acute disseminated encephalomyelitis
  - Acute necrotizing encephalopathy
  - Mild encephalitis or encephalopathy with reversible splenial lesion
  - Longitudinal extensive transverse myelitis
  - Neuromyelitis optica-like syndrome
  - Transversal myelitis
  - Polyneuritis cranialis
  - Optic neuritis
  - Plexopathy
  - Myasthenia gravis
- Pulmonary
  - Interstitial lung disease
  - Post-viral organizing pneumonia
  - Mediastinal lymphadenopathies
  - Pleural effusion
- Cardiac
  - Acute myocarditis
  - Pericardial effusion
  - Cardiac tamponade
- Renal
  - Proximal tubular dysfunction
  - Collapsing glomerulonephritis
  - Focal segmental glomerulonephritis
  - Minimal change disease
  - Crescentic glomerulonephritis
  - ANCA-associated renal vasculitis
  - Membranous glomerulonephritis
  - IgA glomerulonephritis
- Endocrine
  - Clinical hyperthyroidism or thyrotoxicosis
  - Subclinical hypothyroidism
  - Adrenal haemorrhage
  - Adrenal infarction
  - Adrenal insufficiency
- Pancreatic
  - Acute pancreatitis
- Ocular
  - Uveitis
  - Conjunctivitis

frequency of HLH in patients with COVID-19 is probably very small. Of 20 reported cases that were classified as probable HLH, only 20% fulfilled the required five diagnostic criteria for HLH (Supplementary Table 3). In addition, in those studies that used the H-score (a scoring system not validated in prospective studies for HLH diagnosis), only 0–10% of patients achieved the diagnostic cut-off for HLH[41,48]. Consequently, some authors suggest that these patients actually develop ARDS with some HLH features, rather than systemic macrophage activation (the hallmark of HLH)[49,50]. By contrast, other authors contend that all patients with severe COVID-19 should be investigated for underlying HLH[51], considering that most of the reported cases lacked a full evaluation for HLH features (mostly the histopathological criteria and the measurement of natural killer cell function and sCD25 levels were not evaluated), and that macrophage activation syndrome has been reported in 25% of children presenting with MIS-C[52,53]. Probably, HLH related to COVID-19 is a condition with an incidence as rare as the virus-related HLH diagnosed in the pre-pandemic era[38], but owing to the size of the pandemic worldwide reports are yielding a large number of cases. Considering the available evidence, a complete evaluation for possible HLH could be advised for adults with severe COVID-19 who develop cytopenia affecting at least two cell lineages in the peripheral blood (especially including thrombocytopenia), hyperferritinaemia (especially very high levels, that is, >2,000 ng/ml) and hypofibrinogenaemia, as well as in children presenting with life-threatening systemic COVID-19 (MIS-C).

Antiphospholipid syndrome. COVID-19 has been linked with coagulopathy and thrombosis, especially in patients who are severely ill admitted to an ICU[54]. However, SARS-CoV-2 itself does not seem to have intrinsic procoagulant effects, and the abnormal results of coagulation tests frequently detected in patients with COVID-19 seem to be mainly linked to the inflammatory systemic response[1]. However, an additional autoimmune hypothesis emerged when one study reported positivity for lupus anticoagulant in more than 90% of COVID-19 patients[55]. Since then, the number of studies reporting testing for antiphospholipid (aPL) antibodies in patients with COVID-19 has rapidly increased. Among 13 studies that overwhelmingly included patients with COVID-19 admitted to the ICU (Supplementary Table 4), lupus anticoagulant positivity was reported in more than half of the tested patients, although the rate of positive results ranged widely across the studies, from 3%[56] to 91%[55]. Given the common use of heparins for thromboprophylaxis in patients with COVID-19 admitted to hospital, the potential interference of heparin with lupus anticoagulant analysis has been suggested[40]. In addition, lupus anticoagulant antibodies are heterogeneous and are detected in different contexts (including infections and inflammation) that enable the exposure of cell phospholipids that are normally not accessible to the immune system[57]; a positive result is not necessarily linked to the development of APS. With respect to anticardiolipin (aCL) antibodies, among patients with severe COVID-19, the positive rates are lower than those reported for lupus anticoagulant but also widely vary among studies (0–52% positive for IgG-aCL, 3–20% positive for IgM-aCL and 2–32% positive for IgA-aCL antibodies) (Supplementary Table 4), with some studies linking aPL antibody positivity with more severe COVID-19 (REFS[56,58]). Therefore, aPL antibodies are frequently detected in patients with severe COVID-19, as has also been reported in patients with non-COVID-19 ARDS[59]. Owing to the high rates of aPL antibody positivity in patients with COVID-19, it seems rational to consider an autoimmune origin of the thrombosis under the umbrella of APS. Several factors must be considered with

Table 1 | **Summary of reported cases of systemic immune-related manifestations of COVID-19**

Characteristic	MIS-C	Haemophagocytic syndrome	Vasculitis in adults	Myositis	Arthritis
Number of cases reviewed	717	20	19	24	6
Sex ratio (male:female)	4:3	7:3	1:1	7:1	5:0
Mean age (years)	8.5	66.0	55.4	52.4	54.3
Age distribution (%)					
<18 years	717 (100)	0 (0)	0/16 (0)	2/16 (12.5)	0/6 (0)
18–50 years	0 (0)	1 (5)	6/16 (37.5)	5/16 (31.25)	3/6 (50)
>50 years	0 (0)	19 (95)	10/16 (62.5)	9/16 (56.25)	3/6 (50)
Geographical distribution (%)					
Europe	368 (51.3)	13 (65)	15 (78.9)	2 (8.3)	3 (50)
North America	330 (46)	6 (30)	3 (15.8)	17 (70.8)	0 (0)
Asia	19 (2.6)	0 (0)	0 (0)	2 (8.3)	3 (50)
Other	0 (0)	1 (5)	1 (5.3)	3 (12.5)	0 (0)
Virological results (%)					
PCR positive	221/563 (39.3)	20 (100)	15 (78.9)	24 (100)	6 (100)
Serology positive, PCR negative	223/563 (39.6)	0 (0)	2 (10.5)	0 (0)	0 (0)
Negative	60/563 (10.7)	0 (0)	0 (0)	0 (0)	0 (0)
Not tested using any technique	59/563 (10.5)	0 (0)	2 (10.5)	0 (0)	0 (0)
Asymptomatic SARS-CoV-2 infection (%)	184/207 (88.9)	ND	2/18 (11)	4/16 (25)	0 (0)
Time to first symptom after COVID-19 onset (%)					
<7 days	NA	0/2 (0)	6/14 (42.8)	8/12 (66.7)	1/5 (20)
7–14 days	NA	2/2 (100)	3/14 (21.4)	1/12 (8.3)	1/5 (20)
>14 days	NA	0/2 (0)	5/14 (35.6)	3/12 (25)	3/5 (60)
Intensive care (%)	415/582 (71.3)	19 (95)	6 (31.6)	8 (33.3)	0 (0)
Death (%)	11 (1.5)	11/14 (78.6)	3 (15.8)	2 (8.3)	0 (0)

Summary of epidemiological profile, results of virological testing, clinical presentation and outcomes. Details of the selected studies are provided in the Supplementary Tables online. MIS-C, multisystem inflammatory syndrome in children; NA, not applicable; ND, not determined.

respect to a possible role of SARS-Cov-2 as a trigger of APS[42,56,58,60–66] (BOX 2). Although it cannot be discounted that some patients with COVID-19 could present with aPL antibody-related thrombosis, and could therefore be considered as having a COVID-19-related APS, the body of evidence we have reviewed does not support a central role for SARS-CoV-2 as a viral trigger of APS. Because almost all the studies reported to date have been carried out in patients who are severely ill (most with a long stay in ICU), pro-thrombotic factors other than aPL antibodies should always be carefully evaluated.

Systemic vasculitis. Emerging evidence seems to support a potential link between SARS-Cov-2 and systemic vasculitis, including the Kawasaki disease phenotype reported in a considerable percentage of children with MIS-C, the increasing number of reported cases of vasculitis in adults and the post-mortem descriptions of vasculitis involving various organs[67]. In adults, among 15 patients with COVID-19 and vasculitis, cases involved organ-specific involvement of the skin ($n = 9$), central nervous system ($n = 3$), the lungs ($n = 2$) and the gastrointestinal tract ($n = 1$), mainly appearing 2 weeks after the first symptoms of SARS-CoV-2 infection. Histopathological studies confirmed vasculitis in the skin (mainly classified as leukocytoclastic vasculitis) and gastrointestinal tissue (infiltration of small and medium-sized vessels) (Supplementary Table 2).

Vasculitis other than that within the spectrum of Kawasaki disease has been rarely reported in children in association with SARS-CoV-2 infection, and included cutaneous vasculitis[68], retinal vasculitis[69] and a possible central nervous system vasculitis[70]; these children often lack any previous symptoms suggestive of COVID-19 and had negative results of PCR tests but positive IgG serology.

Myositis. Several clinical findings and laboratory abnormalities suggest that a substantial percentage of patients with COVID-19 can have muscular inflammation. Myalgia was reported in approximately 24% of patients included in several large COVID-19 case series (although with a wide range of frequencies, ranging from

1% to 74%), whereas the frequency of raised creatine kinase (CK) levels is ~11% (Supplementary Table 5); myalgias can remain as active, chronic symptoms in ~10–20% of cases of acute COVID-19 (REFS[71,72]). Only two studies have estimated the frequency of myositis in hospitalized patients with COVID-19, finding myositis in 3–11% of patients[73,74], whereas the frequency of rhabdomyolysis is reportedly considerably lower (0.2–1.1%)[73,75]. In these studies, almost all patients with suspected myositic involvement did not undergo myositis-specific tests (such as electromyography, imaging and histopathological studies). Isolated reports of patients with COVID-19 presenting with myositis and/or rhabdomyolysis indicate that most cases occurred in adult males presenting with

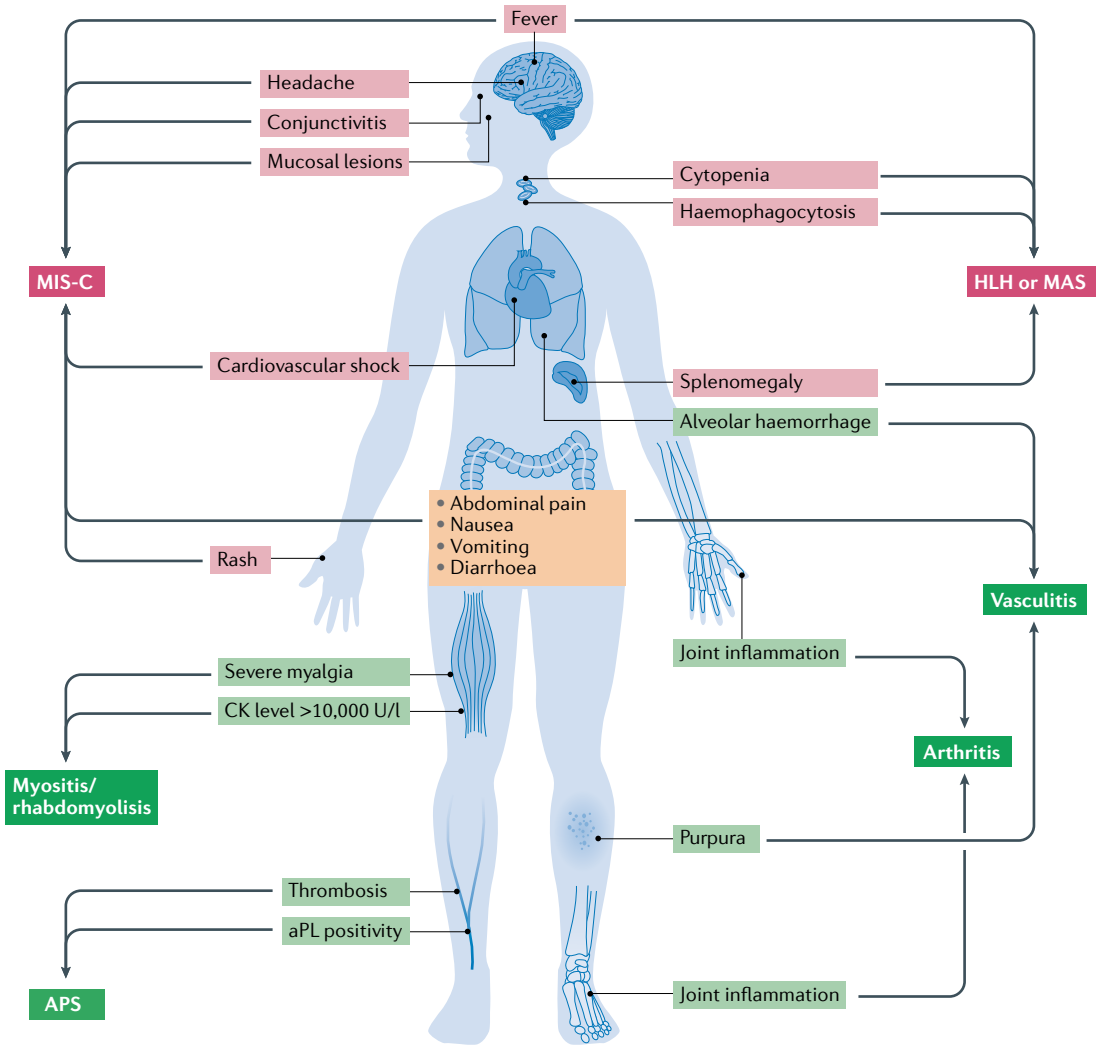


Fig. 1 | **Guiding signs and symptoms of suspected systemic immune-related disease in patients with COVID-19.** The two main systemic inflammatory syndromes associated with COVID-19, multisystem inflammatory syndrome in children (MIS-C) and haemophagocytic lymphohistiocytosis (HLH, including macrophage activation syndrome, MAS) are detailed at the top of the figure. The first sign prompting suspicion of these syndromes is persistent fever without a clear clinical source, together with multisystem organ involvement. The MIS-C phenotype includes Kawasaki disease-like features (conjunctivitis, red cracked lips, swollen hands and feet, and rash), coronary artery enlargement and/or aneurysms, gastrointestinal symptoms (abdominal pain, nausea, vomiting or diarrhoea) and neurological manifestations (headaches and meningitis). With respect to HLH and MAS, the cardinal features are enlarged lymphohaematopoietic organs (lymph nodes, spleen and/or liver) and severely abnormal values for multiple laboratory parameters suggesting involvement of multiple organs (such as severe cytopenia and liver and renal dysfunction). The main signs and symptoms of suspected systemic autoimmune and rheumatic diseases associated with COVID-19 are detailed at the bottom of the figure. Petechial and/or purpuric cutaneous lesions are the main signs prompting suspicion of vasculitis, and the addition of extracutaneous symptoms such as severe abdominal pain, haemoptysis or neurological features could indicate systemic vasculitis. In patients with thrombosis who have antiphospholipid (aPL) antibodies, fulfilment of the classification criteria for antiphospholipid syndrome (APS) should be ruled out. Severe myalgia in association with creatine kinase (CK) levels >10,000 U/l (concurrent with renal failure in some patients) are suggestive of myositis and/or rhabdomyolysis, whereas inflammation of several joints can follow different patterns including symmetric polyarthritis (resembling rheumatoid arthritis), oligoarticular arthritis with cutaneous lesions (resembling psoriatic arthritis) or axial involvement with enthesitis (resembling spondyloarthritis).

Box 2 | **Considering SARS-Cov-2 as a trigger of APS**

Results obtained from different studies[42,56,58,60–65,182] indicate that a number of factors must be considered with respect to a possible role of SARS-CoV-2 in triggering antiphospholipid syndrome (APS).

Extent of association between antiphospholipid (aPL) antibodies and thrombosis:[42,56,58,60,61]

- Using heterogeneous approaches, most studies have discarded a significant association between aPL antibody positivity and thrombosis in patients with COVID-19.
- Combined data from 56 patients admitted to intensive care units in two studies[42,60] depicted a similar frequency of aPL antibody positivity in patients with or without thrombosis:
  - 87% and 83% positive for any aPL antibodies
  - 87% and 76% positive for lupus anticoagulant
  - 47% and 44% positive for anti-cardiolipin (aCL) antibodies
  - 0% and 22% positive for anti-β2 glycoprotein 1 (anti-β2GPI) antibodies

Persistence of aPL antibody positivity:[56,60,61]

- In three studies, aPL antibodies were tested a second time 10–30 days after the first determination.
- In more than 70% of cases, a previous positive result turned negative.

Measurement of aCL antibody titres:[42,60]

- Only 24% of patients with COVID-19 included in two studies[42,60] that detailed individual aCL antibody titres had high titres, and most did not develop thrombosis.
- Most patients with COVID-19 have low-to-moderate aCL antibody titres that are not related to thrombosis.

Detection of multiple aPL antibodies:[42,56,60,61,66]

- The frequency of double and triple positivity (for lupus anticoagulant, aCL antibodies and/or anti-β2GPI antibodies) in patients with COVID-19 was low (24% and 4%, respectively).
- Patients with COVID-19 and triple positivity rarely developed thrombosis.

myalgia (in some cases severe) appearing mainly during the first week of COVID-19, with CK levels being higher than 10,000 U/l in most cases (Supplementary Table 6). The pathogenesis of immune-related muscular damage in COVID-19 is probably multifactorial, and could involve factors linked to critical illness and long ICU admissions, such as critical illness myopathy and superimposed steroid myopathy[73]. In other patients, a direct cytolytic viral effect or damage related to hypercytokinaemia could be involved in the development of an immune-related muscular damage within the spectrum of necrotizing autoimmune myopathy[74], especially in patients presenting with an acute onset of severe muscle weakness with increased inflammatory markers and very high CK levels (in the thousands)[76]. For these patients, a specific muscle-centred diagnostic approach is highly recommended.

Arthritis. Joint pain with no notable evidence of inflammation upon physical examination of the involved joints was reported in ~36% of patients with COVID-19 (REFS[71,77]) (Supplementary Table 5). Although it is not currently known whether COVID-19 will cause an increase in new-onset chronic pain for the population at large, several factors linked to the pandemic (such as psychological distress, epidemiological and socioeconomic factors, poor sleep and reduced physical activity) could be involved in the development of chronic widespread pain[78].

In contrast to non-inflammatory joint pain, arthritis has been reported in isolated cases (affecting predominantly men with a mean age of 54 years), including a wide variety of articular presentations (for example, symmetric polyarthritis, monoarthritis, enthesitis or psoriatic arthritis), and mainly appearing after COVID-19 has resolved (Supplementary Table 7).

Systemic autoimmune diseases. COVID-19 patients can present with several systemic lupus erythematosus (SLE)-related features, including cytopenia (lymphopenia, thrombocytopenia or haemolytic anaemia), arthralgia, serositis, chilblain lesions or aPL antibodies. So far, only one case of probable SLE triggered by SARS-CoV-2 has been reported, which developed in a previously healthy 18-year-old woman who fulfilled the 2019 ACR–EULAR classification criteria for SLE[79].

Sicca symptoms are not usually recorded in COVID-19 clinical studies, and only one small study reported that ~25% of patients mentioned sicca syndrome[71]. Another important feature related to Sjögren syndrome, parotid enlargement, has been reported in 20 patients, who were mostly young people (<30 years old) and were more often women than men (Supplementary Table 8).

Although mediastinal lymphadenopathy has been reported in 3–5% of patients with COVID-19 (REFS[80,81]), only one case of probable sarcoidosis has been reported[82].

Immune-related organ-specific manifestations

In contrast to the above-mentioned systemic presentations that can involve multiple organs, some patients with COVID-19 present with immune-related manifestations involving a single organ, which can mimic a wide range of organ-specific autoimmune diseases (TABLE 2, FIG. 2).

Cutaneous involvement. Symptoms of cutaneous involvement can affect 0.2% to 5% of patients with COVID-19 (REF.[83]), including maculopapular eruptions, urticarial lesions, chilblains and livedoid/necrotic lesions[84]. For maculopapular and urticarial lesions, a predominant drug-induced aetiology is suggested[85], whereas immune-related mechanisms could be postulated for other cutaneous lesions.

The term chilblains (also referred to as pernio) describes a rare inflammatory condition affecting the extremities after exposure to cold, which can cause painful or itchy erythematous or violaceous lesions[86]. An association between chilblains and COVID-19 was initially supported because most reported cases of chilblains in southern Europe occurred during the first peak of the pandemic, and because in one of the largest case series cutaneous lesions appeared after infection onset in two-thirds of patients with symptoms of COVID-19 (REF.[87]). The patient profile derived from more than 1,300 cases of chilblains included in selected studies indicates a clear predominance of young people, with half of the studies reporting only children under the age of 18 years, and the other half including patients with a mean age ranging from 22 to 32 years. However, only 6% of these reported cases had confirmed COVID-19 (although it should be noted

Table 2 | **Summary of reported cases of organ-specific immune-related manifestations of COVID-19**

Characteristic	Chilblains	Erythema multiforme	ITP	Haemolytic anaemia[a]	GBS	Enceph-alitis	Myelitis	Myoca-rditis	Pericardial tamponade	Glomerulo-nephritis	Thyroid-itis	Pancre-atitis
Number of cases reviewed	1,333	17	39	19	73	58	8	25	25	36	85	30
Sex ratio (male:female)	1:1	1:1	1:1	4:3	5:2	1:1	3:1	3:2	4:3	2:1	2:3	1:2
Mean age (years)	21.0	33.3	58.7	55.4	56.35	55.0	45.2	49.1	62.85	57.03	52.4	43.83
Age distribution (%)												
<18 years	9/17 (52.9)	9 (52.9)	1/38 (2.6)	2 (10.5)	3/66 (4.5)	1/57 (1.8)	0 (0)	1 (4)	0 (0)	0/34 (0)	0 (0)	2 (12.5)
18–50 years	8/17 (47.1)	2 (11.8)	8/38 (21.1)	4 (21.1)	15/66 (22.7)	15/57 (26.3)	4 (50)	12 (48)	2 (28.6)	12/34 (35.3)	9 (60)	8 (50)
>50 years	0/17 (0)	6 (35.3)	29/38 (76.3)	13 (68.4)	48/66 (72.7)	41/57 (71.9)	4 (50)	12 (48)	5 (71.4)	22/34 (64.7)	6 (40)	6 (37.5)
Geographical distribution												
Europe	1015 (76.1)	14 (82.4)	32 (82.1)	13 (68.4)	55 (75.3)	42 (72.4)	4 (50)	11 (44)	4 (57.1)	7 (19.4)	55 (64.7)	7 (23.3)
North America	318 (23.9)	0 (0)	3 (7.6)	5 (26.3)	9 (12.3)	5 (8.6)	2 (25)	10 (40)	3 (42.9)	27 (75)	0 (0)	19 (63.3)
Asia	0 (0)	1 (5.8)	4 (10.3)	1 (5.3)	7 (9.6)	11 (19)	2 (25)	4 (16)	0 (0)	2 (5.6)	30 (35.3)	4 (13.3)
Other	0 (0)	2 (11.8)	0 (0)	0 (0)	2 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Virological results												
PCR positive (%)	43/1,262 (3.4)	9 (52.9)	36 (92.3)	19 (100)	56 (76.7)	41 (85.4)	6 (75)	24 (96)	7 (100)	32 (88.9)	83 (97.6)	29 (96.7)
Serology positive, PCR negative (%)	28/1,262 (2.2)	1 (5.9)	0 (0)	0 (0)	6 (8.2)	1 (2.1)	2 (25)	1 (4)	0 (0)	4 (11.1)	2 (2.4)	1 (3.3)
Negative (%)	528/1,262 (41.8)	4 (23.5)	3 (7.7)	0 (0)	7 (9.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Not tested using any technique (%)	663/1,262 (52.5)	3 (17.6)	0 (0)	0 (0)	4 (5.5)	6 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Asymptomatic COVID-19 (%)	675/1,095 (61.6)	1 (5.9)	5 (12.8)	1 (5.3)	3 (4.1)	8/48 (16.7)	0 (0)	1 (4)	0 (0)	5/13 (38.5)	0 (0)	0 (0)
Time to first symptom after COVID-19 onset (%)												
<7 days	12/31 (38.7)	1/3 (33.3)	7/38 (18.4)	3/8 (37.5)	6/59 (10.2)	27/47 (61.4)	3/6 (50)	8/15 (53.3)	3/5 (60)	4/8 (50)	0/7 (0)	9/16 (56.3)
7–14 days	2/31 (6.5)	1/3 (33.3)	12/38 (31.6)	2/8 (25)	22/59 (37.3)	7/47 (14.9)	3/6 (50)	4/15 (26.7)	1/5 (20)	2/8 (25)	1/7 (14.3)	6/16 (37.5)
>14 days	17/31 (54.8)	1/3 (33.3)	19/38 (50)	3/8 (37.5)	31/59 (52.5)	13/47 (27.7)	0/6 (0)	3/15 (20)	1/5 (20)	2/8 (25)	6/7 (85.7)	1/16 (6.2)
Intensive care	0 (0)	1 (5.9)	2 (5.1)	3 (15.8)	20 (27.4)	20 (34.5)	0 (0)	17/21 (81)	7 (100)	0 (0)	0 (0)	11 (36.7)
Death	0 (0)	1 (5.9)	2 (5.1)	1 (5.3)	2 (2.7)	5 (8.6)	0 (0)	6/21 (28.6)	2 (28.6)	4 (11.1)	0 (0)	3 (10)

Summary of epidemiological profile, results of virological testing, clinical presentation and outcomes; details of the selected studies are provided in the Supplementary Tables online. GBS, Guillain–Barré syndrome; ITP, immune thrombocytopenia. [a]Including Evans syndrome.

that testing for the virus was not performed in nearly half of the cases), supporting a weak link between chilblains and COVID-19 (Supplementary Table 9). Potentially, lifestyle changes related to lockdown lead to more inactivity for long periods and this inactivity could contribute to triggering chilblains, especially in predisposed patients (that is, those with a previous history of perniosis, Raynaud syndrome or β-blocker treatment)[88]. Reports of the presence of viral particles in biopsy-obtained skin from children with chilblains and negative results of PCR testing for SARS-CoV-2 might support the need for histopathological studies to confirm a causal relationship between SARS-CoV-2 and these skin lesions[89].

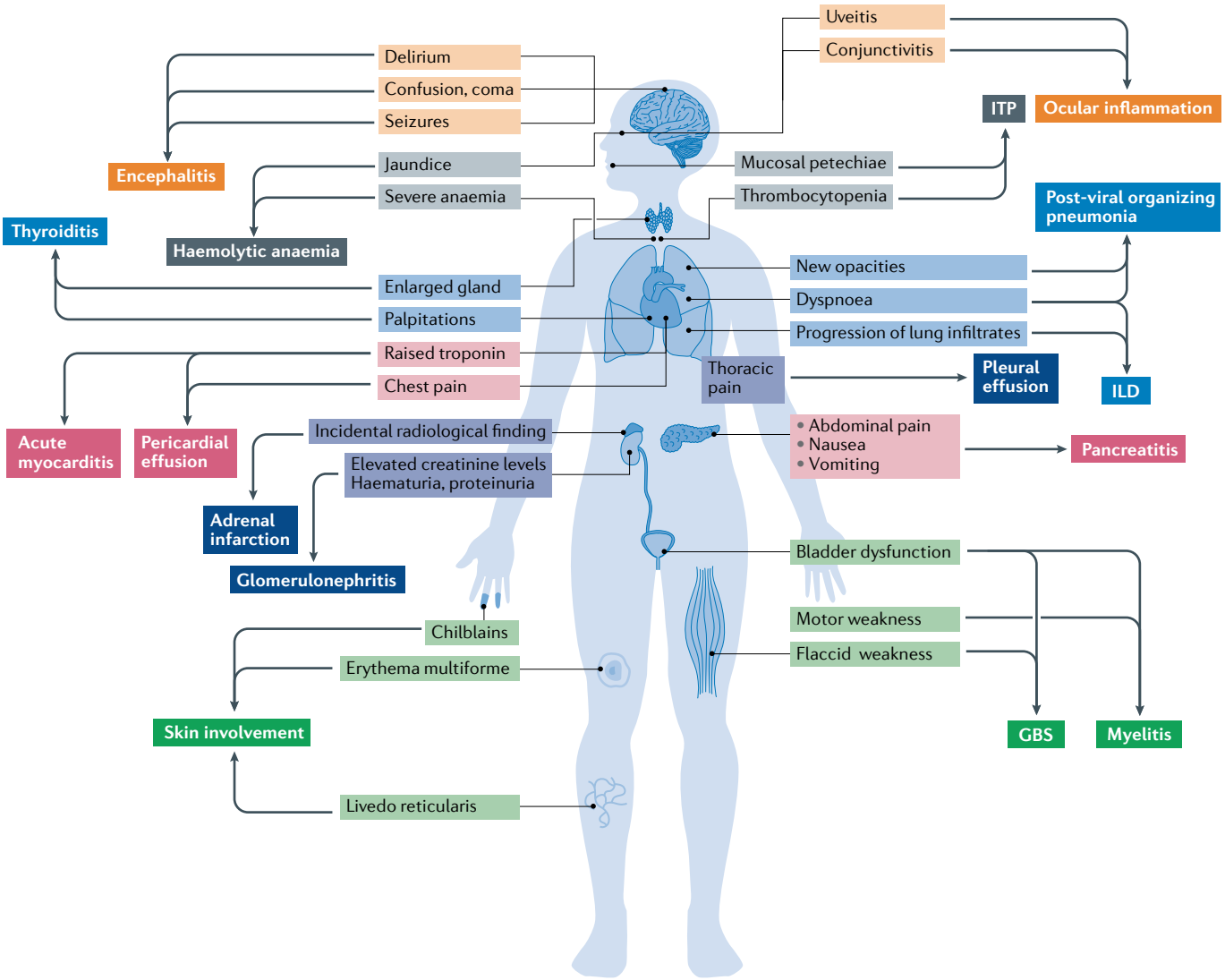


Fig. 2 | **Guiding signs and symptoms of suspected organ-specific immune-related diseases in patients with COVID-19.** The list of clinical symptoms is long, including important features such as dyspnoea (suggestive of interstitial lung disease (ILD) or organizing pneumonia), chest pain (myocarditis, pleuritis and pericarditis), severe acute upper abdominal pain with nausea and vomiting (acute pancreatitis) and neurological features such as confusion, seizures (encephalitis) or weakness with bladder dysfunction (myelitis and Guillain–Barré syndrome (GBS)). Examination is crucial when organ-specific immune-related disease is suspected in patients with COVID-19, paying special attention to eye redness (conjunctivitis and uveitis), jaundice (haemolytic anaemia), cutaneous lesions such as petechiae (immune thrombocytopenia (ITP)) or painful red inflammation on the hands or feet (chilblains), and glandular enlargement in the neck (thyroiditis). Simple laboratory tests such as haemography, biochemical analyses (measuring troponin, pancreatic enzymes, parameters of haemolysis, creatine kinase, haematuria and proteinuria) and determination of thyroid hormone levels could have an important role in diagnosis.

Erythema multiforme is an inflammatory dermatological condition that has been overwhelmingly linked to infectious agents and, less frequently, to drugs[90]. Reports of cases of erythema multiforme in patients with COVID-19 reveal a clearly differentiated age-dependent pattern. Most cases reported in children were associated with chilblain lesions or Kawasaki disease[91,92] and had negative PCR results for SARS-CoV-2, whereas the use of drugs was noted in all reported cases in adults (hydroxychloroquine in all, in most in combination with azithromycin, antivirals and/or antibiotics) (Supplementary Table 10).

Other immune-related skin manifestations have been reported in patients with COVID-19, including livedoid and/or acrocyanotic lesions[84,93–96], retiform purpura[97–99], oral ulcers[84,100], erythema nodosum[101,102], periorbital erythema[103], generalized pustular figurate erythema (in all reported cases, appearing in patients who were being treated with hydroxychloroquine)[94,104,105], drug reaction with eosinophilia and systemic symptoms[94,106] and Sweet syndrome[107].

Haematological involvement. Lymphopenia is a prominent feature of COVID-19, not only because of its high frequency (around half of COVID-19 cases) but also because of its relevance to prognosis (it has been linked with the development of ARDS, a need for intensive care and poor survival)[108–110], whereas thrombocytopenia

and anaemia have been reported in 24% and 59% of COVID-19 cases, respectively (Supplementary Table 5). Cytopenia is overwhelmingly asymptomatic, and symptomatic autoimmune cases (such as thrombocytopenic purpura or haemolytic anaemia) have been infrequently reported in patients with COVID-19 (Supplementary Table 11).

Patients with COVID-19 can present with symptomatic thrombocytopenia, including immune thrombocytopenic purpura (ITP) and thrombotic thrombocytopenic purpura. COVID-19-related ITP predominantly affects people older than 50 years (~75%) presenting with a platelet count below 10,000 per mm^3 (~80%), with the ITP symptoms (purpura and mucosal bleeding) appearing at least 2 weeks after onset of COVID-19 symptoms in nearly half the cases. In two of the three patients with COVID-19 presenting with thrombotic thrombocytopenic purpura, infection was confirmed by positive IgG serology, suggesting a delayed immune-related response. Autoimmune haemolytic anaemia (AIHA; presenting as either warm or cold haemolysis) is also diagnosed predominantly in people older than 50 years (~70%) presenting with a haemoglobin lower than 8 g/l (74%), with the AIHA symptoms (mainly asthenia and jaundice) appearing during the first/second week of COVID-19 (Supplementary Table 11).

Neurological involvement. The neurological manifestations caused by SARS-CoV-2 are diverse and have been related to neuroinvasion or neurotropic damage (including encephalopathy, encephalitis and cerebrovascular pathologies) or to neuroinflammatory damage (Guillain–Barré syndrome (GBS) or acute myelitis)[111,112].

Encephalitis is inflammation of the brain parenchyma, clinical evidence of which includes cerebrospinal fluid pleocytosis, neuroimaging results or focal abnormalities on electroencephalogram[112]. Reported cases of encephalitis in patients with COVID-19 reveal a similar extent of involvement among women and men, with a mean age at diagnosis of 55 years (including cases in patients ranging from 11 to 84 years old) (Supplementary Table 12). In one-third of cases, neurological symptoms started at least 2 weeks after COVID-19 onset. Although several cases were classified as non-specific viral encephalitis or meningoencephalitis, some specific clinical entities were identified in other patients, including autoimmune encephalitis associated with anti-NMDA receptor autoantibodies, acute disseminated encephalomyelitis, acute necrotizing encephalopathy and mild encephalitis/encephalopathy with a reversible splenial lesion (Supplementary Table 12). The pathogenesis of COVID-19-associated encephalitis is unknown, although one study has suggested that patients with COVID-19 can develop neurological manifestations that share notable similarities with those of CAR-T cell-related encephalopathy, involving different pathophysiological mechanisms including CRS, endothelial activation, blood–brain barrier dysfunction and immune-related damage[113].

GBS is a typical post-infectious disorder, with more than two-thirds of patients reporting symptoms of respiratory or digestive tract infections within the 6 weeks prior to GBS onset[114]. Therefore, that SARS-CoV-2 could be a potential new viral trigger of GBS is not unexpected, but the frequency of COVID-19-related GBS is unknown, with only one large study estimating a frequency of GBS of ~0.1% among hospitalized patients with COVID-19 (REF.[73]). So far, almost all cases of GBS related to SARS-CoV-2 have been reported as isolated cases and in one small series; these cases mainly affected men aged >50 years (90% of cases) and were diagnosed at least 2 weeks after the onset of COVID-19 respiratory symptoms in two-thirds of reviewed cases. The clinical presentation and severity of GBS in these cases was similar to that in non-COVID-19 GBS; the electrodiagnostic pattern was classified as demyelinating in most cases (although other phenotypic variants, such as Miller Fisher syndrome and acute motor and sensory axonal neuropathy, have also been reported), serum anti-ganglioside antibodies were absent in most patients tested and cerebrospinal fluid, when assessed, was negative for SARS-CoV-2 (REFS[115,116]) (Supplementary Table 13).

Several cases of myelitis have been reported in patients with COVID-19, mainly in men and with two discrete age peaks, one at ~30 years old and the other at ~60–70 years old (Supplementary Table 14); additional reports of other immune-related neurological manifestations include cranial neuropathies and optic neuritis[73,117–120], plexopathy[121] or myasthenia gravis[122].

Pulmonary involvement. Among studies of COVID-19 pneumonia to date, few have evaluated the long-term natural history of pulmonary damage, and they often have a short follow-up period (~1 month after starting COVID-19 symptoms). These studies have reported that a substantial percentage of patients have abnormal pulmonary findings, including abnormal pulmonary function test (PFT) results in 54% of patients and abnormal CT imaging studies in 40–94%[123–126]. One small study has reported that PFT results remain abnormal in ~25% of patients evaluated 3 months after diagnosis of COVID-19 (REF.[127]), suggesting the development of a post-pneumonia interstitial lung disease. Some studies have reported individual cases of patients who developed severe, bilateral pulmonary fibrosis after COVID-19 (REFS[128–130]). Owing to the large number of patients affected by severe COVID-19 pneumonia, long-term respiratory complications can be expected and could cause substantial population morbidity[131].

Although several post-mortem studies have suggested diffuse alveolar damage as the predominant pathological lung damage caused by SARS-Cov-2, other studies suggest a more heterogeneous pathological scenario, including a predominant pattern suggestive of organizing pneumonia in some patients[47,132]. A late development of new respiratory symptoms and opacities (>2 weeks after the first symptoms of COVID-19), especially if these features were not detected in previous CT studies, could suggest the late development of organizing pneumonia, as has been reported in influenza infection[133].

Pleural involvement has also been linked to COVID-19, with an estimated frequency of 27% for pleural thickening and 5–6% for pleural effusion[80,81]. Some patients

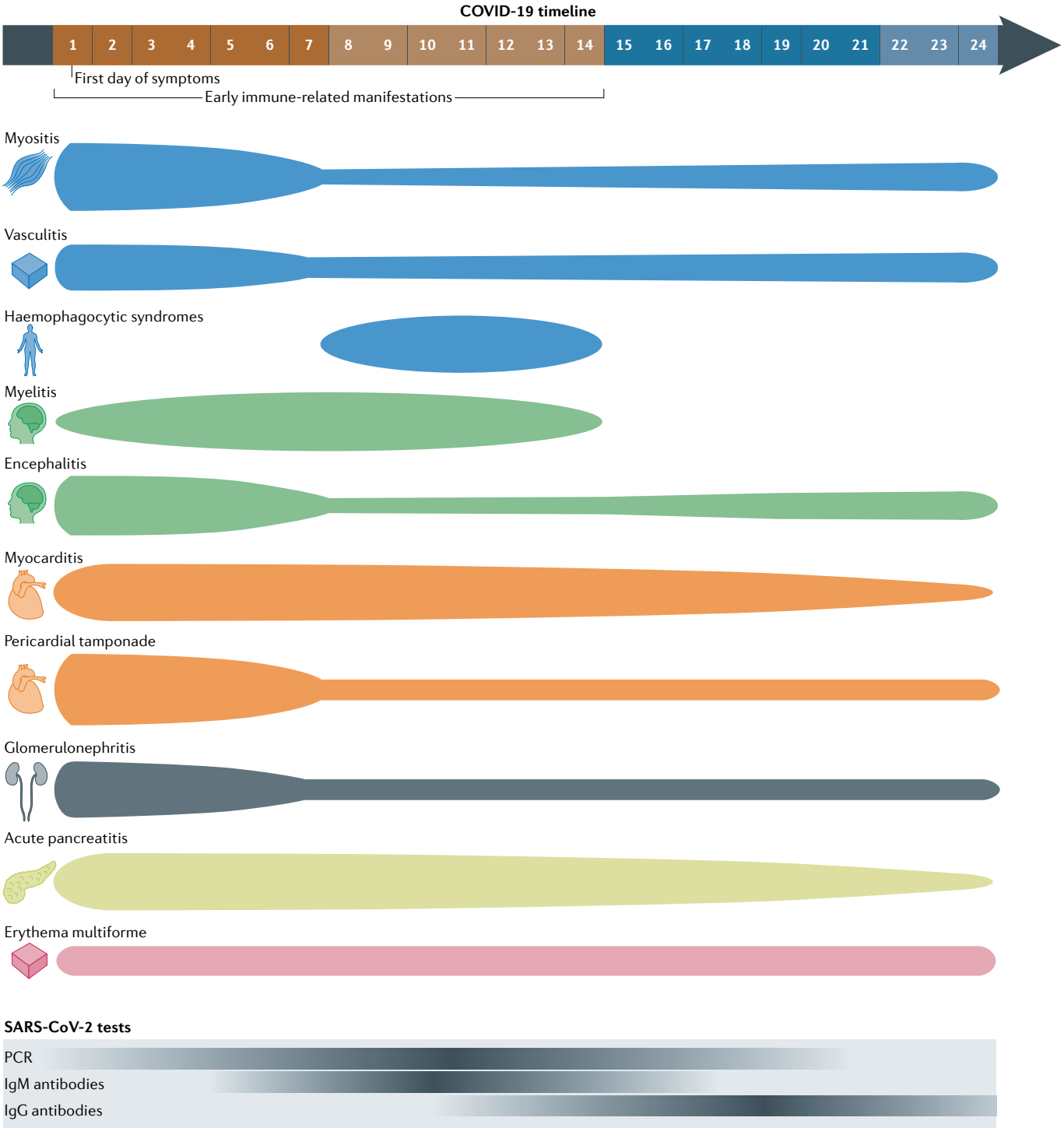


Fig. 3 | **Immune-related manifestations predominantly diagnosed during the first 2 weeks of COVID-19 (early features).** This figure illustrates the distribution of reported cases of immune-related manifestations predominantly diagnosed within 2 weeks of the onset of symptoms of acute COVID-19, as summarized in TABLE 3. The thickness of each segment corresponds to the proportion of cases reported in each time period (within the first 7 days of onset, between 8 and 14 days after, and 15 or more days after; the last period includes cases diagnosed in patients with asymptomatic infection). The bottom of the figure illustrates the representative positivity rate of the main microbiological tests from the first day of symptomatic infection; the intensity of colour corresponds to a higher rate of positive test results.

can have symptoms of pleurisy as the initial manifestation of COVID-19 (REF.[134]) and others might develop pleural effusion even though it was absent in the initial examination[135].

Cardiovascular involvement. In patients with COVID-19, development of myocardial damage is indicated by abnormal laboratory parameters, cardiac imaging studies, and in vivo and post-mortem histopathological data.

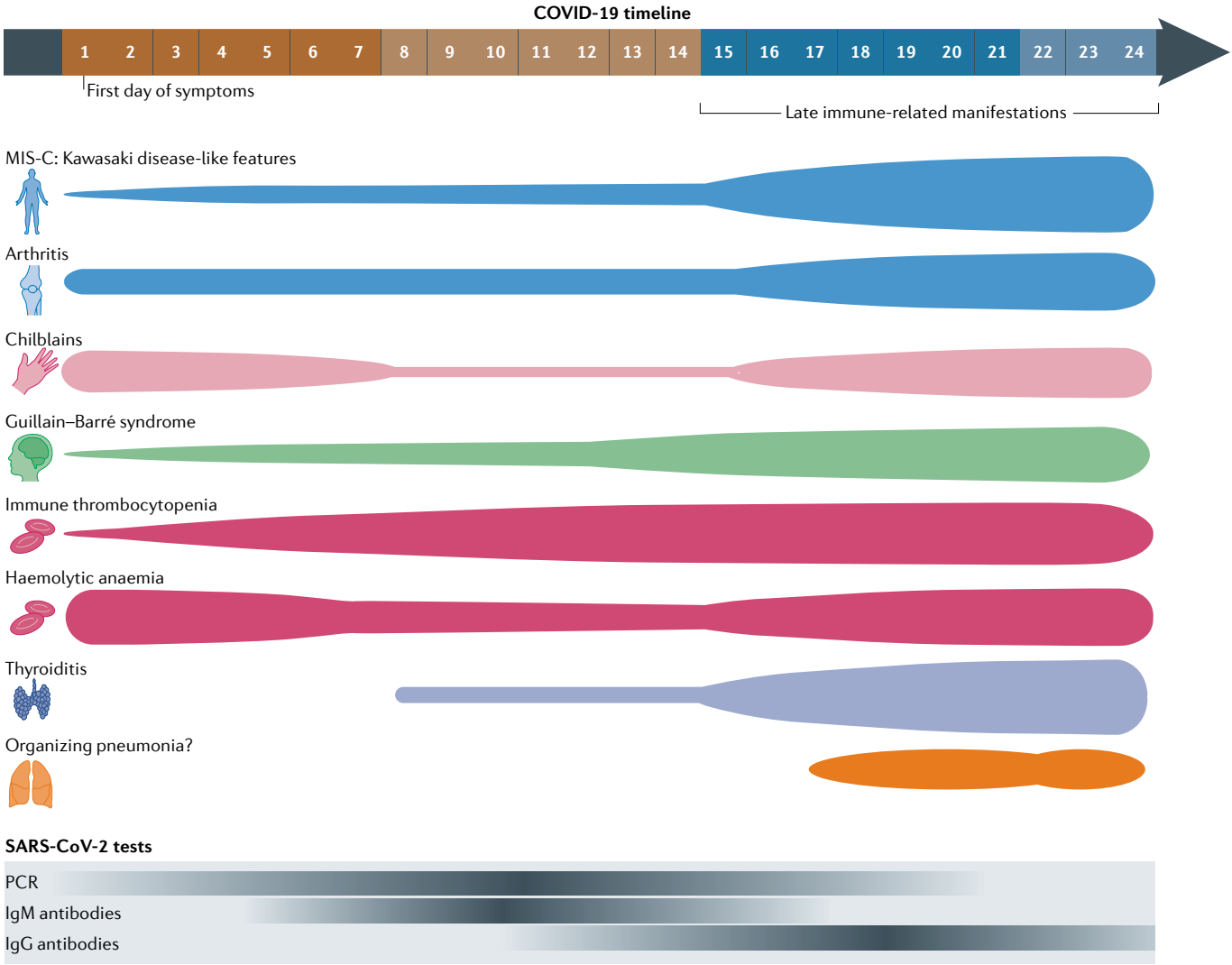


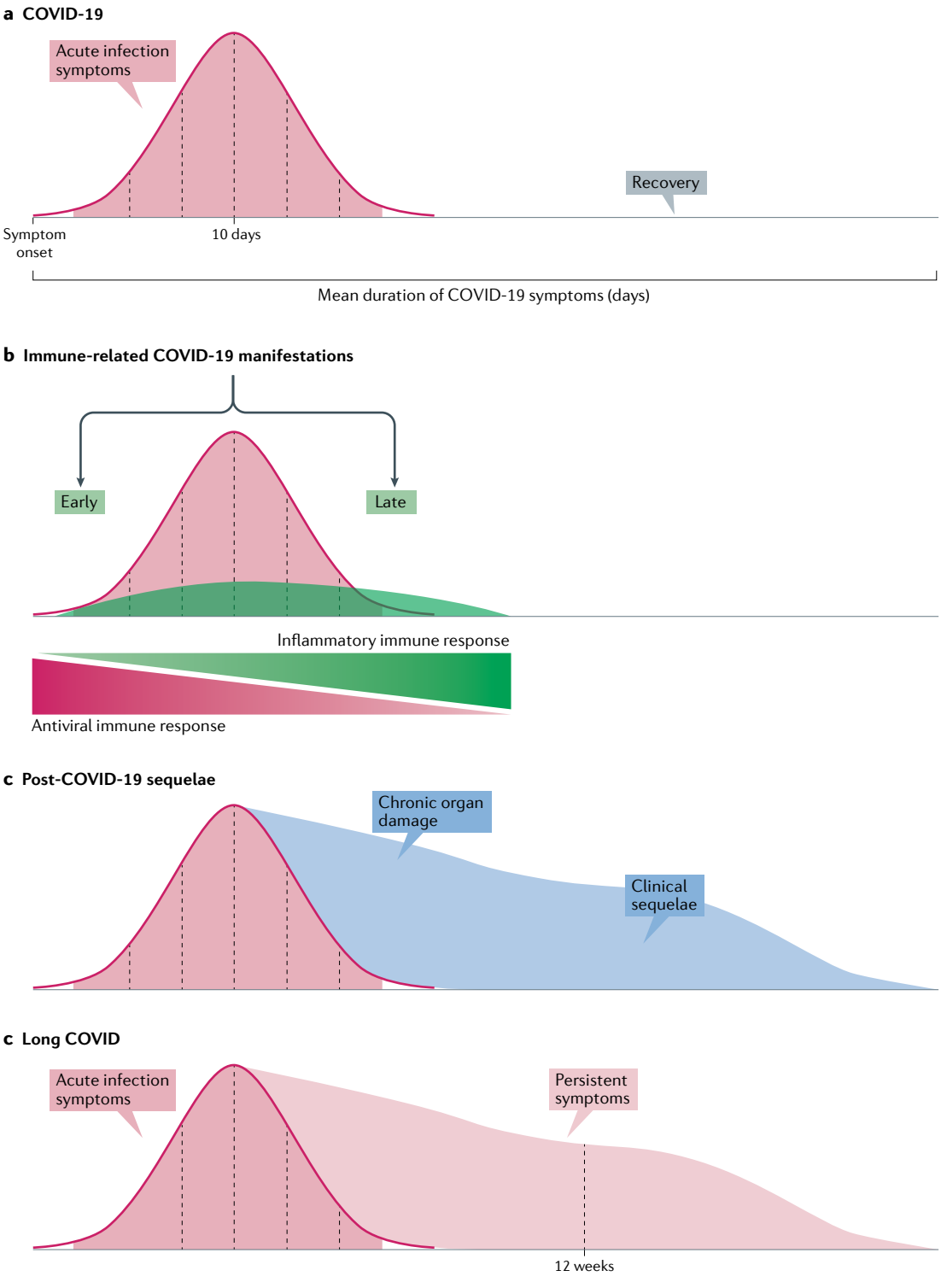
Fig. 4 | **Immune-related manifestations predominantly diagnosed after the first 2 weeks of COVID-19 (late features).** The top part of the figure illustrates the distribution of reported cases of immune-related manifestations predominantly diagnosed more than 2 weeks after the onset of symptoms of acute COVID-19, as summarized in TABLE 3. The thickness of each segment corresponds to the proportion of cases reported within each time period (the first 7 days, between 8 and 14 days after onset, and 15 or more days after onset; the last period includes cases reported in patients with asymptomatic SARS-CoV-2 infection). The bottom part of the figure illustrates the representative positivity rate of the main microbiological tests from the first day of symptomatic infection; the intensity of colour corresponds to a higher rate of positive test results. MIS-C, multisystem inflammatory syndrome in children.

Around 40–80% of patients with COVID-19 can have raised troponin-I levels[136,137], cardiac MRI identified cardiac involvement in 78%[137], and several studies have reported myocardial interstitial infiltration by mononuclear cells and lymphocytic infiltration[138] with evidence of active viral replication[139,140]; a myocyte-specific upregulation of ACE2 has been suggested as a putative pathogenic mechanism for SARS-CoV-2-associated viral myocarditis[141]. Acute myocarditis is often categorized into the histologically defined entities of lymphocytic, eosinophilic and giant cell myocarditis and sarcoid heart disease[142]. To date, acute myocarditis related to COVID-19 has been overwhelmingly described as lymphocytic and rarely as eosinophilic, in contrast to SARS-CoV-2-associated myocarditis, which did not exhibit lymphocytic infiltration[143,144]. Reports of cases of acute myocarditis in patients with COVID-19 show that a wide range of ages are involved (from 17 to 79 years), more frequently affecting men than women, with the main symptoms (thoracic pain and dyspnoea) being presented mainly during the first 2 weeks of COVID-19, although several cases have been described some weeks after the infection is resolved. Only around half cases had a confirmatory MRI study (the remaining underwent only cardiac ultrasonography) and most required ICU admission, with a mortality rate of ~30% (Supplementary Table 15).

A study[137] in 100 patients evaluated a mean of 2 months after confirmed COVID-19 diagnosis showed that raised levels of high-sensitivity troponin were detected in 76% and that cardiac MRI showed cardiac involvement in 78%, including evidence of active

myocardial inflammation in 60%. In comparison with healthy volunteers, the patients who had recovered from COVID-19 had lower left ventricular ejection fractions and higher left ventricular volumes; moreover, 32% manifested myocardial late gadolinium enhancement and 22% had pericardial involvement. The clinical relevance of these findings remains unclear, although the findings demonstrating chronic inflammation and left ventricular dysfunction a couple of months after the clinical onset of COVID-19 could represent an increased risk of developing new-onset heart failure and other cardiovascular complications[145].

Pericardial effusion has been reported in ~5% of patients with COVID-19 (REF.[81]), and it seems that patients with suspected myocarditis could have a higher rate of pericardial effusion (22–75%)[137,146]. Cardiac tamponade was reported in 11 (1%) of 1,216 patients with available echocardiographic findings[147], and



several additional cases have been reported, mainly diagnosed in the first 7–10 days of COVID-19 illness (Supplementary Table 16).

Renal involvement. COVID-19 has been associated with both tubular and glomerular renal damage. Proximal tubule dysfunction has been reported in a subset of patients with COVID-19 presenting with low-molecular-weight proteinuria, neutral aminoaciduria and defective handling of uric acid[148]. With respect to glomerular disease, several studies have reported patients with biopsy-proven glomerulonephritis presenting with acute renal failure (in some cases accompanied by haematuria and/or nephrotic syndrome), with a clear differentiation in profile between podocytopathies and other types of glomerulonephritis (Supplementary Table 17). Most renal pathology in patients with COVID-19 falls within the spectrum of podocytopathies (with most classified as collapsing glomerulonephritis, and some as focal segmental glomerulosclerosis or minimal change disease), primarily affecting men of African ancestry carrying high-risk *APOL1* genotypes. The next most frequently reported type of glomerulonephritis after podocytopathies in patients with COVID-19 is pauci-immune crescentic glomerulonephritis associated with autoantibodies, which in all cases but one affected women. Other types of glomerulonephritis have been also reported, including membranous and IgA glomerulonephritis. In some patients, acute renal disease appeared more than 2 weeks after onset of COVID-19 symptoms, showing negative PCR results and positive serological tests. Patients with COVID-19 presenting with glomerulonephritis have a poor prognosis, and more than half of the reported cases required dialysis (most even after being discharged from the hospital) (Supplementary Table 17).

Endocrine involvement. Studies have reported abnormal thyroid function potentially related to SARS-CoV-2 infection. A retrospective study in patients hospitalized with COVID-19 found thyrotoxicosis in 20% and hypothyroidism in 5%[149], whereas another study found low concentrations of thyroid-stimulating hormone in 56% of patients[150]. To date, all reported cases of COVID-19-associated thyroid dysfunction are overwhelmingly consistent with overt hyperthyroidism (defined as low levels of thyroid-stimulating hormone plus high levels of free T4), often presenting with clinical symptoms of thyrotoxicosis and enlarged painful thyroid gland in physical and ultrasonography examinations; cases presenting with subclinical hypothyroidism are rare. From a pathogenic point of view, some findings seem to suggest that thyroid dysfunction could be a transient phenomenon related to the hyperinflammatory biological scenario (correlating with increased concentrations of IL-6 and infection severity, with abnormal values reverting after infection recovery). Most patients who were tested for anti-thyroid antibodies had negative results. Adrenal involvement can include acute adrenal infarction (as an incidental CT finding in one quarter of patients), adrenal haemorrhages and micro-infarctions and, rarely, adrenal insufficiency[47,151,152] (Supplementary Table 18).

Pancreatic involvement. Several patients with COVID-19 presenting with abdominal pain and elevated concentrations of pancreatic enzymes have been diagnosed with acute pancreatitis, most frequently women (Supplementary Table 19). The clinical and epidemiological scenario is wide, and includes involvement of children and older people, patients presenting without clinical symptoms, post-mortem studies, family cases, or patients with underlying predisposing factors. Compared with patients without COVID-19, patients with COVID-19 presenting with acute pancreatitis showed a similar epidemiological profile but a worse bedside index for severity in acute pancreatitis (BISAP) score, a higher frequency of persistent organ failure and a worse survival rate[153] (Supplementary Table 19). Several pathogenic mechanisms have been suggested to explain the putative association between acute pancreatitis and COVID-19, including direct viral damage to pancreatic cells, endothelial damage and ischaemic and/or thrombotic mechanisms[154].

Ocular involvement. Some inflammatory ocular diseases have been diagnosed in patients with COVID-19, including one reported case of bilateral anterior uveitis[155] and conjunctivitis, which has been reported in more than 50 adult patients, mostly from Asian countries[156].

Fig. 5 | **Time-dependent clinical scenarios in patients with symptomatic SARS-CoV-2 infection. a** | Acute infection. The mean duration of symptoms in patients with symptomatic SARS-CoV-2 infection has been reported as ~11 days[76,180], and as long as 13–28 days in patients with COVID-19 requiring hospitalization[74,123,181–187]. Complete recovery is reported in >85% of patients 4 weeks after the onset of symptoms[180]. **b** | Immune-related manifestations of COVID-19. Most immune-related COVID-19 manifestations are diagnosed during the first 4–6 weeks after symptom onset. Some immune-related manifestations tend to appear during the first 2 weeks of infection (early immune-related features of COVID-19), whereas others tend to emerge in a late post-infectious stage or even in asymptomatic patients (late immune-related features of COVID-19). **c** | Post-COVID-19 sequelae. Symptoms related to organ-specific sequelae caused by the viral infection — affecting internal organs such as the lungs (interstitial lung disease in patients with severe pneumonia), the heart (chronic heart failure in patients with myocarditis) or the kidneys (chronic renal failure in patients with glomerulonephritis) — can emerge after resolution of the acute infection. **d** | Long COVID. One or more of the symptoms related to the acute viral infection, such as fatigue, pain, chills, anosmia, dysgeusia or headaches (as the most frequently reported), can persist for more than 12 weeks, a situation often referred to as 'long COVID'. These symptoms can affect any bodily system, are not explained by an alternative diagnosis and, in some patients, may follow a relapsing–remitting pattern, possibly fluctuating and changing over time[188].

Probing the underlying pathogenesis

An increasing number of studies are reporting about collateral manifestations related to an excessive response of the immune system against SARS-CoV-2, breaking the natural self-tolerance maintained by the immune system, as has been previously described in other acute and chronic viral infections[157–160] or that has been related to the administration of biologic drugs[161,162]. Immune-related manifestations related to COVID-19 were initially described in hospitalized patients, especially in those with severe disease, but have also been described in patients with an already resolved infection or even in asymptomatic patients. The clinical phenotype seems to be modified by epidemiological factors such as age (manifestations clearly differentiated between

Table 3 | **Persistent symptoms and organ-specific sequelae reported in patients with COVID-19**

Features	Weeks after first symptom of acute COVID-19		
	4 weeks[a]	8 weeks	12 weeks
General features[71,72,127,176]			
Fever	4%	0%	–
Chills	5%	–	–
Fatigue	35%	53%	16%
Musculoskeletal features[71,72,176]			
Arthralgia	10–15%	16–27%	–
Myalgia	–	6%	–
Myalgia, headache and/or fatigue	36%	21%	–
Pulmonary features[71,72,127,176–178]			
Dyspnoea	11–27%	8–43%	14%
Chest pain	20%	22%	–
Cough	43%	18%	2%
Sputum production	–	8%	2%
Abnormal pulmonary function tests[b]	47–54%	–	25%
Ear, nose and throat features[71,72,127,176]			
Rhinitis and/or congestion	28%	15%	–
Sore throat	15%	7%	–
Anosmia	23%	17%	–
Dysgeusia	24%	10%	–
Anosmia and/or ageusia	28%	23%	4%
Cardiovascular features[137,179,180]			
Raised troponin levels	–	78%	–
Imaging myocardial inflammation	–	60%	–
Imaging pericardial enhancement	–	22%	–
Late myocardial gadolinium enhancement	–	32%	–
Post-discharge thrombosis	0.5–2.5%	–	–
Gastrointestinal features[71,72,127,176]			
Abdominal pain	15%	–	–
Nausea	10%	–	–
Vomiting	4%	–	–
Diarrhoea	–	3%	–
Diarrhoea or vomiting	17%	11%	31%
Lack of appetite	–	8%	–
Weight loss >5%	16%	17%	–
Neurological features[71,127,176]			
Headache	14%	9%	18%
Confusion	21%	–	–
Vertigo	–	6%	–
Other features[71]			
Sicca syndrome	–	16%	–
Red eyes	–	10%	–

[a]Tenforde et al.[176] evaluated features at 2–3 weeks. [b]At least one abnormal parameter.

children and adults)[163], sex (myositis, arthritis, GBS, myelitis and glomerulonephritis are reported mainly in men, whereas thyroiditis and pancreatitis occur more frequently in women) or ethnicity (although the role of socioeconomic factors must always be assessed)[164,165] (TABLES 1 and 2), and some pathogenic mechanisms could be specifically involved in certain epidemiological subsets of patients. For instance, in comparison with adults, children with COVID-19 predominantly generate IgG antibodies specific for the SARS-CoV-2 spike protein, targeting mainly the S2 subunit, but not for the nucleocapsid protein, a virus-related pathogenic mechanism that could help to explain the differentiated phenotype reported in children and adults[166,167]. The severity of the manifestations of COVID-19 is also very wide, ranging from completely benign and self-limiting manifestations (for example, perniosis) to systemic syndromes (such as MIS-C or HLH) that can lead to the need for intensive care and potentially to death. Multidisciplinary management of patients with COVID-19 is mandatory, including experts in the corresponding systemic and organ-specific autoimmune diseases, and should always follow a holistic diagnostic approach owing to the large variety of multisystem symptoms that patients with COVID-19 might present (FIGS 2 and 3). In the absence of specific data about the therapeutic management of immune-related COVID-19 manifestations, following an approach similar to that used in the corresponding non-COVID-19 diseases could be a reasonable option, as has been suggested for neuroinflammatory COVID-19 with the use of corticosteroids, intravenous immunoglobulin and plasma exchange[121,168].

Little is known about the pathogenesis of these manifestations[169], although involvement of specific responses by the acquired immune system seems to be of little consequence if we consider that serum autoantibodies (one of the main pathogenic hypotheses of autoimmune diseases) are absent in most patients tested for them. Therefore, the use of terms such as 'immune-related' or 'immune-mediated' could be more appropriate than the term 'autoimmune' to refer to these manifestations, as has been proposed for terminology of immune-related manifestations associated with checkpoint inhibitors[161]. Studies centred on investigating the role of interferon-related pathways in COVID-19, an important mechanism also involved in the pathogenesis of several autoimmune diseases[170], have reported the presence of autoantibodies against type I interferon or inborn errors of type I interferon immunity[171,172] in patients with severe COVID-19. In the literature we reviewed, there seems to be a certain pattern of occurrence of many immune-related manifestations in relation to the onset of SARS-CoV-2 infection: some features tend to appear in the first 2 weeks of infection (FIG. 3), whereas others tend to emerge in a late post-infectious stage or even in asymptomatic patients (FIG. 4). This differentiated temporal distribution along the different stages of SARS-CoV-2 infection could suggest the involvement of different aetiopathogenic mechanisms triggered by a common aetiological agent[173,174], with some features being predominantly linked to early viral immune responses and other features with

subsequent inflammatory responses emerging once the virus has been eliminated.

Immune-related manifestations of COVID-19 should be distinguished from other clinical scenarios with a different pathogenic basis (FIG. 5). In patients with severe COVID-19, some vital internal organs can be severely damaged by the inflammatory process. In a considerable percentage of patients with COVID-19 pneumonia, results of pulmonary function evaluations can remain abnormal several weeks after acute infection, including abnormal PFT results in 54% of patients and abnormal CT imaging studies in 40–94%[123–126]. Several cases of pulmonary fibrosis have also been reported[128–130], suggesting that long-term respiratory complications could cause substantial morbidity in the population[131,175]. Other studies have reported chronic cardiac inflammation and left ventricular dysfunction a couple of months after the clinical onset of COVID-19, findings that could increase the risk of developing new-onset heart failure and other cardiovascular complications[145]. Another clinical scenario that has been scarcely explored is the persistence over time of acute symptoms of COVID-19, such as fatigue, pain, chills, anosmia, dysgeusia and headaches (referred to as 'long COVID')[71,72,127,137,176–180] (TABLE 3). Some patients also develop a syndrome that can resemble myalgic encephalomyelitis/chronic fatigue syndrome or fibromyalgia[181]. To date, no study has demonstrated that immune-related mechanisms could be involved in the pathogenesis of these symptoms[144].

The novelty of these manifestations and the large number of different specialties involved make it very difficult at present to have consensus on a diagnostic definition for most of them, and two different approaches have been followed to date. The first has been to propose a new syndrome, as has been done with the MIS-C in children, to separate it from other known diseases with which this syndrome has notable similarities (in the case of MIS-C, Kawasaki disease). The second has been to include SARS-CoV-2 within the multi-aetiological spectrum often reported in patients affected by the classical syndrome or disease, considering that patients with COVID-19 might have a different clinical phenotype but under the umbrella of the same syndrome (such as HLH, vasculitis, autoimmune cytopenia, GBS or glomerulonephritis).

As knowledge of COVID-19 pathogenesis remains limited, there remain many more doubts than certainties, such as knowing why immune-related manifestations affect only certain people with COVID-19, why the phenotype is so different in children from that in adults, why some manifestations emerge especially in the acute infection phase and others when the infection is overcome, why some manifestations vary greatly with respect to geography and/or ethnicity of the affected patients, why a substantial proportion of patients remain symptomatic several months after being exposed to the virus, or why the number of reported cases of each immune-related manifestation of COVID-19 is clearly imbalanced, given that only two manifestations (MIS-C and chilblains being precisely those manifestations that particularly affect children and young people) make up two-thirds of the total reported cases.

Conclusions

Immune-related manifestations are increasingly recognized in patients with COVID-19, with a protean clinical presentation affecting a wide range of organ systems in both children and adults. The body of evidence consists predominantly of case series and uncontrolled studies that had reported ~3,000 cases worldwide as of August 2020, including more than 70 different systemic and organ-specific disorders. Unsurprisingly, therefore, diagnostic and therapeutic decision-making are often based on scarce clinical experience and expert opinion. Without being able to offer solid conclusions and plausible aetiopathogenic explanations, the main objective of this Review is to awaken the interest of the scientific community in this emerging group of manifestations and thus facilitate the development of studies specifically devoted to investigating the pathogenic mechanisms that could help enable the early detection and adequate management of immune-related manifestations of COVID-19.

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Review criteria
A search for original articles published without date limitation was performed in PubMed in August 2020. Search terms included "SARS-CoV-2", "COVID-19" and the individual immune-related disorders that have been reported in patients with COVID-19 and that are detailed in Box 1.

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Epithelial–immune cell interplay in primary Sjögren syndrome salivary gland pathogenesis

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Abstract | In primary Sjögren syndrome (pSS), the function of the salivary glands is often considerably reduced. Multiple innate immune pathways are likely dysregulated in the salivary gland epithelium in pSS, including the nuclear factor-κB pathway, the inflammasome and interferon signalling. The ductal cells of the salivary gland in pSS are characteristically surrounded by a $CD4^+$ T cell-rich and B cell-rich infiltrate, implying a degree of communication between epithelial cells and immune cells. B cell infiltrates within the ducts can initiate the development of lymphoepithelial lesions, including basal ductal cell hyperplasia. Vice versa, the epithelium provides chronic activation signals to the glandular B cell fraction. This continuous stimulation might ultimately drive the development of mucosa-associated lymphoid tissue lymphoma. This Review discusses changes in the cells of the salivary gland epithelium in pSS (including acinar, ductal and progenitor cells), and the proposed interplay of these cells with environmental stimuli and the immune system. Current therapeutic options are insufficient to address both lymphocytic infiltration and salivary gland dysfunction. Successful rescue of salivary gland function in pSS will probably demand a multimodal therapeutic approach and an appreciation of the complicity of the salivary gland epithelium in the development of pSS.

Nearly 100 years ago, Henrik Sjögren described a disease whereby patients had a reduced functionality of the salivary glands (xerostomia) and lacrimal glands (xerophthalmia), with accompanying disturbances of the cornea and the conjunctiva (keratoconjunctivitis sicca)[1]. Sjögren syndrome, named after his work, has since been extensively characterized and is now considered a systemic autoimmune disease. Primary Sjögren syndrome (pSS) typically refers to the occurrence of Sjögren syndrome as the first clinically presenting autoimmune condition in a patient. In terms of clinical presentations, pSS is associated with dryness of the mouth, eyes and vagina, chronic pain and fatigue, and numerous possible extraglandular organ manifestations (including neuropathies, pulmonary manifestations and nephritis)[2].

pSS is mirrored in pathological and biological analysis by the presence of lymphocytic infiltration in the salivary glands (focal lymphocytic sialadenitis) and autoantibodies in the blood. The salivary gland biopsy and its analysis have an important role in the diagnosis and classification of pSS[3]. Introduced in 1974, the focus score (that is, the number of lymphocytic foci per 4 mm^2 tissue) has been relied upon heavily for the histological assessment of salivary gland involvement in pSS[4]. Infiltrating cells, congregated in foci around the striated ducts, consist mostly of $CD4^+$ T cells and B cells, although other immune cells, including (but not limited to) myeloid dendritic cells, plasmacytoid dendritic cells (pDCs) and follicular dendritic cells, might also be present[5,6]. These infiltrates can develop into ectopic lymphoid structures, even comprising ectopic germinal centres[7]. Particular attention has been paid to the role of (glandular) B cells in pSS pathogenesis. These cells are hyperactive in pSS and responsible for the formation of autoantibodies, hypergammaglobulinaemia, lymphoepithelial lesions (LELs) and pSS-related mucosa-associated lymphoid tissue (MALT) lymphoma[8–11].

Regarding autoantibodies, anti-SSA/Ro (and to a lesser extent anti-SSB/La) antibodies are present in the majority of patients with pSS[3]. Serum levels of anti-SSA/Ro and anti-SSB/La antibodies correlate with the number of corresponding plasma cells in the minor salivary glands of patients with pSS, suggesting that salivary glands form an important niche for autoantibody-secreting cells[12]. The SSA/Ro antigen comprises two ribonucleoproteins, Ro52 and Ro60, which are both present in the cytoplasm of all mammalian cells. Although a direct contribution of autoantibodies to exocrine gland dysfunction in humans has not been proven, they have been linked to glandular immune activation[13].

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Key points

- Dysregulation of the functional machinery of acini, activation and apoptosis of ductal cells and defects in progenitor cell homeostasis all contribute to salivary gland dysfunction in primary Sjögren syndrome (pSS).
- Unknown trigger(s) in salivary gland epithelial cells might activate innate immune responses and result in adaptive immune responses towards self-antigens, making epithelial cells both mediators and targets of the response.
- Dysregulation of innate immune signalling pathways in salivary gland epithelial cells and consequent pro-inflammatory cytokine production by the epithelium probably contributes to salivary gland dysfunction.
- Direct or indirect interference of autoantibodies with innate immune responses could perpetuate type I interferon activity in salivary gland epithelial cells and immune cells.
- Increased activation of nuclear factor-κB and expression of pro-survival factors, together with enhanced proliferation, might predispose certain intraepithelial B cells to neoplastic changes, promoting mucosa-associated lymphoid lymphoma development.
- The restoration of salivary gland function in patients with pSS might require treatment tailored to glandular pathology and multimodal therapeutic approaches, for example, a combination of immunotherapy and cell therapy.

The fact that autoantibodies, encompassing anti-SSA/Ro antibodies, anti-SSB/La antibodies and rheumatoid factor, can be present years before pSS diagnosis suggests that an additional trigger is needed in the development towards clinical disease[14]. The journey towards impaired function of the salivary glands in pSS, resulting in reduced saliva production and a notable decrease in patient quality of life, is most probably multifactorial and still somewhat enigmatic.

Far from being only a site where infiltrating immune cells assemble, the ductal epithelium has a central role in disease pathogenesis. In this Review, we discuss how interactions between the epithelium and the immune system in pSS contribute to both disease initiation and throughout different stages of disease development in patients with pSS. We focus on new insights into the development of abnormalities in epithelial cells, as well as innate immune signalling and T cell and B cell activation within the salivary glands. All studies cited are based on human samples, unless specifically otherwise stated.

Salivary gland epithelium abnormalities

A complete discussion of the deterioration of salivary gland function in pSS necessitates comprehension of the foundation of the salivary gland: the epithelium. The salivary gland epithelium comprises several cell types, namely acinar, myoepithelial and ductal cells (FIG. 1a). Acinar cells produce and secrete either watery or mucous-rich saliva (referred to as serous or mucous acinar cells, respectively). This secretion is facilitated by the contraction of myoepithelial cells that envelope the acinar cell clusters. The secreted saliva is channelled and simultaneously modified through small intercalated ducts into striated ducts (both consisting of basal and luminal cell types), and finally through the larger excretory ducts into the mouth. This architecture can be applied to both the minor and major (parotid, submandibular and sublingual) salivary glands. Although all salivary glands can be affected in pSS, the minor glands of the lower lip (the labial salivary glands (LSGs)) are most often used in diagnosis and research owing to their relative ease of access. Notably, minor salivary glands, including the LSGs, account for less than 10% of unstimulated whole saliva production[15]. This caveat aside, the literature suggests that the volume of LSG lymphocytic infiltration correlates only weakly with the reduction in salivary gland function in pSS[16,17], implying that additional mechanisms or epithelium-specific deficits are probably important in decreased salivary gland function in pSS.

Changes in acinar cells in pSS

Acinar cells are the saliva-producing workhorses of the salivary glands, controlling both the volume and protein content of saliva. All the acinar cells in the parotid gland, the major gland responsible for saliva production after stimulation, are serous cells. The action of chewing or exposure of taste receptors to acidic substances stimulates these cells to secrete the digestive enzyme α-amylase. pSS is associated with a decrease in the amount and activity of α-amylase, strongly implying that serous acinar cells have a less than optimal function in pSS[18–20]. Despite a notable reduction in saliva production in pSS, the number and gross morphology of acinar cells in both the LSGs and the parotid salivary gland often appears normal, implying that, even if defective, acinar cells remain viable in pSS. In support of this idea, various data show that the levels of FAS–FAS ligand (FASL)-induced apoptosis in acinar cells is low in patients with pSS, although the levels are higher than those of control populations (such as healthy individuals)[21–23]. However, data from studies in mice suggest that TNF is capable of inducing apoptosis of major salivary gland acinar cells; notably, acinar cells from the NOD mouse, a model of pSS, are more likely to undergo TNF-induced apoptosis than cells from age-matched BALB/c (control) mice[24]. Conversely, treatment of human LSG acinar cells with TNF and IFNγ seems to induce the expression of the anti-apoptotic genes *ATF6* and *ERAD*[25]. To complicate the situation further, levels of sex hormones might also affect acinar cell apoptosis[26]. Taken together, no firm conclusions can be drawn from the available data regarding the degree of acinar cell apoptosis that occurs in the salivary glands in pSS.

The secretion of saliva by acinar cells begins with the engagement of the muscarinic 3 receptors on acinar cells by muscarinic neurotransmitters such as acetyl choline. Downstream of this event, an increase in cytosolic levels of Ca^{2+} in these cells occurs, originating from both outside the cells and from intracellular internal endoplasmic reticulum stores[27]. The presence of autoantibodies directed against extracellular loops of the muscarinic 3 receptor implies that these very first stages of secretion might already be disturbed in pSS, or at least targeted by the immune system[28] (FIG. 1b). Muscarinic receptor binding and the subsequent increase in cytosolic Ca^{2+} culminates in inositol 1,4,5-trisphosphate receptor (IP3R) signalling, via the secretory machinery components phosphatidylinositol 4,5-bisphosphate (PIP2) and synaptotagmin 1. Notably, the levels of IP3R and the expression of synaptotagmin 1 are decreased in salivary gland acinar cells of patients with pSS compared with

in healthy individuals[29,30]. Furthermore, both PIP2 and synaptotagmin 1 seem to localize to the acinar cell basolateral membrane in pSS rather than to the apical membrane, suggesting at least dysfunction of the secretory machinery[30]. Hence, various critical components of the secretory machinery in acinar are dysregulated in pSS.

Another mechanism proposed to interfere with the calcium signalling phase of saliva production in pSS

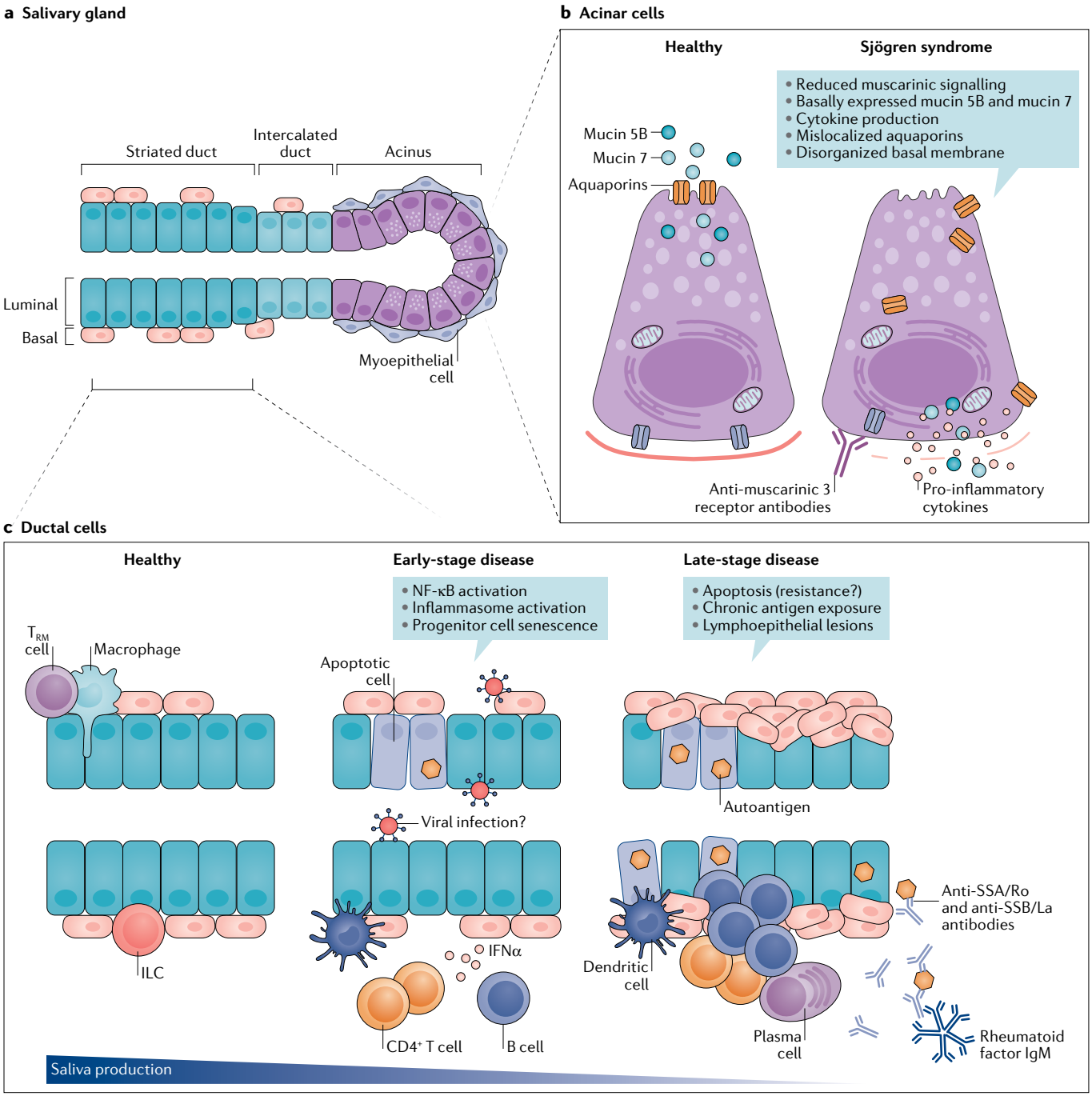

Fig. 1 | **The salivary gland epithelium in health and in pSS. a** | The salivary gland epithelium is made up of several cell types, including acinar cells (that cluster to form the acinus), ductal cells (consisting of basal and luminal cell types) that form the striated and intercalated ducts, myoepithelial cells and progenitor cells. Saliva is produced by acinar cells and secreted upon myoepithelial cell contraction. Saliva is then channelled and modified through the intercalated and striated ducts and flows via larger excretory ducts into the mouth. **b** | Acinar cells contain multiple defects in primary Sjögren syndrome (pSS). **c** | A number of pathogenic events could take place in the ductal epithelium during pSS development. In a healthy situation, several immune cells (for example, macrophages, innate lymphoid cells (ILCs) and tissue resident memory (T_{RM}) cells) are present for immune surveillance to enable a fast response to injury or infection. In pSS, an unknown trigger (for example, viral infection or tissue damage) might cause activation of innate immune pathways, epithelial cell apoptosis and senescence, which is exacerbated in more severe stages of disease. Late-stage disease is characterized by chronic antigen exposure, local production of autoantibodies, accumulation of $CD4^+$ T cells and B cells, complete loss of progenitor cells and the formation of lymphoepithelial lesions. These lesions are characterized by basal cell hyperplasia and the presence of intraepithelial lymphocytes.

involves the microRNA miR-142-3p. This microRNA is present in secreted exosomes derived from infiltrating T cells in the glands and is transferred to acinar cells. miR-142-3p targets important components of intracellular Ca^{2+} signalling, ultimately resulting in decreased saliva production[31]. In healthy scenarios, successful Ca^{2+} signalling results in the activation of aquaporin (AQP) water channels, and water is transported out of the apical membrane of acinar cells. Notably, the expression of the various AQPs is increased (AQP5) or decreased (AQP1, AQP3 and AQP5) in patients with pSS compared with healthy individuals, and the ability of these channels to respond to muscarinic stimuli are decreased compared with individuals without pSS[32,33].

The function of saliva depends not only on water secretion but also on its protein content. Mucins, produced by the salivary gland mucous acinar cells, provide essential lubrication to facilitate swallowing, and are secreted via the apical pole of acinar cells[34]. Mucous-producing acinar cells are found in the LSGs, as well as in the submandibular and sublingual major salivary glands[34]. In pSS, the salivary mucin components MUC5B and MUC7 are found outside the basal pole of LSG mucous acinar cells[35,36]. Interestingly, MUC5B and MUC7 are capable of Toll-like receptor 4 (TLR4)-mediated autocrine stimulation of CXCL8, TNF, IFNα, IFNβ, IL-6 and IL-1β production by LSG acinar cells[36]. Consequently, basally mislocalized mucins might contribute to glandular inflammation. However, the direction of causality between mucin disruption and acinar cell inflammation remains to be clarified, as does the relevance of mucin disruption in the larger, non-mucin-producing parotid salivary gland. The basal lamina, which is normally strictly localized to the basal pole of acinar cells to provide anchorage and polarization, is also disorganized in the salivary glands in pSS[37]. This change might contribute to the secretion of mucins at the wrong cellular pole, although further evidence is needed to support this idea. Intriguing further data suggest that the cytoplasm and endoplasmic reticulum are overloaded with a different mucin, MUC1, in LSG acinar cells in pSS, which might cause cellular stress and potentially increase the likelihood of apoptosis[35,36,38]. In a separate study, incubation of LSG acinar cells with TNF or IFNγ triggered both increased nuclear factor-κB (NF-κB) activity and overexpression and aberrant localization of MUC1 (REF.[38]), implying that the aberrant distribution of mucins might be a consequence of the inflammatory environment in the salivary glands in pSS, potentially culminating in cellular stress and cell death.

pSS is associated with the presence of autoantibodies, most often with antibodies directed against SSA/Ro proteins. However, autoantibodies directed against the acinar cell-associated proteins parotid secretory protein, salivary protein 1 and carbonic anhydrase 6 have also been reported in some patients with pSS, in addition to those antibodies against the muscarinic 3 receptor mentioned above[39–41]. These findings open up debate as to whether acinar-specific autoantibodies have a potential role in the induction of cell death in acinar cells, or whether these antibodies are a downstream consequence of that process.

Acinar cells might thus contribute to the expression of pro-inflammatory cytokines in pSS and are grossly dysregulated in terms of the functional machinery necessary for saliva production (FIG. 1b). The extent of apoptosis of the cells, and its relation to the development of salivary gland disease, requires further clarification. Despite the observed abnormalities in acinar cells of patients with pSS, these abnormalities do not cumulatively result in appreciable numbers of immune cells surrounding the acini, as the infiltrating cells accumulate mostly around the striated ducts. However, it remains possible that cytokines produced by periductal infiltrates affect acinar cells. Furthermore, the potentially pathogenic role of the small number of interstitial immune cells and immune cells located within the acini (for example, $CD8^+$ T cells)[37] needs further investigation.

Changes in ductal cells in pSS

Complementing the acinar cells responsible for making saliva are the ductal cells. Aside from the channelling of saliva into the mouth, ductal cells are also responsible for the extraction of sodium from, and the addition of potassium to, the saliva. This process, mediated via calcium-activated sodium and potassium channels, culminates in attainment of the hypotonic sodium and potassium concentrations necessary for the slightly acidic antimicrobial functions of saliva, and for the prevention of mineral loss from dental hard tissues[42]. In patients with pSS, the concentrations of sodium and chloride in the saliva are higher than in healthy individuals, potentially owing to a reduction in ion resorption by ductal cells and/or because the volume of water secreted from acinar cells is reduced[19,20,43,44]. The investigation of the role of ductal cells in pSS has centred historically around the striated ducts in histology-based studies or in vitro culture systems of salivary gland epithelial cells (SGECs). Although cultured SGECs might represent ductal cells in general (that is, both striated and intercalated ductal cells), whether they represent striated duct cells specifically has never been proven[45]. For the purpose of this Review, however, we will treat SGEC cultures as a model for striated duct cells, referred to as SGECs (to mean LSG-derived SGECs) throughout unless mentioned otherwise.

SGECs from patients with Sjögren syndrome respond aberrantly to extrinsic stimuli. For example, SGECs from patients with pSS are more sensitive to FAS/FASL-mediated apoptosis in the presence of TNF and IFNγ than SGECs from healthy individuals[46,47]. These cells are also more susceptible to other mechanisms of programmed cell death, such as IFNγ-induced or poly (I:C)-induced anoikis (that is, apoptosis due to loss of attachment to the basal membrane)[48]. However, data from the literature suggest that striated duct cells[22,49] and SGECs[50] are both resistant and susceptible to apoptosis.

In addition to disturbed apoptosis, SGECs express various TLRs (TLR1, TLR2, TLR3, TLR4 and TLR7) and other pattern recognition receptors (PRRs), implying that these cells have an ability to process pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs).

Furthermore, TLR3 activation can stimulate the expression of pSS-associated autoantigens SSA/Ro60, SSA/Ro52 and SSB/La by SGECs[51]. SGECs can also express receptors for cytokines, including receptors for type I interferons and TNF[52–54]. The chemokine receptor CXCR3 might function as a scavenging receptor in the healthy salivary gland, preventing a build-up of surplus pro-inflammatory chemokines such as CXCL9, CXCL10 and CXCL11. In pSS, the function of this receptor is thought to be impaired in the ductal cells; indeed, in SGECs from patients with Sjögren syndrome, intracellular levels of CXCL10 are reduced compared with SGECs from healthy individuals, which is presumed to be a consequence of impaired CXCR3 functionality permitting the accumulation of these chemokines[55]. In addition to responding aberrantly to extrinsic stimuli, striated duct cells[56,57] and SGECs[52,58] from patients with Sjögren syndrome also express many cytokines and chemokines (for example, IL-1, IL-6, IL-7, IL-18, TNF, B cell-activating factor (BAFF), CXCL10, CXCL12 and CXCL13) at higher levels than those observed in healthy individuals. Thus, striated ducts and their presumed in vitro counterpart SGECs are capable of regulating the immune response (as discussed in more detail in the section on epithelial cell–lymphocyte crosstalk).

Considering the involvement of striated duct cells in, for example, LELs, the NF-κB pathway and inflammasome activation (as discussed in detail in later sections), we would argue that the role of striated duct cells in the development of pSS pathology cannot be understated. The further roles of smaller, intercalated ducts remain until this point less well understood, and will require further investigation.

Salivary gland homeostasis disruption

Mouse studies have shown that a small number of immune cells, including but potentially not limited to tissue-resident memory T (T_{RM}) cells, macrophages, group 1 innate lymphoid cells (ILC1s), natural killer cells and dendritic cells, patrol the salivary glands, scanning for hallmarks of infection and/or epithelial cell damage[59,60] (FIG. 1c). T_{RM} cells and macrophages, in particular, have been identified in close association with the mouse acinar epithelium[60]. In patients with pSS, this homeostatic surveillance system seems to be disturbed, and the salivary gland immune landscape is unbalanced owing to an infiltration of mainly $CD4^+$ T and B cells as well as, in lower abundance, pDCs and other immune cells[9]. Besides their immune function, these infiltrating cells can affect salivary gland homeostasis in multiple ways, including crosstalk with the salivary gland epithelium (which will be discussed in more detail in the section on epithelial cell–lymphocyte crosstalk).

In normal salivary gland homeostasis, the aforementioned immune cell sentinels are complemented by salivary gland progenitor cells (SGPCs). SGPCs proliferate and differentiate into fresh acinar and ductal cells, to replenish damaged cells and cells reaching the end of their lifespan[61–64]. In the major salivary glands, the ductal compartment (both the striated and intercalated ducts) has been proposed to house SGPCs, although progenitor cells might also be present in the acinar compartment[61,65–67]. These progenitor cells can be studied in vitro through organoid cultures[61,65,68]. The number of SGPCs is much lower in patients with pSS than in healthy salivary glands, and the few that remain have a lower differentiation capacity[68]. One explanation for this finding is that progenitor cells residing in the basal layer of the parotid gland striated ducts become senescent prematurely in pSS, as a result of the mitotic effects of pro-inflammatory cytokines[68]. The telomeres of SGPCs from patients with pSS are shorter, suggesting that some SGPCs have indeed undergone replicative senescence[68]. In one study, the degree of senescence of cells located in the basal layer of the striated ducts (where progenitor cells probably reside), as inferred from the expression of the senescence marker p16, correlated with the production of saliva by the parotid salivary gland, the extent of $CD45^+$ cell infiltration and the ultrasound score[69]. The expression of p16 by all acinar and ductal cells combined also correlated with saliva production and $CD45^+$ cell infiltration, but to a lesser extent[69]. Senescent cells in general have a senescence-associated secretory phenotype (SASP), consisting of the expression of a panel of pro-inflammatory cytokines (including IL-1α, IL-1β, IL-6, IL-7, IL-13, IL-15, TGFβ, GM-CSF and TNF), chemokines (including IL-8, GROα, GROβ, CCL2, CCL3, CCL5, CCL16, CCL26 and CCL20), growth factors (including HGF and FGF), matrix metalloproteases and proteases[70–72]. Most interestingly and potentially detrimentally, this SASP enables the active spreading of senescence directly to neighbouring cells[72]. Therefore, senescent cells might not only reduce the ability of the salivary gland to function, by disabling a potential progenitor cell population, but also augment inflammation and disease progression. Coupled with this effect is the strange persistence of functionally defective acinar cells, whose clearance would otherwise presumably trigger replacement with fresh counterparts by SGPCs, a curious dynamic that requires more investigation.

Of further interest, disruption of salivary gland homeostasis also occurs in patients undergoing immune checkpoint inhibitor (ICI) therapy for the treatment of cancer. Approximately 5% of ICI-treated patients will experience some form of salivary gland dysfunction[73–76]. Among these patents, 60% will progress to technically fulfil the ACR–EULAR 2016 criteria for pSS[3], owing to the presence of sicca symptoms, immune foci in the salivary glands and/or autoantibody positivity[73–76]. The potential mechanism of ICI-induced salivary gland dysfunction is highlighted in BOX 1.

Virus–epithelial cell interactions

Microorganisms are implicated as a potential trigger in the development of pSS. Activation of TLRs that recognize components of bacteria (such as TLR1, TLR2 and TLR4) or viruses (such as TLR3, TLR7 and TLR9) on salivary gland ductal cells, for example, might represent a first step in the initiation of inflammation in the salivary gland in the early stages of disease. However, evidence that conclusively demonstrates the contribution of specific bacteria to salivary gland dysfunction in pSS is lacking[77–81]. Indeed, the most likely hypothesis is that alterations in the bacteriome in the oral cavity

Box 1 | **Salivary gland dysfunction following checkpoint inhibition**

Both the major and minor salivary glands contain a T cell-rich inflammatory infiltrate following immune checkpoint inhibitor (ICI) use, but the glands do not resemble classical salivary glands of patients with pSS on the infiltration level[74,203]. Some evidence suggests that ICI therapy induces a considerable shift in the organization of the parenchyma (specifically, a loss of typical saliva-producing acinar cells)[203]. The mechanism underpinning this effect is as yet unclear, but might encompass acinar cell death followed by aberrant salivary gland progenitor cell compensation, or increased plasticity of salivary gland epithelial cell types. This plasticity could be induced, for example, by the presence of the type II interferon signature associated with cytotoxic T cell activation and checkpoint inhibitor therapy (as opposed to the type I interferon signature most commonly associated with classical pSS), and might explain the notably different morphology observed[204]. Although presenting potentially with a different phenotype to pSS, deciphering how this epithelial skewing occurs will probably shed light on the mechanism underpinning salivary gland dynamics in general.

represent a consequence, rather than a cause, of salivary gland dysfunction in pSS[82]. Nevertheless, the triggering of specific TLRs and other (cytosolic) PPRs on SGECs by viral material might result in type I interferon production, and conceivably contribute to the well-known type I interferon signature associated with pSS. Although intrinsic and other extrinsic triggers might also be responsible for interferon production by epithelial cells (as discussed in the later section on interferon pathways and as reviewed elsewhere[83]), this common feature of pSS suggests the presence of an initial immune response against viral infection.

To date, infection with three different viruses has been associated with the development of pSS. First, some data suggest that past infection with hepatitis C virus (HCV) is associated with the development of pSS, with estimates suggesting that approximately 14% of patients with pSS test positive for antibodies against HCV in certain pSS populations[84–86]. However, it should be noted that HCV infection (latent or active) is an exclusion criterion in the 2016 ACR-EULAR classification criteria for pSS and debate exists as to whether this patient subgroup truly represents pSS or a Sjögren syndrome-like condition, especially as HCV infection rates vary widely geographically[86]. Second, chronic infection with human T cell lymphotropic virus type 1 (HTLV1) has also been associated with low-grade salivary gland damage in pSS and increased serum concentrations of TNF and IFNγ, although this virus is not necessarily associated with pSS development[87,88]. Patients with sicca syndrome and HTLV1 antibody serum positivity, for example, do not tend to have anti-SSA autoantibodies[87,88]. Third, various evidence points to an association between Epstein–Barr virus (EBV) and pSS, although it should be noted that this virus is very common and present in up to 95% of the general population[89]. The EBV remains latent in resting memory B cells and requires B cell stimulation and plasma cell differentiation for lytic replication[89–91]. This ability to remain dormant in memory B cells means that the virus poses a persistent threat. EBV DNA has been detected at increased levels in the salivary glands of patients with pSS compared with healthy individuals, with Ro52-reactive perifollicular plasma cells being frequently infected[92,93]. Furthermore, patients who were also positive for anti-SSA and/or anti-SSB antibodies had higher titres of IgG antibodies against the EBV early antigen than those patients without anti-SSA and anti-SSB antibodies[91].

EBV is capable of directly infecting epithelial cells; in these cells, the EBV glycoprotein BMRF2 mediates transport of the virus to the basolateral membrane and seems to facilitate spreading of progeny virions[94]. In one study, levels of the EBV-derived microRNA ebv-miR-BART13-3p were increased in the salivary glands of patients with pSS compared with healthy individuals[95]. Interestingly, ebv-miR-BART13-3p was suggested to downregulate the expression of stromal interaction molecule 1 (STIM1), a component of the acinar cell secretory machinery, providing a plausible link between EBV and saliva production[95]. This microRNA can transfer between cells, such as between B cells (the preferred target cells of EBV) and SGECs, via microvesicles[95]. Notably, the EBV protein Epstein–Barr nuclear antigen 2 (EBNA2) has a marked degree of protein sequence similarity to Ro60 autoantigens and likewise the protein sequences of Epstein–Barr virus (EBV)-encoded RNA 1 (EBER1) and EBER2 are strikingly similar to La autoantigens[96,97]. Furthermore, antibodies to Ro60 can cross-react with EBNA1 (REF.[98]), suggesting that molecular mimicry might trigger immune system activation to self-antigens in pSS. However, no correlation has been found between EBV reactivation periods and the development of pSS symptoms[96]; furthermore, the molecular mimicry hypothesis does not explain why such a common infection as EBV results in pSS development in only a small proportion of these individuals[89].

How viral infections might contribute to pSS remains unclear. In addition to the often touted classical hypotheses, including molecular mimicry or enhanced apoptosis of epithelial cells, evidence would also suggest that viruses are capable of inducing epigenetic changes in epithelial cells[99], an exciting new interaction that remains to be investigated in pSS. Virus-induced epigenetic changes might, for example, repress the expression of pro-apoptotic genes. Impaired viral clearance as a mechanism for pSS development has also been inferred from the presence of a less efficient splice variant of the classical type I interferon downstream mediator OAS1 in patients with pSS[100,101].

With respect to SARS-CoV-2 viral infections, it is worth considering that a potential pool of patients with sicca complaints might be emerging. The involvement of the salivary gland as a reservoir of this virus has been proposed, following the identification of the ACE2 receptor (an entry receptor for SARS-CoV-2) on SGECs[102,103]. Close monitoring of this patient pool over the coming years might provide crucial evidence regarding the role of viral infections in the triggering of sicca symptoms and possibly also in the triggering of pSS.

Innate immune function of the epithelium

As discussed in the previous sections, the salivary gland epithelium fulfils innate immune functions that are mainly mediated by the expression of PRRs (for example, TLRs)[53,54] and the secretion of cytokines. In this section, we discuss the different innate immunological pathways that contribute to dysfunction of the salivary

gland epithelium in pSS. Although the initial events that cause innate immune activation in the salivary gland epithelium remain to be identified, several possibilities have been raised. These include the involvement of exogenous antigens (for example, PAMPs from ineffectively cleared viruses) or aberrant expression of endogenous factors (for example, retroelements) that stimulate innate immune responses. Another possibility is that these responses are triggered by DAMPs because of inefficient removal of epithelial cell debris. Interestingly, a patient's genetic background might predispose them to impaired clearance of cell debris. The genotype and copy gene number of the complement component C4 has a high sex bias and has been linked to pSS susceptibility[104]. The lower gene copy number and expression of C4A reported for women that increase the risk of SLE or pSS might reduce the capacity of the immune system to clear cell debris, thereby prolonging exposure of intracellular proteins to the immune system[104]. However, data on the relationship between the C4 genotype and salivary gland pathology are not yet available.

NF-κB signalling

The NF-κB family is a group of transcription factors capable of activating an array of inflammatory downstream targets, including pro-inflammatory cytokines[105]. The canonical NF-κB pathway is activated by binding of PAMPs to the TLRs and by pro-inflammatory cytokines themselves binding to their respective receptors[105]. Engagement of TLR and cytokine receptors triggers activity of the IκB kinase complex, culminating in phosphorylation of IκBα. In the inactive state, IκBα is in complex with canonical NF-κB members RelA and p50, whereas phosphorylation of IκBα results in degradation of this inhibitor, enabling the release of the RelA–p50 heterodimer and its subsequent translocation to the nucleus, where the dimer activates target gene transcription[105]. Studies have reported NF-κB activity in the epithelial cells of the salivary glands of patients with pSS, reflected by the expression of NF-κB downstream target genes encoding cytokines such as IL-1α, IL-6 and TNF[57]. NF-κB is also important for the regulation of cell–cell interactions and is capable of disrupting tight junction integrity[106]. Mutations that result in overactivity of the NF-κB pathway have been reported in patients with pSS: for example, mutations in genes encoding IκBα or the inhibitor TNFα-induced protein 3 (TNFAIP3; also known as A20)[107–109]. Interestingly, in mice, constitutive activation of the NF-κB pathway via knockout of TNFAIP3 in cytokeratin 14–expressing epithelial cells (including those cells of the striated duct) is sufficient to result in reduced saliva production and infiltration of a T cell-rich infiltrate into the salivary glands[110]. This finding emphasizes the role of epithelial cells and the NF-κB pathway in pSS development, and the apparent ability of activated epithelial cells to recruit immune cells.

The inflammasome

The inflammasome is an intracellular complex of oligomers that detect and respond to DAMPs and PAMPs to incite inflammation, functioning as an important innate immune system receptor and sensor. Inflammasomes require priming via classical innate immune activation pathways, such as the microbe-activated TLR pathway[111]. Subsequent to this signal, a second activation signal of inflammasome components via stimuli such as lysosomal disruption, reactive oxygen species production and release of oxidized mitochondrial DNA or microbial nucleic acids is required[111]. Inflammasomes can be broadly divided into four types: the absent in melanoma 2 (AIM2) inflammasome, the NOD-, LRR- and pyrin domain-containing protein 1 (NLRP1) inflammasome, the NLRP3 inflammasome and the NLRP4 inflammasome. Inflammasome activation ultimately facilitates processing of pre-formed pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18 into their final active forms. Stimulation of the AIM2 inflammasome occurs following binding by cytosolic free DNA (cfDNA) of viral, bacterial or mammalian origin, but not by other triggers[112]. Some data suggest that the AIM2 inflammasome is highly active in SGECs from patients with pSS, which could be because of defective functioning of DNase 1 resulting in the accumulation of cfDNA[113]. Patients with pSS and MALT lymphomas, and those patients with pSS judged to be at a high risk of developing MALT lymphomas, have high serum levels of cf DNA and extranuclear DNA accumulations in salivary gland tissue, mostly localized between the striated ducts and lymphocytic infiltrates[114]. This extracellular DNA, if transported into striated duct cells, might be an important stimulus for the AIM2 inflammasome.

Oligomerization of NRLP1, NRLP3 or NRLP4 results from upregulation of NRLP transcription following signals induced by cfDNA, as is described for AIM2, as well as signals induced by a panoply of DAMPs, PAMPs and other triggers, such as reactive oxygen species, oxidized mitochondrial DNA and lysosomal disruption. Various data suggest that the salivary glands of patients with pSS express higher levels of NLRP3 than healthy individuals, and P2X7, a component of the NRLP3 machinery, is also upregulated in the salivary gland tissue of patients with pSS[114,115]. However, which specific cell types express the upregulated NRLP3 is unclear, as indeed is the expression of the NRLP1 and NRLP4 inflammasomes in pSS[115]. However, although perhaps less well-understood than the role of the TLR and NF-κB systems, a central role for the inflammasome in pSS is emerging. Mirroring the NF-κB pathway, the activity of the inflammasome in the salivary gland epithelium reinforces the role of the epithelium, and its crosstalk with the immune system, in pSS. The upregulated inflammasome might contribute to the sustained glandular inflammatory process in pSS, and represents a potential alternative therapeutic target.

Interferon pathways

Important signalling pathways that contribute to the disruption of the salivary gland epithelium in pSS involve interferons. The interferon family consists of type I (IFNα and IFNβ), type II (IFNγ) and type III (IFNλ) interferons, which have distinct as well as common functions. Interferons stimulate a wide variety of genes important in innate and adaptive immune responses[116]. In particular, type I interferons create an anti-viral state by promoting immune responses to viruses[117]. The important role of

interferons in the pathogenesis of pSS is reflected by the elevated transcript levels and altered epigenetic regulation of interferon-stimulated genes (ISGs) in both the blood and salivary gland tissue of patients[118–120]. These ISGs are upregulated in both SGECs and striated ducts, in addition to in immune cells[121,122], together accounting for the interferon signature of the glands. Indeed, both type I and type II interferon-induced proteins (IFN-induced protein with tetratricopeptide repeats 3 (IFIT3) and guanylate-binding protein 2 (GBP2), respectively) are expressed in ductal epithelial cells, whereas infiltrating immune cells mainly expressed GBP2 (REF.[122]). In the following subsections, we discuss both the pathways that result in interferon production (for example, PRR signalling and the overexpression of endogenous retroelements), and the pathways that result from stimulation by interferon (for example, pro-inflammatory cytokine production) in the salivary glands.

Type I interferons. pDCs are a potent source of IFNα within the inflamed salivary gland[6]. Activated pDCs are able to migrate to the salivary glands in response to chemokines such as CXCL12, CXCR3 ligands and CCR5 ligands[123,124]. Within the glands, type I interferon production by pDCs can be sustained in the presence of TLR7 ligands, TLR9 ligands and immune complexes of autoantibodies and autoantigens that contain TLR7 ligands in the form of autoantigen-associated RNA[125]. Although a strong correlation exists between the presence of Ro/La autoantibodies and the expression of ISGs in the blood and tissue of patients with pSS, the sequence of appearance of these features and their contribution to dysfunction of the salivary gland epithelium need further investigation.

In addition to pDCs, epithelial cells themselves can produce type I interferon after stimulation via PRRs, as demonstrated, for example, in studies of mice and of a human submandibular gland cell line[36,126]. Furthermore, SGECs respond to TLR signalling by producing IFNβ[127,128]. Interestingly, interferon production can be augmented by endogenous virus-like genomic repeat elements ('retroelements')[129]. The retroelement long interspersed nuclear element 1 (LINE-1) is overexpressed in the minor salivary gland tissue of patients with pSS, probably owing to hypomethylation in the promoter region of the gene encoding LINE-1 (REFS[129,130]). Importantly, LINE-1 and IFNβ are expressed in the ductal epithelium[129,130]. Mechanistically, LINE-1 retroelements bind to cytosolic nucleic acid sensors, such as RIG-I-like receptors, and consequently trigger type I interferon production[131]. Vice versa, signalling downstream of extrinsic interferons can modulate the transcription of retroelements. For example, transcriptional modifications of Alu retroelements, induced by interferon signalling, enables this retroelement to bind to intracellular Ro60 (REFS[129,132]). Ro60 probably has an inhibitory role in type I interferon receptor signalling, as data have shown that deletion of Ro60 in an EBV-transformed B cell line increases interferon-induced pro-inflammatory cytokine production and the expression of retroelements[132].

Similar to Ro60, Ro52 (later identified as TRIM21) has a multifaceted role in the innate immune response. TRIM21 primarily functions simultaneously as a cytosolic Fc receptor and as an E3 ubiquitin-protein ligase[133,134]. An important anti-viral role of TRIM21 is binding the Fc part of intracellular antibodies, directing endocytosed antibody-bound viral particles to the proteasome for degradation and activation of innate immune signalling[134]. Conversely, TRIM21 can also function as a negative regulator of TLR signalling by mediating ubiquitination (protein inactivation) of interferon-regulating factors (IRFs), such as IRF3, IRF5 and IRF7, thereby inhibiting interferon production[135–137]. An inhibitory role for TRIM21 is supported by studies of TRIM21-deficient C57BL/6 mice, which develop features of systemic autoimmunity upon local tissue injury[138]. Furthermore, the amount of anti-dsDNA antibody production and plasmablast formation is higher in TRIM21-deficient MRL/lpr mice than in wild type MRL/lpr mice, indicating enhanced B cell activation in the absence of TRIM21 (REF.[139]). Anti-Ro52/TRIM21 antibodies from patients with pSS can specifically recognize the RING domain of the TRIM21 protein. This RING domain is essential for the E3 ubiquitin ligase activity of TRIM21 and anti-Ro52 antibodies could interfere with this activity in vitro[140]. Although direct interference of anti-Ro52/TRIM21 antibodies (via their antigen-binding fragment (Fab) domain) with intracellular TRIM21 has not been proven in vivo, and is difficult to reconcile, aberrant function of TRIM21 upon uptake of autoantibody-containing immune complexes cannot be ruled out. For example, a study of mice with lupus-like disease provided evidence of possible leakage of IgG antibodies from phagolysosomes containing endocytosed immune complexes into the cytosol, which stimulated the TRIM21-mediated immune responses[141]. Furthermore, studies of patients with pSS have shown that TRIM21 can be expressed on the cell surface of antigen-presenting cells[142], enabling transport of aggregated immunoglobulins and immune complexes into the cell. In conclusion, aberrant expression of retroelements and (indirect) interference of autoantibodies with the regulation of innate immune responses by TRIM21 (and possibly also Ro60) might result in a feed-forward loop of type I interferon activity in ductal epithelial cells, pDCs and B cells (FIG. 1c). A main consequence for the glandular epithelium is that type I interferons enhance the production of pathogenic chemokines and cytokines (for example, CXCL10, IL-7 and BAFF) by ductal epithelial cells, resulting in the recruitment and activation of T cells and B cells and thereby amplifying the inflammatory response[58,143,144].

Type II interferons. In addition to type I interferons, type II interferons have also been implicated in pSS-associated salivary gland pathology. IFNγ is mainly produced by natural killer cells and T cells as part of the innate and adaptive immune responses, respectively. In the salivary glands of patients with pSS, IFNγ is abundantly produced by immune cells and the salivary gland tissue often has a mixed type I and type II interferon signature[57,122]. IFNγ is not only produced by bona fide T_H1 cells in the salivary gland but also by T follicular helper (T_{FH})-like cells that express both

programmed cell death protein 1 (PD1) and inducible T cell costimulator (ICOS) and by CCR9$^+$ T helper cells[145,146]. IFNγ exerts pro-inflammatory effects on SGECs (including both labial and parotid gland-derived SGECs) by stimulating the production of cytokines and chemokines[58,143]. IFNγ also induces the expression of MHC class II and co-stimulatory molecules on SGECs[47,147,148]. Collectively, these effects might result in immune cell activation[46]. IFNγ can also induce FAS-mediated apoptosis and anoikis in SGECs[47,48], although an opposite effect was observed in acinar cells and in an immortalized salivary gland ductal cell line, where IFNγ induced apoptotic resistance[25,46]. IFNγ has further been implicated in reducing the integrity of the glandular epithelium (that is, the tight junction barrier function), as has been observed in minor salivary gland tissue from patients with pSS[106]. Together, these results suggest that IFNγ negatively affects SGECs in various ways, dependent on the glandular cell type, and might contribute to salivary gland dysfunction in patients with SS.

Type III interferons. Type III interferon has been added to the interferon family more recently[149]. Downstream effector genes of type III interferons are similar to those downstream of type I interferons and the functions of both interferon pathways largely overlap. However, an important difference is restricted expression of the type III interferon receptor by epithelial cells of mucosal surfaces and pDCs (reviewed elsewhere[150]). In the ductal epithelium of patients with pSS, IFNλ2 is upregulated compared with individuals with non-Sjögren syndrome sicca symptoms[151]. Similar to type I and type II interferons, IFNλ can promote the production of cytokines (for example, IL-7, BAFF and CXCL10) by the salivary gland epithelium[58,151]. Whether type III interferons notably contribute to salivary gland pathology, either independent or in synergy with other interferons, remains to be elucidated.

Epithelial cell–lymphocyte crosstalk

In healthy salivary glands, a small number of lymphocytes (in the form of T_{RM} cells) are present for immune surveillance[60]. By comparison, in pSS, CD8$^+$ T cells are located in close association with acini or ducts with a disrupted basal lamina and CD4$^+$ T cells are located in association with the ducts[37]. B cells can also infiltrate the ductal epithelium, a phenomenon that is specific to pSS[10]. Such histological findings suggest that crosstalk between the salivary gland epithelium and lymphocytes has a critical role in salivary gland pathology. In this section, we focus on crosstalk between epithelial cells of the salivary glands and lymphocytes (specifically, CD4$^+$ T cells and intraepithelial B cells) in pSS.

Crosstalk with CD4$^+$ T cells

The presence of MHC class II in combination with CD80 and CD86 molecules on the surface of SGECs upon immune activation (for example, following stimulation with IFNγ) infers the ability of the epithelial cells to process antigens for presentation to CD4$^+$ T cells[52,152]. In the presence of T cell receptor stimulation, constitutive expression of CD86 by SGEC lines could promote CD4$^+$ T cell proliferation by engaging with CD28 (REF.[153]). SGECs from patients with pSS are also capable of expressing other co-stimulatory molecules such as CD40 and ICOS ligand (ICOSL)[49,152,154]. In SGECs from patients with pSS, CD40 is spontaneously expressed (at higher levels than in SGECs from patients with sicca symptoms but not diagnosed with pSS) and this expression can be enhanced in the presence of IFNγ or IL-1β[148]. Ligation of CD40 on SGECs activates the non-canonical NF-κB pathway, resulting in pro-inflammatory cytokine production and either FAS-dependent apoptosis or apoptotic resistance[49,155], probably depending on the epithelial cell type and microenvironmental cues. However, although SGECs seem to express all molecules required for antigen presentation, no formal proof is yet available showing that human SGECs actively present antigen to T cells via cognate interaction in vivo.

In addition to the provision of co-stimulatory signals to T cells, SGECs can produce several cytokines and chemokines, as shown for both labial and parotid gland-derived SGECs. These can include chemokines that attract T cells (for example, CCL19 and CXCL10), as well as cytokines that promote local T cell proliferation and/or differentiation[56,58,143,154]. For example, SGECs can produce IL-6, a pleiotropic cytokine that can promote the differentiation of T_{FH} cells (as well as B cells)[156]. IL-6 production by SGECs, together with ICOSL expression on these SGECs, can support the differentiation of T_{FH} cells in vitro[154]. This finding is notable as the expansion of both glandular and circulating T_{FH} cells in pSS is associated with more severe disease, that is, increased ESSDAI scores, IgG antibody production and ectopic lymphoid structure formation[146,157,158].

Another potentially relevant cytokine for salivary gland epithelium–T cell crosstalk is IL-7. This cytokine is produced by non-haematopoietic cells and has an important role in T cell homeostasis[159]. Although a histological study in minor salivary glands found that IL-7 was mostly expressed by cells with a fibroblast morphology in the interstitium, and not by acinar or ductal cells[160], more recent findings suggest that IL-7 is also expressed by SGECs[58,121]. Cultured SGECs from patients with pSS produce IL-7 after stimulation with poly(I:C), IFNα or IFNγ[58]. Data from the B6.NOD-Aec mouse model of pSS suggest that IL-7 also has an indirect role in salivary gland pathology, by enhancing T_H1 responses and IFNγ-dependent CXCL10 expression in the salivary gland[161]. Thus, activation of T_H1 cells by IL-7 can propagate IFNγ production, creating a pro-inflammatory loop. Although the epithelial source of IL-7 production (for example, ductal or acinar cells) has not been studied, various data suggest that the IL-7–IL-7Rα axis contributes to T cell-driven autoimmune pathology in pSS. For example, the amount of IL-7R-positive T cells in the salivary glands of patients with pSS correlates with IL-7 expression and the severity of sialadenitis[162]. In addition, CCR9$^+$ T helper cells typically express high levels of the IL-7 receptor and stimulation of these cells with IL-7 induces IFNγ, IL-17 and IL-21 production, thereby also supporting B cell activation[145].

Crosstalk with B cells

Available evidence indicates that the salivary gland epithelium is able to promote B cell hyperactivity in pSS and forms a niche for autoantibody-producing plasma cells. Chronic B cell hyperactivity in the salivary glands is demonstrated, amongst others findings, by the frequent occurrence of ectopic lymphoid structures in the glandular tissue, which might even contain germinal centres[7], and by the high risk of patients with pSS developing B cell non-Hodgkin lymphomas in these glands[163]. Several mechanisms underlying B cell hyperactivity in pSS have been described and reviewed elsewhere[8,9]. In this section, we focus on the crosstalk between epithelial cells and B cells and highlight novel insights into the development of LELs and MALT lymphoma in pSS.

B cell activation by the salivary gland epithelium. Epithelial cells can contribute to B cell activity through the production of cytokines, including IL-6 and BAFF[121,154,164]. Both IL-6 and BAFF are involved in B cell activation and homeostasis, and BAFF is critically important for B cell survival[156,165,166]. Previous reports have shown that type I interferon is an important promoter of BAFF production by SGECs[6,144,167]. The expression of interferon-inducible genes was indeed increased in EpCAM-positive epithelial cells in biopsy samples from patients with pSS compared with biopsy samples from individuals with non-Sjögren syndrome sicca symptoms[121]. In one study that employed co-cultures of B cells and SGECs, poly(I:C) stimulated the production of soluble factors by SGECs from patients with pSS, which enhanced the survival of the B cells[121]. Surprisingly, blockade of a proliferation-inducing ligand (APRIL) or BAFF alone had no effect on B cell survival[121], suggesting that other factors might be responsible for enhanced B cell survival under this experimental condition.

The epithelium not only affects B cells, but B cells can also, vice versa, have effects on epithelial cells. For example, B cells can induce epigenetic modifications in SGECs[168]. B cell-induced transcriptional changes in epithelial cells might contribute to the formation of LELs, as discussed in the next section.

Lymphoepithelial lesion development. The intimate relationship between the salivary gland epithelium and B cells in pSS is most clearly demonstrated by the presence of LELs (FIG. 2). These LELs develop exclusively in the striated ducts and are a characteristic histological feature of pSS[11]. LELs are formed in close association with the periductal infiltrate[10,11]. Although these lesions are found in both the minor and major salivary glands, they are more pronounced in the major (parotid) glands[10]. LELs consist of hyperplastic duct cells in the epithelial cell lining and infiltrating lymphocytes within the contour of the basement membrane[11]. This hyperplasia might result in complete occlusion of the ducts, potentially contributing to hyposalivation. Evidence suggests that LELs nearly always harbour B cells in between the hyperplastic basal cells and that a small proportion of the striated ducts without hyperplasia already contain some B cells[10]. In addition, some intraepithelial T cells are also found in LELs; however, unlike B cells, which are completely absent from the salivary gland ducts of patients with non-Sjögren syndrome sicca symptoms, the presence of intraepithelial T cells is not specific to pSS[10]. Together these findings suggest that LEL formation starts with the infiltration of B cells into the ductal epithelium. The severity of the LEL (stage of duct occlusion) increases with the absolute and relative number of intraepithelial B cells[10]. How B cells are attracted into the epithelium is not fully understood, but the expression of CXCR3 probably has an important role, as intraepithelial B cells express CXCR3 and the salivary gland epithelium produces CXCL10 and other CXCR3 ligands upon activation[143]. These intraepithelial B cells, which are probably already activated before they migrate to the epithelium, can expand locally, as demonstrated by the high proportion of Ki67-staining cells and the expansion of clonal B cells within the striated ducts[169,170]. The latter finding suggests that additional activation and proliferation signals might be derived from epithelial cells, and possibly also from other intraepithelial immune cells, such as T cells and dendritic cells.

Interestingly, the majority, if not all, of the intraepithelial B cells in the minor (labial) and major (parotid) glands express the inhibitory Fc-receptor like 4 (FcRL4) protein (FIG. 3), which is abundantly expressed by MALT lymphoma B cells of patients with pSS[169], and is also expressed on activated B cells[171]. Gene expression profiling of FcRL4$^+$ B cells from parotid gland tissue of patients with pSS has further revealed that these cells express transcripts of chronic activation markers, such as T-bet

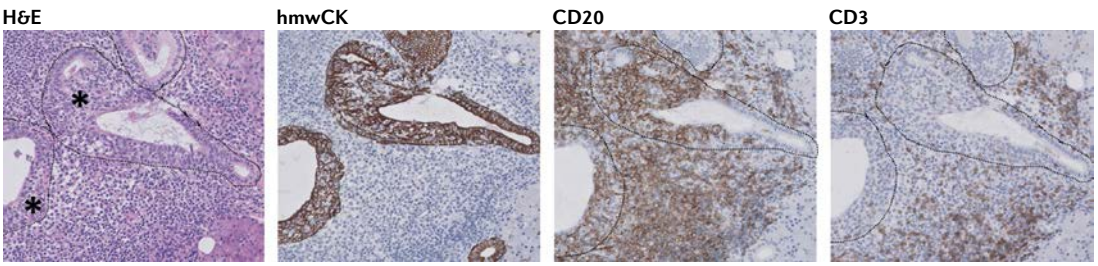

Fig. 2 | **Lymphoepithelial lesions in the salivary glands of a patient with primary Sjögren syndrome.** Consecutive sections of parotid gland tissue from a patient with primary Sjögren syndrome are shown. Lymphoepithelial lesions are indicated by an asterisk on the haematoxylin and eosin (H&E)-stained section. Consecutive sections were stained with antibodies to reveal either epithelial cells (using antibodies against high molecular weight cytokeratin (hmwCK)), B cells (using antibodies against CD20) or T cells (using antibodies against CD3). B cells dominate the lymphoepithelial lesions, whereas T cells are rarely detected within these structures. Image courtesy of M.S. van Ginkel and B. van der Vegt.

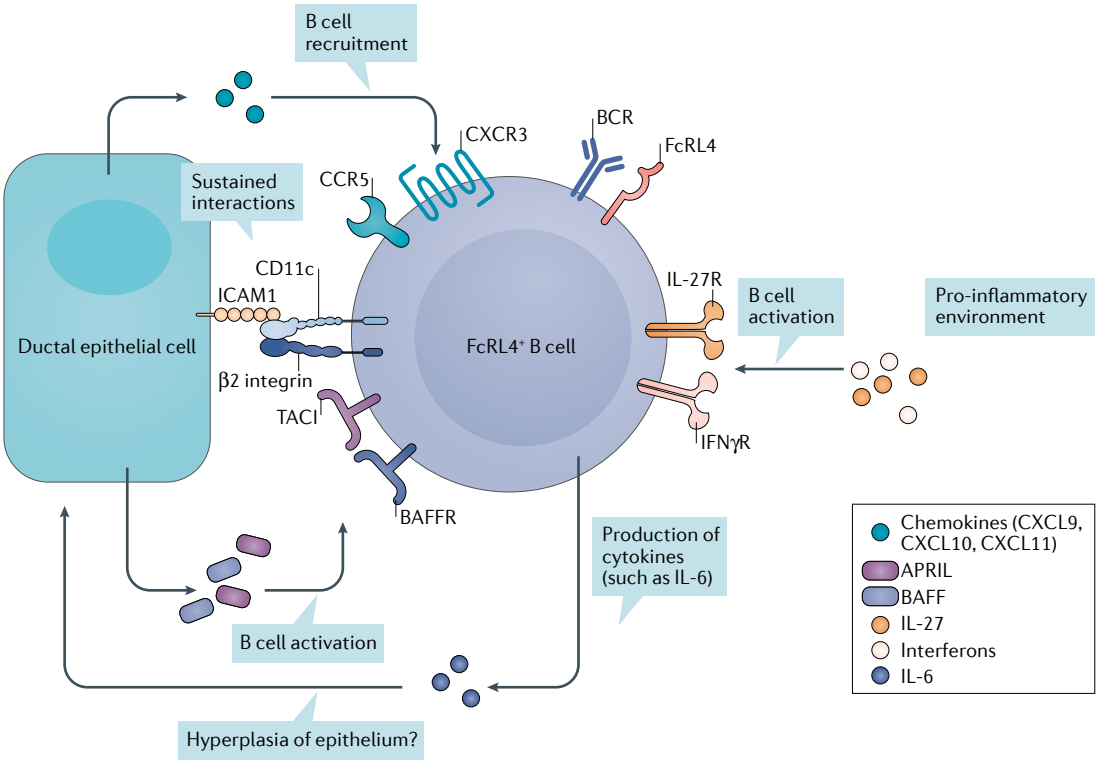

Fig. 3 | **Interactions between FcRL4⁺ B cells and the ductal epithelium in primary Sjögren syndrome. a** | Activated epithelial cells secrete CXCL10 and attract Fc-receptor like 4-positive (FcRL4⁺) B cells. These cells are further activated by B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), secreted by epithelial cells, and other stimuli from the pro-inflammatory environment. Upon activation, FcRL4⁺ B cells express CD11c, which can form an integrin together with the β2 integrin. This integrin can bind to intercellular adhesion molecule 1 (ICAM1) on epithelial cells and sustain their interaction. The production of IL-6 and possibly also other pro-inflammatory cytokines by FcRL4⁺ B cells might result in hyperplasia of the epithelium.

and CD11c[172]. T-bet expression might be the result of a type II interferon response, as the expression of *IFNGR1* and *IL27RA* is upregulated in FcRL4⁺ B cells[172]. Indeed, signalling downstream of the IFNγ receptor and IL-27 receptor in B cells induces the expression of T-bet, via STAT1, and such signalling pathways have been implicated in the development of autoimmunity in mice[173–176]. FcRL4⁺ B cells also have increased expression of *TACI* (encoding the receptor for BAFF and APRIL), *CXCR3*, *CCR5*, NF-κB-related genes (*NFKB1* and *MAP3K14*) and *IL6*, and reduced expression of negative regulators of NF-κB (*NFKBIA* and *NFKBID*, encoding IκBα and IκBNS, respectively), *CD40* and *CXCR5* compared with FcRL4⁻ glandular B cells. These phenotypical characteristics suggest that FcRL4⁺ B cells are activated cells that persist in inflamed tissue and are sustained by pro-inflammatory cytokines (in particular IFNγ, IL-27, BAFF and APRIL) and CD40-independent stimulation.

Glandular FcRL4⁺ B cells resemble double-negative (IgD⁻CD27⁻) B cells (called DN2 B cells), a cell type also characterized by T-bet and CD11c expression that is associated with the pathogenesis of SLE[177,178]. In patients with SLE, CD11c⁺T-bet⁺ B cells are poised to differentiate into plasmablasts outside of the follicles[177,178]. However, glandular FcRL4⁺ B cells in patients with pSS lack the expression of plasma cell markers, such as B lymphocyte-induced maturation protein 1 (BLIMP1; also known as PRDM1)[169]. Available evidence indicates that binding of soluble IgA to FcRL4 results in a switch from B cell receptor (BCR)-mediated activation to TLR-mediated activation[179]. This functional switch of the B cells probably results in enhanced NF-κB pathway activation and cytokine production. Secreted cytokines of intraepithelial B cells, such as IL-6, might affect epithelial homeostasis and lead to the proliferation of epithelial cells and ultimately to LEL formation. Direct proof that B cells are involved in LEL formation comes from trials of rituximab (a B cell-depleting agent) in patients with pSS. Treatment of patients with pSS with rituximab not only led to a strong reduction of B cells within the salivary gland, including B cells located within the epithelium, but concomitantly also led to a reduction in the severity of the LELs and the partial restoration of the epithelium after 12 weeks of treatment[180]. Notably, salivary gland B cell depletion was more variable at week 24 in this study[181], suggesting that in some patients, B cells have started to repopulate the glands at this time point. Although rituximab treatment had clear effects on salivary gland histopathology, in particular at early time points, these effects unfortunately did not translate into rituximab having a proven clinical efficacy in pSS[182–184].

The effects of B cell depletion therapy on the epithelium using rituximab, together with the cytokine profiles of intraepithelial B cells and SGECs, suggest that some

crosstalk occurs between B cells and striated duct epithelial cells, leading to sustained activation and proliferation of both cell types. Upregulation of CXCR3 and CD11c, and possibly also other integrins, on the B cell surface might have a role in keeping the cells within the epithelial layer. However, in addition to being present in ductal areas, B cell clones are also present, to some extent, in the periductal areas, suggesting that some cellular exchange occurs between these areas[170].

The lack of mRNA and protein expression of plasma cell markers by glandular FcRL4$^+$ B cells suggest that these cells rarely differentiate into plasmablasts and instead the cells are maintained in a state of chronic activation and continue to proliferate at a high rate[169,172]. These cells might undergo extrafollicular somatic hypermutation; indeed, activation induced deaminase (AID), the enzyme responsible for initiating diversity in immunoglobulin genes during somatic hypermutation (and class switching), is expressed by FcRL4$^+$ B cells in the tonsils (and possibly also in salivary glands)[185]. Notably, AID expression, which can be induced by T-bet[186], might not only result in hypermutation of immunoglobulin variable region genes, but also in off-target mutations (that is, non-immunoglobulin genes)[187]. Thus, a pathogenic combination of increased NF-κB activation, pro-survival factors, proliferation and possibly also AID expression in FcRL4$^+$ intraepithelial B cells could make these cells prone to neoplastic changes and promote progression towards MALT lymphoma, as discussed in the next section.

MALT lymphoma and rheumatoid factor-expressing B cells. Non-Hodgkin lymphomas arise in 5–10% of patients with pSS, and the majority of these lymphomas are MALT lymphomas that develop preferentially within the parotid glands[163]. A hallmark of salivary gland MALT lymphomas is their association with LELs, highlighting the dependency of these lymphomas on epithelial cells. MALT lymphomas are considered to arise as a consequence of chronic B cell stimulation and, in pSS, these neoplastic cells often express stereotypic rheumatoid factors that have a high affinity for the Fc region of IgG antibodies[188–190]. In patients with pSS, non-neoplastic rheumatoid factor-expressing B cells are enriched within the circulating CD21$^{-/low}$ B cell population[191], a phenotype that is associated with impaired BCR stimulation (similar to FcRL4$^+$ B cells) and frequent polyreactivity or self-reactivity[192,193]. The phenotype of CD21$^{-/low}$ B cells partially overlaps with DN2 B cells[194]. Notably, IL-21 and TLR7 ligands together promote the expansion and differentiation of DN2 B cells[178] and are both readily available in the inflamed salivary gland in patients with pSS[146]. While the frequency of rheumatoid factor-expressing B cells in the inflamed salivary glands of patients with pSS without MALT lymphoma seems to be low[195,196], these cells might expand after dual engagement of the BCR and TLRs with immune complexes that contain RNA-associated autoantigens[197]. Although the role of the BCR in lymphomatous escape remains enigmatic, rheumatoid factor expressed on the surface of B cells might simply trap immune complexes of autoantibodies and RNA-associated autoantigens, which stimulate TLRs, with or without further BCR-mediated activation of the cells. In the inflamed salivary gland, these immune complexes might provide chronic B cell stimulation and a strong selection advantage of incidental stereotypic rheumatoid factor-expressing B cells. Simultaneous engagement of FcRL4 on these B cells might inhibit differentiation towards plasma cells and in turn enable ongoing proliferation.

Rheumatoid factor-expressing B cells frequently express IGHV1-69, a immunoglobulin heavy-chain variable region variant that can be detected by the monoclonal antibody G6 (REF.[198]). Compared with healthy individuals, patients with pSS and cryoglobulinaemic vasculitis have an increased frequency of G6-positive memory B cells on the periphery, which exclusively express kappa light chains[191]. Interestingly, all MALT lymphomas that have rheumatoid factor activity are IgM clones with kappa light chains[188,190], suggesting that G6$^+$ B cells might form a pool of potential precursor cells to MALT lymphomas. Furthermore, researchers have shown that G6$^+$ B cells incidentally contain somatic mutations in genes involved in B cell proliferation, such as *TNFAIP3* (REF.[191]). A specific germline polymorphism of *TNFAIP3* is associated with MALT lymphoma development in pSS[199], underlining the importance of intact NF-κB regulation in preventing B cell dysregulation. Variations in B cell-regulating genes, such as *TNFAIP3*, might result in the escape of autoreactive cells from tolerance checkpoints[191]. Although the presence of G6$^+$ B cells that contain lymphoma-associated mutations in the peripheral blood of patients with pSS has not been linked directly to MALT lymphoma development, these cells might migrate to the salivary glands, where they could be further activated.

Taken together, an attractive hypothesis is that the highly proliferative, intraepithelial FcRL4$^+$ B cells are the glandular counterparts of DN2 B cells, incidentally displaying rheumatoid factor reactivity and forming a pool of lymphoma precursor cells in the salivary glands of patients with pSS. The notion that FcRL4$^+$ B cells are more frequently observed in parotid glands compared with labial glands might explain the preferential development of MALT lymphoma in parotid glands[169]. Although the critical steps towards neoplastic dysregulation of glandular B cells remain unknown, somatic mutations within and outside of the immunoglobulin variable region genes are probably involved. We postulate that therapeutic targeting of intraepithelial B cells might prevent the development of MALT lymphoma in patients with pSS.

Future directions

Over the past decade, the attenuation of sicca symptoms and, more recently, the attenuation of systemic disease activity have evolved as main treatment targets in pSS. As the epithelium is the functional backbone of the salivary gland, its restoration must be a central determinant of future therapies aimed at targeting hyposalivation in pSS. We hypothesize that therapies that target, for example, only the immune system might reduce glandular inflammation but not necessarily rescue salivary gland function, as demonstrated in existing clinical trials[200].

Although the targeting of the immune system is a valid approach in pSS, considering the typical (auto)immune manifestations and systemic nature of the disease, more attention should be paid to the correction of epithelial defects. Multifaceted approaches that consist of both an anti-inflammatory and a pro-epithelial component — for example, immunotherapy combined with cell therapy — might provide the crucial regenerative stimuli required to correct these defects.

With regard to cell therapy, fresh, patient-matched induced pluripotent stem cells might be used to derive untainted salivary gland acinar and/or ductal cells, which could subsequently be transplanted back into the patient. The relatively new drug class senolytics (a class of drugs that selectively induce death of senescent cells) might also prove therapeutically useful in the depletion of senescent epithelial progenitor cells, limiting further damage that would otherwise be inflicted by these cells[201,202]. The timing of therapy application is probably an important consideration, given the general early loss of salivary gland function in pSS. More advanced stages of glandular disease are characterized by the dominance of B cells and the presence of LELs, and in these patients, glandular B cells are an important treatment target. As previously highlighted, B cell depletion therapy with rituximab reduced the severity of LELs[180], but whether other B cell-directed therapies also target these pathogenic structures remains to be examined. In-depth histological evaluation of glandular tissue in clinical trials is, in this context, a valuable outcome measure of clinical trials.

Dissection and identification of specific defects at the individual patient level might be the key to successful treatment, given the variety of inflammatory pathways involved in the pathogenesis of pSS. Therefore, treatment decisions guided by the clinical picture of the salivary gland hold promise. To define the clinical picture of the salivary gland in individual patients, imaging and in-depth histopathological examinations of salivary gland biopsy samples are needed, going beyond measuring the focus score. In addition, tissue transcriptomics, in particular at the single-cell level, might be useful to identify dysregulated pathways. Lastly, the scientific community is advancing with the establishment of in vitro models of salivary gland disease (for example, co-cultures of organoids or explants with immune cells)[58,68,121] to screen for new drug targets and to examine the effect of immunomodulatory treatments on salivary gland function.

Conclusions

Overall, a multitude of pathways, systems and processes are probably dysregulated in the salivary gland in pSS, including defects that affect the epithelium, innate immune signalling and adaptive immune activation. In an ideal world, each defect would be corrected, and the salivary gland restored to its naive state. Although the initial triggers that activate the salivary gland epithelium remain undetermined, the nature of the local immune response, including dominant type I interferon activity, hints at the involvement of a viral infection. Subsequent dysregulation of the immune response, possibly owing to interference of type I interferon signalling by autoantibodies and chronic autoantigen exposure by apoptotic epithelial cells, results in a pro-inflammatory feedback loop. Various immune cells (such as lymphocytes, dendritic cells and macrophages) might interact with the epithelium and contribute to the inflammatory response. In particular, crosstalk between the ductal epithelium and B cells, and consequently the formation of LELs, negatively affects salivary gland morphology and might result in MALT lymphoma development. Finally, as a scientific community, we need to think more abstractly, and consider combining immunotherapy with cell therapies to provide an unfettered source of SGECs, although preventing continual resurgence of the same epithelial problems will remain a challenge.

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Interferon lambda in inflammation and autoimmune rheumatic diseases

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Abstract | Interferons are potent antiviral cytokines that modulate immunity in response to infection or other danger signals. In addition to their antiviral functions, type I interferons (IFNα and IFNβ) are important in the pathogenesis of autoimmune diseases. Type III interferons (IFNλs) were initially described as a specialized system that inhibits viral replication at epithelial barrier surfaces while limiting inflammatory damage. However, evidence now suggests that type III interferons have complex effects on both innate and adaptive immune responses and might also be pathogenic in systemic autoimmune diseases. Concentrations of IFNλs are increased in blood and tissues in a number of autoimmune rheumatic diseases, including systemic lupus erythematosus, and are further associated with specific clinical and laboratory parameters. This Review is aimed at providing a critical evaluation of the current literature on IFNλ biology and how type III interferons might contribute to immune dysregulation and tissue damage in autoimmunity. The potential effects of type III interferons on treatment strategies for autoimmune rheumatic diseases, such as interferon blockade, are also considered.

Interferons are a group of cytokines that are produced in response to infection or other inflammatory stimuli. Functionally, these cytokines have potent antiviral effects and modulate immune cell function. Interferons are classified into three subgroups: type I interferons (IFNα, IFNβ, IFNε, IFNκ and IFNω), type II interferon (IFNγ) and type III interferons (four IFNλ subtypes). The type III interferons are a relatively new addition to the interferon family and are especially important in immune defence at barrier surfaces[1–3]. Although type III interferons are structurally distinct from type I interferons, they have overlapping functions, and both signal through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway to induce transcription of interferon-stimulated genes (ISGs) and promote antiviral activity.

Interferons are critical for host defence, but can also contribute to disease processes in autoimmune and inflammatory diseases. Indeed, dysregulated type I interferon responses are a major feature of systemic lupus erythematosus (SLE) and a number of other systemic autoimmune diseases[4]. Mutations in genes associated with the type I interferon pathway can also result in monogenic autoinflammatory diseases[5,6]. Chronic activation of the type I interferon system has myriad effects on both innate and adaptive immune responses. For example, type I interferons can modulate antigen-presenting cell (APC) function, promote B cell activation and antibody production, and induce the production of chemokines that lead to tissue inflammation[7]. Given the importance of this pathway, biologic agents that target either IFNα or IFNα receptor (IFNAR), the main receptor for type I interferons, have emerged as a potential therapeutic strategy for systemic rheumatic diseases such as SLE[8]. However, some of these agents have had mixed efficacy in clinical trials, highlighting the complexity and heterogeneity of immune derangement in systemic autoimmunity.

In addition to type I interferons, type III interferons might also contribute to autoimmune and chronic inflammatory diseases. Although type III interferons were initially described as an anti-inflammatory counterpart to the type I interferon system, data suggest that IFNλ biology is more complex than suspected and that excessive and chronic activation of the IFNλ pathway can in fact be detrimental to the host. In this Review, we summarize new insights into IFNλ biology and how type III interferons compare with the type I interferon system. We also discuss potential roles for type III interferons in the immunopathology of systemic rheumatic diseases and explore how this information can be applied to current and future treatment strategies.

IFNλ biology and signalling

Four subtypes of IFNλ have been identified in humans: IFNλ1 (IL-29), IFNλ2 (IL-28A), IFNλ3 (IL-28B) and IFNλ4. Several *IFNL* pseudogenes are located in the vicinity of the genes encoding IFNλs 1–3 (REF.[9]), and a common dinucleotide polymorphism in the *IFNL* locus can result in a frameshift mutation that enables the

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Key points

- Type III interferons (IFNλs) are critical for immune defence against pathogens at epithelial barrier surfaces and were initially described as an anti-inflammatory counterpart to the type I interferon system.
- IFNλs have complex effects on both innate and adaptive immunity and can promote inflammation in certain contexts.
- Similar to type I interferons, type III interferon concentrations are increased in the blood and affected tissues of patients with autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE).
- Concentrations of IFNλs correlate with clinical and immunological parameters and seem to have non-redundant effects on cell-specific and tissue-specific disease processes in SLE.
- Current biologic therapies that target IFNα or its receptor do not block the effects of IFNλs.
- Additional research is needed to fully characterize the context-dependent effects of IFNλs and to optimize treatment for patients with autoimmune rheumatic diseases.

expression of a functional *IFNL4* gene product[10]. In contrast to humans, only IFNλ2 and IFNλ3 are expressed in mice[11].

IFNλs signal through a unique heterodimeric receptor complex comprising IFNλ receptor 1 (IFNLR1) and IL-10 receptor subunit-β[12,13]. An important difference between type I and type III interferons is the expression of their respective receptor complexes. IFNAR is widely expressed on almost all cell types in the body, whereas expression of the IFNλ receptor (IFNLR) is more limited, being highly expressed on epithelial cells and some immune cells, such as neutrophils in mice and B cells in humans[1–3]. This distribution enables the IFNλ system to have specialized effects at barrier sites.

In target cells, the IFNLR complex signals through the JAK–STAT pathway (FIG. 1). IFNα, IFNβ and IFNλs can all activate JAK1 and non-receptor tyrosine-protein kinase TYK2, resulting in the phosphorylation of STAT proteins and the formation of STAT1–STAT2 heterodimers[1–3]. Interferon regulatory factor 9 (IRF9) interacts with these STAT1–STAT2 heterodimers to form the interferon stimulated gene factor 3 (ISGF3) transcription factor complex. ISGF3 then translocates to the nucleus, where it can bind to interferon-stimulated regulatory element sequences located in the promoters of ISGs such as *MX1*, *IFIT1* and *ISG15* (REF.[14]).

Although type I and type III interferons share downstream signalling machinery, some differences exist in the kinetics of different types of interferon responses. Type III interferons induce longer-lasting expression of ISGs at lower amplitude than type I interferons[15,16]. This difference might be caused by differential negative regulation by Ubl carboxyl-terminal hydrolase 18, which preferentially inhibits type I interferon signalling but not type III interferon signalling[17–19]. Nevertheless, the transcriptional profiles induced by type I interferons and type III interferons are remarkably similar, and a unique signature for IFNλs has not been identified. Despite these similarities, studies in IFNLR-deficient (*Ifnlr1*$^{-/-}$) mice indicate that IFNλs have non-redundant functions in immunity and that type III interferons are particularly important for immune responses at mucosal surfaces[20–25].

Current thinking suggests that IFNλs restrict viral replication in epithelial cells without inducing inflammatory pathology[26]. One potential mechanism for the non-inflammatory effects of type III interferons compared with type I interferons is related to chemokine production. IFNβ induces the expression of the chemokines CXCL9, CXCL10 and CXCL11 to a greater extent than IFNλs, owing to insufficient induction of IRF1 by IFNλs[27]. These chemokines can recruit CXCR3^{+} leukocytes and are important in the development of tissue inflammation. IFNβ promotes the formation of STAT1 homodimers that bind to the *IRF1* promoter and induce IRF1 expression (FIG. 1). By contrast, IFNλs do not induce sufficient IRF1 expression to enable the production of chemokines. Notably, IRF1 induction is dependent on the expression of IFNLR1, as overexpression of IFNLR1 increases the amount of CXC chemokines produced in response to IFNλs to similar levels to those elicited by IFNβ. These findings[27] suggest that IFNLR1 density is an important determinant of IFNλ function. As such, IFNλs could theoretically promote inflammation if IFNLR1 expression is sufficiently high to induce IRF1 expression. The way in which IFNLR1 expression is regulated, particularly in autoimmune diseases, might therefore explain the context-dependent effects of IFNλs (discussed in the following sections).

IFNλs can also signal through a variety of non-canonical mechanisms. Data from mouse neutrophils show that IFNλs can activate JAK2 and inhibit reactive oxygen species (ROS) production in a model of intestinal inflammation[28]. This effect was not mediated through traditional STAT1-dependent signal transduction but rather through JAK2-mediated inhibition of RAC-alpha serine/threonine-protein kinase (AKT). Whether this JAK2–AKT pathway is present and operational in other cell types is currently unclear. IFNλs can also activate the mitogen-activated protein kinase pathway[29] and modulate cell–cell tight junctions[30], further highlighting the complexity of their biology.

IFNλs in host immunity

IFNλs have direct effects on epithelial cells, inducing a variety of cell-intrinsic mechanisms that restrict viral replication and inhibit viral transmission[26]. However, evidence indicates that IFNλs have additional functions in orchestrating innate and adaptive immune responses. These functions can be separated into direct effects on IFNλ-responsive cells (TABLE 1) and indirect effects on non-responsive cell types. The effects of IFNλs on different cell types have been reviewed elsewhere[3,31]. In this section, we focus specifically on aspects of the IFNλ response axis that are relevant for inflammation and autoimmunity.

Innate immunity. IFNλs have direct effects on various innate immune cell populations (TABLE 1). Multiple studies report that IFNλs can activate mouse neutrophils to induce STAT1 phosphorylation and ISG expression[25,28,32]. IFNλs can also increase ROS production by mouse neutrophils, and in vivo experiments show that mice with neutrophil-specific deletion of *Ifnlr1* are more susceptible to *Aspergillus* infection[33], indicating that IFNλs

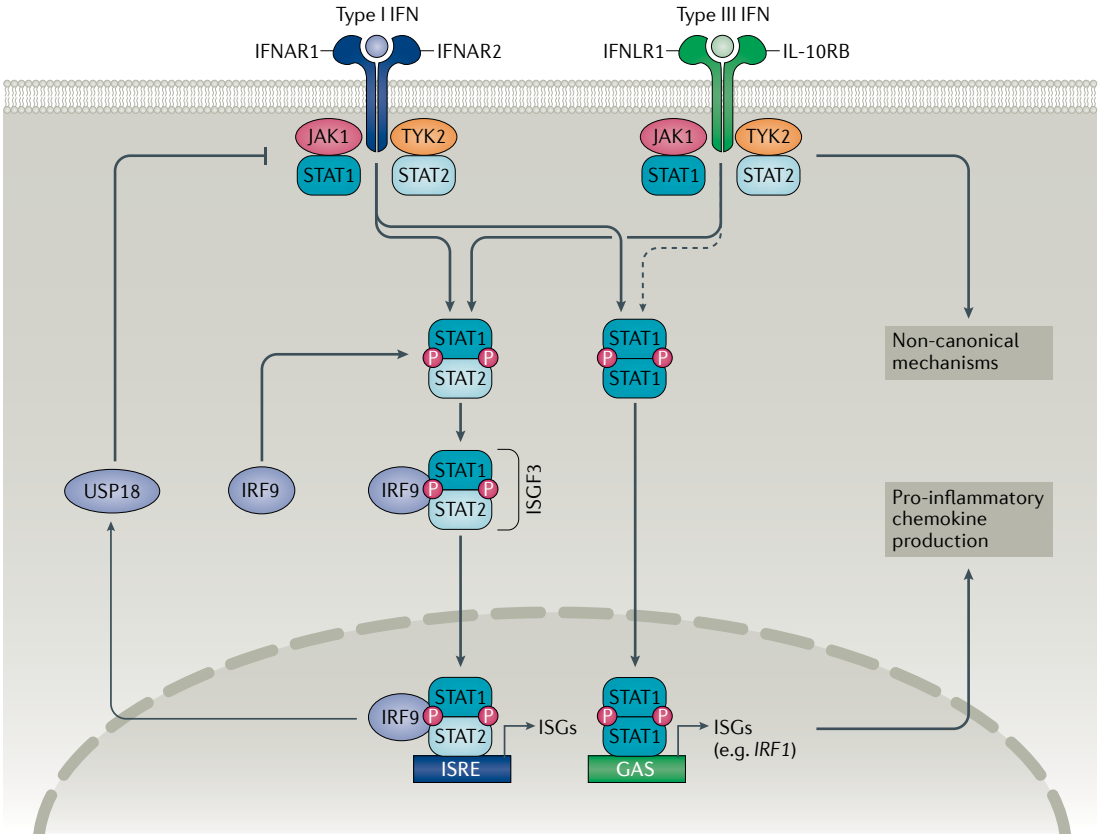

Fig. 1 | **Type I and type III interferon signalling pathways.** Type I and type III interferons can activate both Janus kinase 1 (JAK1) and non-receptor tyrosine-protein kinase TYK2 (TYK2), leading to signal transducer and activator of transcription (STAT) phosphorylation and the formation of STAT1–STAT2 heterodimers. These heterodimers can interact with interferon regulatory factor 9 (IRF9) to form the interferon stimulated gene factor 3 (ISGF3) transcription factor complex. ISGF3 translocates to the nucleus, where it can bind to interferon-stimulated regulatory element (ISRE) sequences and promote the expression of interferon-stimulated genes (ISGs). Type III interferons comparatively induce lower amplitude expression of ISGs over a longer period of time than type I interferons, possibly owing to differential negative regulation by Ubl carboxyl-terminal hydrolase 18 (USP18). Type I and type III interferons can also promote the formation of STAT1 homodimers, which upregulate IRF1 expression and lead to pro-inflammatory chemokine production. IFNλ can also signal through a variety of non-canonical mechanisms. GAS, IFNγ-activated sequence; IFN, interferon; IFNAR, IFNα receptor; IFNLR1, IFNλ receptor 1; IL-10RB, IL-10 receptor subunit-β.

regulate antifungal immunity through specific effects on neutrophil function. By contrast, IFNλs can inhibit ROS production and degranulation in mouse neutrophils during intestinal inflammation through a non-translational, STAT1-independent pathway[28]. Whether human neutrophils are similarly responsive to IFNλs is unclear. Human neutrophils express IFNLR1 and upregulate it in response to inflammatory stimuli such as lipopolysaccharide or fungal infection[33]. IFNλs can also inhibit TNF-induced ROS production in human neutrophils[28] and suppress neutrophil extracellular trap (NET) formation in response to activated platelets or platelet-derived inorganic polyphosphate[34]. These data are somewhat contradicted by reports that IFNλs do not induce ISG expression in human peripheral blood neutrophils[35,36], leading to uncertainty about whether and how these cells respond to IFNλs in different settings.

Dendritic cell (DC) subsets are also an important part of the IFNλ response network. IFNλs can increase type I interferon and chemokine production by human plasmacytoid DCs (pDCs)[37–39] and can upregulate the expression of class I and II MHC molecules and co-stimulatory molecules on pDCs, which could promote T cell activation[37,39]. By contrast, IFNλs seem to induce a more regulatory phenotype in human monocyte-derived DCs, which promote the expansion of FOXP3$^+$ regulatory T cells[40]. Other reports suggest that IFNλs are involved in T cell polarization in vitro[41] and that they can skew T cells towards a T helper 1 cell response in a mouse model of allergic asthma through effects on lung DCs[41,42]. Despite the progress made by these studies, the effects of IFNλs on DC function have not been fully characterized, and it is probable that only certain DC subsets respond directly to this cytokine.

In addition, IFNλs can activate human monocyte-derived macrophages and promote a pro-inflammatory phenotype, leading to chemokine production and the upregulation of pathways related to antigen presentation, co-stimulation, phagocytosis and cytotoxicity[43–46]. By contrast, natural killer cells do not seem to respond to IFNλs directly[35,36,43,47,48]; however, IFNλs can modulate natural killer cell function indirectly through their effects on macrophages[43,48,49].

Table 1 | **Direct effects of IFNλs on immune cell populations**

Species	Neutrophils	Macrophages	Dendritic cells	NK cells	B cells	T cells	Refs
Mouse	Increased ISG expression; ROS production can increase or decrease; decreased migration and IL-1β production	Increased ISG expression; increased stimulation of NK cell proliferation; some studies report no effects	Increased stimulation of T helper 1 cell polarization; increased stimulation of CD8⁺ T cell responses; increased ISG expression (pDCs and BMDCs); increased antigen presentation and co-stimulatory molecule expression (BMDCs); some studies report no effects	No effects	No effects	No effects	25,28,32,33,35, 42,47,49–52,58,150
Human	Increased IFNLR1 expression on activated neutrophils; decreased ROS production; decreased NET formation; some studies report no effects	Increased ISG expression; increased antigen presentation and co-stimulatory molecule expression; increased cytokine and chemokine production; increased phagocytosis and cytotoxicity; increased activation of NK cells	Increased ISG expression (pDCs); increased cytokine and chemokine expression (pDCs); increased antigen presentation and co-stimulatory molecule expression (pDCs and moDCs) increased migration (moDCs); increased stimulation of regulatory T cell proliferation (moDCs); some studies report no effects in moDCs	No effects	Increased ISG expression; increased TLR7-mediated antibody production; increased plasmablast differentiation	Increased ISG expression (CD8⁺ T cells and activated CD4⁺ T cells); increased IFNLR1 expression (activated CD4⁺ T cells); some studies report no effects	28,33–40,43–46, 48,53–55,57

BMDC, bone marrow-derived dendritic cell; IFNLR1, IFNλ receptor 1; ISG, interferon-stimulated gene; moDC, monocyte-derived dendritic cell; NET, neutrophil extracellular trap; NK, natural killer; pDC, plasmacytoid dendritic cell; ROS, reactive oxygen species; TLR7, Toll-like receptor 7.

Adaptive immunity. IFNλs also have direct effects on some adaptive immune cells (TABLE 1). Although IFNλs do not seem to affect mouse B cells and T cells[35,50–52], data indicate that human lymphocytes can respond to IFNλs. The reasons behind the discrepancies between mouse and human responses remain unclear. Human B cells express IFNLR, and stimulation with IFNλs promotes ISG expression in these cells[35,36,53]. Moreover, IFNλs increase Toll-like receptor 7 (TLR7)-mediated and TLR8-mediated antibody production and plasmablast differentiation in human B cells[54,55]. Pre-treatment with IFNλs can also inhibit influenza-induced IgG production in human peripheral blood mononuclear cells (PBMCs)[56]. However, it is worth noting that this inhibitory effect was observed in a mixed cell population and might result from decreased production of T helper 2 cell cytokines rather than from a direct effect on B cell function. This idea is consistent with other reports that IFNλs promote T helper 1 cell skewing via effects on DCs[41,42].

The effects of IFNλs on human T cells are less obvious than the effects on B cells. Several reports indicate that human T cells do not express IFNLR1 and are not responsive to IFNλ stimulation[35,53,57]. By contrast, a 2020 study has indicated that CD8⁺ T cells can respond to IFNλs and upregulate ISGs[36]. Activation of T cells with anti-CD3 and anti-CD28 antibodies also upregulated IFNLR1 on CD4⁺ T cells, allowing the induction of ISGs by IFNλs[36]. Therefore, T cells could potentially acquire responsiveness to IFNλs in the context of antigen-specific immune responses.

In addition to direct effects, IFNλs also coordinate adaptive immunity through indirect mechanisms. *Ifnlr1*$^{-/-}$ mice have decreased antibody and CD8⁺ T cell responses following infection with influenza virus[51]. This effect is dependent on thymic stromal lymphopoietin (TSLP) production by microfold cells in the upper airway. IFNλs induce TSLP production in these cells, leading to CD103⁺ DC migration to the draining lymph nodes. These CD103⁺ DCs subsequently promote follicular helper T cell expansion and germinal centre responses in the lymph node, thereby generating a robust adaptive immune response. Whether this IFNλ-induced TSLP-mediated mechanism is specific to the respiratory tract and is also present in humans, or whether it can boost adaptive immune responses against self-antigens, remains unclear. A separate study further demonstrated that IFNλs are required for APC migration to the draining lymph nodes and are critical for the development of effective antiviral CD8⁺ T cell responses during influenza infection in mice[58], highlighting another mechanism through which IFNλs can potentiate adaptive immune responses.

In summary, these data suggest that IFNλs can modulate immune responses through a variety of direct and indirect pathways. Although these mechanisms have primarily been identified and studied in response to infection, they might also be relevant for autoimmunity. Considerable differences in the IFNλ response also exist between mouse and human cells. These differences will be important to consider when evaluating IFNλs in the context of human diseases.

IFNλs in autoimmune rheumatic diseases

Concentrations of IFNλs are increased in blood and affected tissues in a number of autoimmune rheumatic diseases, including SLE, rheumatoid arthritis (RA), primary Sjögren syndrome (pSS) and systemic sclerosis (SSc). Increased amounts of IFNλs are also associated with increased disease severity, increased

autoantibodies, increased inflammatory markers and/or specific manifestations in these diseases (TABLE 2). In this section, we summarize the main findings in these diseases and discuss potential mechanisms of immune dysregulation.

Systemic and cutaneous lupus erythematosus. SLE is a complex autoimmune disease that can affect multiple organ systems, including the skin, kidneys, joints and vasculature. The role of type I interferons is well established in SLE pathogenesis, and many patients with SLE display a characteristic type I interferon signature in blood and affected tissues[59–61]. Functionally, type I interferons lead to the aberrant activation of immune cells[62] by promoting autoantibody production and immune complex formation that result in tissue damage. Type I interferons can also prime neutrophils, modify APC activity and regulate T cell function to further promote autoimmune tissue damage in SLE.

In addition to type I interferons, evidence suggests that type III interferons are dysregulated in SLE. Several studies have reported that serum IFNλ1 and IFNλ3 concentrations are increased in patients with SLE compared with healthy individuals[63–71]. *IFNL1* transcripts are increased in PBMCs and *IFNL2* and *IFNL3* transcripts are increased in activated $CD4^+$ T cells from patients with SLE relative to those from healthy individuals[63,72]. Moreover, increased serum concentrations of IFNλs are associated with disease severity and clinical laboratory values. Specifically, higher serum concentrations of IFNλ correlate with higher SLE Disease Activity Index scores[63–66], higher anti-double-stranded DNA (dsDNA) autoantibody titres[63,64] and lower amounts of complement proteins C3 and C4 (REFS[63–66]). Increased circulating concentrations of IFNλs are also associated with the presence of specific disease manifestations in SLE, including arthritis, nephritis, serositis and skin involvement[63,65,71]. Genetic studies further implicate IFNλs in SLE pathogenesis. *IFNL3* and *IFNL4* variants are risk factors for lupus nephritis among patients with SLE in a Taiwanese cohort[66], and the rs4649203 single nucleotide polymorphism in *IFNLR1* is associated with an increased risk of SLE in a Chinese Han population[73].

IFNλs have also been detected in affected tissues in patients with SLE. Immunohistochemistry analysis of skin tissue showed that IFNλs and IFNLR1 are substantially increased in patients with chronic discoid lupus erythematosus or subacute cutaneous lupus erythematosus (CLE) relative to healthy individuals or patients with other inflammatory skin diseases (such as atopic dermatitis or psoriasis)[69]. The detection of IFNλs in the skin of patients with CLE is most prominent in the epidermis, with some additional staining of mononuclear cells in the dermis. Patients with CLE also have increased serum IFNλ1 concentrations, particularly in those patients with disseminated lesions compared with those with more localized disease, and a case report from a single patient found that serum IFNλ1 concentrations declined during clinical remission following treatment with glucocorticoids and hydroxychloroquine[69]. In addition to skin, IFNλs and IFNLR1 are also detectable in kidney tissue from patients with lupus nephritis[66,70]; IFNλs were mostly observed in glomerular crescents and areas with inflammatory infiltrates, and glomerular IFNλ staining decreased in repeat biopsy-retrieved samples from patients who achieved a histological response to treatment[70]. However, these studies did not include kidney tissue samples from healthy individuals or disease-matched controls. Overall, these findings indicate that IFNλs might be involved in SLE-associated skin and kidney disease.

Data from mouse models support a mechanistic role for IFNλs in SLE. One study investigated the effects of IFNλs in a TLR7-induced lupus model, whereby mice are repeatedly exposed to the TLR7 agonist imiquimod. Serum concentrations of IFNλ2 and IFNλ3 were increased in imiquimod-treated mice, and IFNLR1 deficiency substantially reduced splenomegaly and leucocytosis compared with wild-type controls[35]. *Ifnlr1*$^{-/-}$ mice were fully responsive to IFNα, suggesting that IFNλs

Table 2 | **IFNλs in autoimmune rheumatic diseases**

Disease	Expression in blood	Expression in tissue	Disease activity	Antibodies	Inflammatory markers	Associated disease manifestations	Refs
SLE	Increased IFNλ1 and IFNλ3; increased *IFNL1* mRNA (PBMCs); increased *IFNL2* and *INFL3* mRNA ($CD4^+$ T cells)	Increased IFNλs in skin and kidneys	Not associated with SLAM; contradictory results for SLEDAI and SDI	Associated with anti-nucleosome antibodies; not associated with ANAs; contradictory results for anti-dsDNA antibodies	Associated with a decrease in complement proteins C3 and C4; not associated with ESR; contradictory results for CRP	Arthritis, nephritis, serositis and skin involvement	63–72
Rheumatoid arthritis	Increased IFNλ1 and IFNλ2; increased *IFNL1* mRNA (PBMCs)	Increased IFNλ1 in synovial fluid	Contradictory results for DAS28	Associated with anti-MCV antibodies; contradictory results for RF and ACPAs	No association with CRP or ESR	Knee joint involvement	86–89
Primary Sjögren syndrome	Increased IFNλ1	Increased IFNλ1 in minor salivary glands	ND	ND	ND	Exocrine gland involvement	94,95
Systemic sclerosis	Increased IFNλ1 and IFNλ3	ND	ND	ND	ND	Myositis and pulmonary fibrosis	96,97

ACPA, anti-citrullinated protein antibody; ANA, antinuclear antibody; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; dsDNA, double-stranded DNA; ESR, erythrocyte sedimentation rate; MCV, mutated citrullinated vimentin; ND, not determined; PBMC, peripheral blood mononuclear cell; RF, rheumatoid factor; SDI, SLICC Damage Index; SLAM, Systemic Lupus Activity Measure; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

have important and non-redundant functions in systemic immune dysregulation. Further investigation of splenic immune cell populations revealed that IFNλs promote myeloid cell expansion and T cell activation following in vivo TLR7 stimulation, potentially through a combination of direct and indirect effects on these cells. By contrast, IFNλs did not modulate B cell responses in TLR7-induced lupus, as the number of plasma cells and levels of B cell activation markers did not differ between *Ifnlr1*$^{-/-}$ mice treated with imiquimod and wild-type controls. IFNLR1 deficiency also had no effect on the amounts of circulating antinuclear antibodies or anti-dsDNA autoantibodies. These results[35] are somewhat contradictory to data in humans, which indicate that increased concentrations of IFNλs correlate with higher levels of autoantibodies in SLE[63,64,67,68]. This discrepancy could potentially be related to differences in B cell responsiveness to IFNλs between mice and humans. Mouse B cells are unresponsive to IFNλs, whereas human B cells can respond directly to this cytokine by increasing TLR7-mediated antibody production and plasmablast differentiation[54,55]. Therefore, additional research is needed to determine how IFNλ affects autoantibody production in the context of human SLE.

Mouse models of lupus also support a role for IFNλs in the pathogenesis of skin and kidney manifestations in SLE. *Ifnlr1*$^{-/-}$ mice had substantially reduced skin inflammation in the TLR7-induced lupus model[35]. This decrease in skin inflammation corresponded with a decrease in tissue expression of pro-inflammatory genes such as *Il6*, *Cxcl9* and *Cxcl10*. In vitro experiments show that mouse and human keratinocytes respond to IFNλs and can upregulate CXCL9, CXCL10 and CXCL11 chemokines[35]. Moreover, culture supernatants from IFNλ-stimulated human keratinocytes can induce the in vitro migration of mononuclear immune cells[35,69]. These data suggest that IFNλs might, at least in part, promote SLE-associated skin disease by increasing pro-inflammatory chemokine production in keratinocytes (FIG. 2). Notably, co-treatment of keratinocytes with IFNα and IFNλ1 induced greater chemokine expression than either cytokine alone[35], indicating that type I and type III interferons could have an additive effect in promoting skin inflammation. IFNλs also increase the expression of MHC class I molecules by human keratinocytes, which can in turn promote pathogenic CD8^{+} T cell responses[74]. In the same TLR7-induced lupus model, *Ifnlr1*$^{-/-}$ mice also had decreased immune complex deposition, glomerulosclerosis and ISG expression in the kidneys[35]. IFNλs were able to induce ISGs and chemokine production in mouse mesangial cells, suggesting that IFNλs could have an important effect on structural cells in the kidney. Other kidney cells, in particular those of epithelial origin, can also potentially respond to IFNλs[75]. Further analysis is required to identify and characterize how IFNλs can affect other tissues, such as the lung, brain and joints, that are commonly involved in SLE; however, unlike type I interferons, type III interferons do not seem to have any effects on vascular disease in mice, as IFNLR1 deficiency did not improve endothelium-dependent vasorelaxation in the TLR7-induced lupus model[35].

Interferon production in SLE occurs through a variety of mechanisms involving nucleic acids, immune complexes and the engagement of various intracellular sensors (FIG. 2). pDCs are a major source of type I interferons in SLE[76] and are also involved in IFNλ production. These cells accumulate in the skin of mice with lupus and produce IFNλs in response to TLR7 agonists[35]. In humans, RNA-containing immune complexes can induce the production of type III interferons in a subset of pDCs that also produce type I interferons[77]. The production of IFNλs by pDCs in vitro was attenuated in the presence of hydroxychloroquine or an IL-1 receptor associated kinase 4 (IRAK4) inhibitor, indicating that RNA-containing immune complexes induce the production of IFNλs through the endosomal TLR–myeloid differentiation primary response protein (MyD88) system. Additional research is needed to determine whether other immune stimuli that trigger type I interferon production, such as NETs[78,79], can also contribute to the production of IFNλs in SLE. In addition to pDCs, keratinocytes seem to be a potential source of IFNλs in the skin. Epidermal explants and cultured human keratinocytes produce considerable amounts of IFNλs in response to synthetic TLR3 agonists[69]. A follow-up study demonstrated that endogenous nucleic acids isolated from keratinocytes, in combination with the cathelicidin LL-37, were able to induce the production of IFNλs[80]. These results are consistent with the finding that keratinocyte cell death and increased amounts of nuclear debris perpetuate inflammation in SLE skin lesions[81] (FIG. 2). Keratinocytes can also upregulate *IFNL* transcripts after stimulation with IFNα[35], highlighting another potential feed-forward pro-inflammatory loop whereby type I interferon amplifies the type III interferon pathway in skin. Overall, current data indicate that IFNλs are potentially pathogenic in SLE, causing cell-specific and tissue-specific effects.

Rheumatoid arthritis. RA is a systemic autoimmune disease that leads to chronic inflammation, cartilage damage and bone erosion in synovial joints. Pro-inflammatory cytokines such as TNF and IL-6 are important in the pathogenesis of RA[82]; however, blocking these cytokines is not effective in all patients with RA, suggesting that additional pathways are involved. Similar to SLE, a subset of patients with RA display a type I interferon signature in blood[83]. pDCs and type I interferons have also been detected in RA synovium[84,85], further indicating that interferons might contribute to RA immunopathology.

Notably, IFNλs are also upregulated in RA. IFNλ1 is substantially increased in serum from patients with RA compared with serum from healthy individuals or patients with ankylosing spondylitis[86–89]. *IFNL1* transcripts are also increased in PBMCs from patients with RA and, in addition, IFNλ1 is increased in synovial fluid from patients with RA compared with synovial fluid from patients with osteoarthritis[88]. Despite there being increased amounts of IFNλs in blood and synovial fluid in RA, data on associations between IFNλs and clinical features in RA are mixed. Several studies have reported no correlations between serum IFNλ1

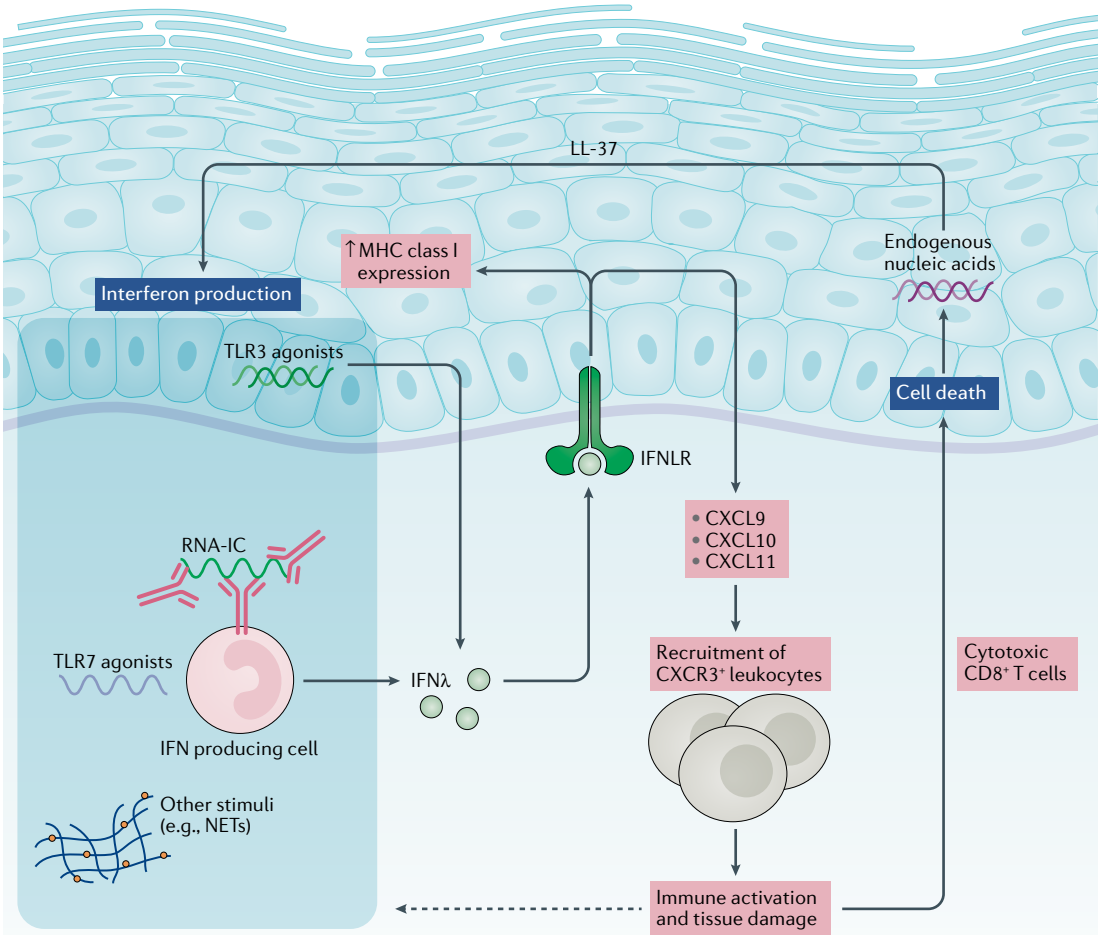

Fig. 2 | **IFNλs in skin disease in systemic lupus erythematosus.** Danger signals, including Toll-like receptor 7 (TLR7) agonists and RNA-containing immune complexes (RNA-IC), can induce the production of IFNλs by plasmacytoid dendritic cells. TLR3 agonists can also induce the production of IFNλs by keratinocytes. IFNλs can subsequently activate keratinocytes to upregulate the expression of the chemokines CXCL9, CXCL10 and CXCL11, as well as surface MHC class I molecules. These chemokines recruit CXCR3$^+$ leukocytes to the skin, where they promote tissue damage; in particular, cytotoxic CD8$^+$ T cells can cause keratinocyte cell death. The release of endogenous nucleic acids (in combination with the cathelicidin LL-37 in experimental models) can induce further production of IFNλs, resulting in a feed-forward loop that perpetuates inflammation in the skin. Whether inflammatory stimuli can also upregulate IFNλ receptor (IFNLR) expression on keratinocytes is unclear. NET, neutrophil extracellular trap.

concentrations and clinical parameters, including the 28-joint Disease Activity Score (DAS28), circulating inflammatory markers (such as C-reactive protein) or RA-associated autoantibodies (rheumatoid factor and anti-citrullinated protein antibodies)[86,88]. Although IFNλ1 was not associated with the presence of autoantibodies, it was associated with knee joint involvement[86]. By contrast, a separate study reported that serum IFNλ1 concentrations correlated with the presence of RA-associated autoantibodies, and also correlated with worse DAS28 scores in patients positive for anti-citrullinated protein antibodies[89]. Moreover, IFNλ1 concentrations decrease following 6 months of treatment with DMARDs[89]. IFNλ1 concentrations have also been associated with the presence of anti-mutated citrullinated vimentin autoantibodies, and IFNλ2 concentrations are only increased in patients with active RA (defined by a DAS28 score of >2.6)[87], suggesting that each IFNλ might contribute to specific disease processes. Further assessment of whether this cytokine modulates human B cell function and autoantibody production in RA will be important.

In the synovium, IFNλ1 co-localizes with CD68$^+$ cells and FGF2$^+$ cells[88], suggesting that macrophages and synovial fibroblasts might be relevant sources of IFNλs in RA. In vitro experiments also indicate that synovial fibroblasts can respond to IFNλs. A human RA synovial fibroblast cell line expresses IFNLR1, and stimulation of these cells with recombinant IFNλ1 upregulates *IL6*, *IL8* and *MMP3* expression[88]. IFNλs also upregulate the expression of TLRs 2, 3 and 4 in the same synovial fibroblast cell line, thereby amplifying TLR-mediated IL-6 and IL-8 production[90]. These results suggest that IFNλs might promote joint inflammation and damage in RA.

Other data indicate that IFNλs actually have the opposite effect and are protective against inflammatory arthritis. In one study, treatment with recombinant IFNλ2 suppressed neutrophil infiltration and IL-1β production in mice with collagen-induced arthritis[32]. Another study showed that IFNλ1 can inhibit osteoclast

formation in vitro[91], suggesting that IFNλ1 might be protective against bone erosion in RA. Overall, it is still unclear if IFNλs are pathogenic in RA, and further research is needed to better characterize how IFNλs could contribute to immune dysregulation and joint damage in this disease. Notably, variability in responses to type III interferons by human and mouse cells could complicate interpretation of data in the context of animal models of RA in future studies.

Other autoimmune rheumatic diseases. pSS is a systemic autoimmune condition that targets exocrine glands, resulting in a dry mouth, dry eyes and several systemic manifestations. pSS is characterized by an exaggerated type I interferon response in blood and affected glands[92,93], and evidence indicates that IFNλs might also contribute to the immunopathology of pSS. Immunohistochemistry analysis of minor salivary glands has demonstrated that expression of IFNλs is increased in tissue from patients with pSS compared with tissue from individuals with non-pSS sicca symptoms[94,95]. Serum IFNλ1 concentrations are similarly increased in patients with pSS[94].

Salivary gland epithelial cells might contribute to both the production of IFNλs and the IFNλ response in pSS. TLR3 agonists induce the production of IFNλs by salivary gland epithelial cells, which in turn upregulates *CXCL10* and *BAFF* (which encodes B cell activating factor (BAFF)) expression in these cells[94,95]. Co-treatment with IFNα and IFNλ1 can further enhance STAT1 phosphorylation and cytokine expression in salivary gland epithelial cells, suggesting that type I and type III interferons could have combined effects in pSS. BAFF is a known pathogenic factor in pSS and promotes B cell hyperactivity and autoantibody production[92]. On the basis of these findings, it will be important to further investigate the potential link between IFNλs and aberrant B cell responses in pSS.

IFNλs also have potential effects in SSc, an autoimmune disease characterized by vasculopathy and widespread fibrosis in the skin, lungs and other organs. The amount of IFNλ1 is increased in the serum of patients with SSc (both diffuse and limited cutaneous subtypes) compared with healthy controls[96]. Concentrations of IFNλs are highest in patients with SSc who have muscle involvement and correlate positively with concentrations of IFNγ, suggesting that IFNλs might interact with other cytokine networks to amplify pathogenicity in SSc. Notably, the rs12979860 variant of *IFNL3* is associated with an increased risk of pulmonary fibrosis in SSc[97], whereas no associations have been reported between this variant and skin fibrosis. Serum IFNλ3 concentrations are also higher in patients with SSc who have pulmonary fibrosis than in those patients with SSc who do not develop this complication, and *Ifnl3* transcripts are increased in lung tissue in a mouse model of pulmonary fibrosis[97]. However, additional research is needed to identify the cellular targets and pathways responsible for the pro-fibrotic effects of IFNλs in SSc.

Preliminary evidence also exists suggesting that IFNλ expression is dysregulated in other autoimmune and inflammatory diseases. The expression of IFNλs is increased in skin samples from patients with dermatomyositis[69]. By contrast, expression of IFNλ1 is decreased in the ocular fluid of patients with juvenile idiopathic arthritis-associated uveitis[98]. No further investigation has been carried out into how IFNλs might relate to pathogenesis, disease severity or other immunological parameters in these diseases. IFNλs have also been implicated in psoriasis and inflammatory bowel disease[99,100], which are beyond the scope of this Review, and it remains unclear if IFNλs are involved in seronegative spondyloarthritis, which is associated with these conditions.

Are IFNλs protective or harmful?

IFNλs seem to have considerable pro-inflammatory and anti-inflammatory effects that are highly context dependent. As discussed in previous sections, concentrations of IFNλs are increased and could have pathogenic effects in autoimmune diseases such as SLE. Data obtained during the COVID-19 pandemic also indicate that persistent IFNλ signalling can disrupt epithelial barrier function in the lungs and predispose individuals to bacterial superinfection[101,102], further highlighting the possibility of IFNλ-mediated tissue damage. By contrast, compelling data suggest that IFNλs can be protective against inflammation by regulating neutrophil function in mouse models of arthritis, colitis and thromboinflammation[28,32,34], as well as promoting mucosal healing in the gastrointestinal tract[100].

One explanation for these seemingly contradictory effects of IFNλs is the expression level of IFNLR. As discussed in a previous section, IFNLR density on epithelial cells seems to regulate the pro-inflammatory effects of IFNλs. Specifically, cells expressing high amounts of IFNLR1 are able to induce sufficient IRF1 expression to produce pro-inflammatory chemokines such as CXCL10 (REF.[27]). Therefore, it is possible that local or systemic inflammatory processes in SLE and other autoimmune rheumatic diseases can increase IFNLR1 expression above the threshold necessary for IRF1 induction. For example, IFNLR1 staining is increased in the epidermis of patients with CLE[69], and *Ifnlr1* expression is substantially upregulated in the skin of mice with TLR7-induced lupus compared with healthy controls[35] (FIG. 2). Data from primary human hepatocytes suggest that IFNα can upregulate *IFNLR1* expression and that this effect is dependent on the *IFNL3* genotype of the cells[103]. Such interactions between type I and type III interferons could also be important in autoimmune rheumatic diseases such as SLE. Additional research is needed to better understand how IFNLR is expressed and regulated in autoimmunity.

Another possibility is that there are cell-specific and tissue-specific differences in IFNLR1 expression, both during homeostasis and in the context of inflammatory pathology. These differences might explain why concentrations of IFNλs correlate with clinical phenotypes in some tissues (such as the skin and kidneys), but not in others. At present, limited data exist on how individual cell types in a tissue respond to IFNλs. Single-cell and spatial transcriptomic analyses will enable better characterization of type III interferon responses in various tissues.

Such approaches will help investigators to identify IFNλ-responsive cell types from bulk samples, rather than having to sort individual cell types or use genetically engineered models. Similarly, single-cell approaches will also help researchers to investigate the levels of sensitivity and/or distinct patterns of transcriptional responses to IFNλs in individual cell types or in cells at different stages of differentiation. For example, human neutrophils might gain responsiveness to IFNλs under certain conditions, such as fungal infection[33]. Although no differences were detected in IFNλ responses between neutrophils from patients with SLE and those from healthy individuals in peripheral blood[35], it will be necessary to study leukocyte responses in situ, as these cells could be regulated by local environmental factors in inflamed tissues.

Additionally, IFNλ subtypes could potentially have different immunoregulatory functions. Concentrations of IFNλs 1–3 are all increased in patients with autoimmune rheumatic diseases (TABLE 2); however, mechanistic studies have largely focused on genetic deletion of *Ifnlr1*, which abrogates signalling by all IFNλ subtypes. Although there are currently insufficient data to define mechanisms for potential differences between IFNλ subtypes, one possible explanation is their different affinities for IFNLR[104]. Specifically, IFNλ1 seems to bind to IFNLR with the highest affinity of the IFNλ subtypes, which could generate differences in signalling output, leading to distinct biological potencies of IFNλs[105,106]. Differential kinetics and magnitudes of IFNλ subtype induction, different stability, bioavailability and tissue distribution of the proteins, and different sensitivity to negative regulators might also result in distinct activities of IFNλ subtypes.

More broadly, IFNλs could have additional roles in immune homeostasis via effects on central tolerance and T cell education in the thymus[107]. IFNλs are constitutively expressed in medullary thymic epithelial cells and promote MHC class I molecule expression in thymic epithelial cells. IFNλ-induced MHC class I expression seems to be crucial for effective T cell selection, as *Ifnlr1*$^{-/-}$ mice have impaired negative selection of T cells[107]. Functionally, this lack of negative selection results in *Ifnlr1*$^{-/-}$ mice developing spontaneous autoimmune manifestations such as immune cell infiltration in the lung and kidneys. *Ifnlr1*$^{-/-}$ mice also have increased amounts of total IgG antibodies and develop some tissue-reactive autoantibodies[107]. Together, these data indicate that IFNλs have myriad effects in different health and disease states.

Implications for treatment

Interferons are important factors underlying the immunopathogenesis of autoimmune rheumatic diseases. Accordingly, the interferon pathway has been an attractive therapeutic target, and several drug candidates (both interferons themselves and interferon-inhibiting therapies) are currently under investigation for SLE and other diseases. Pegylated-IFNλ has been studied as a novel treatment for viral hepatitis and is being tested for COVID-19 (REFS[108–110]). Data from mouse models also show that recombinant IFNλ1 suppresses joint inflammation in mice with collagen-induced arthritis by inhibiting neutrophil recruitment[32]. Accordingly, IFNλ1 has been proposed as a potential treatment for controlling neutrophil-mediated pathology in rheumatic diseases. However, it is still unclear if IFNλ1 has similar effects on human neutrophils to its effects on mouse neutrophils, and it is important to consider off-target effects of IFNλs that might actually worsen autoimmune disease.

The interferon-inhibiting therapies can be categorized into three main groups: drugs that target interferons (both the cytokine and the receptor); drugs that inhibit downstream JAK–STAT signalling; and drugs that inhibit interferon production. Current therapies that target interferons and their receptors only block the type I interferon pathway, whereas therapies that target JAK–STAT signalling components or interferon production can block the effects of both type I and type III interferons (FIG. 3, TABLE 3).

Targeting interferons. Several neutralizing antibodies that recognize IFNα, including sifalimumab and rontalizumab, have been tested in clinical trials for SLE. A phase II trial of sifalimumab met its primary end point for efficacy of an SLE Responder Index (SRI) response in patients with moderate-to-severe active SLE[111]. In addition to producing an SRI response, sifalimumab was moderately effective at reducing tissue-specific disease activity in the skin and joints. By contrast, a phase II trial of rontalizumab failed to meet its primary and secondary end points for reducing disease activity in patients with SLE[112]. The development programmes for both sifalimumab and rontalizumab have since been discontinued, and these therapies are no longer being developed for SLE[113]. Sifalimumab was also tested in a phase I trial for dermatomyositis and polymyositis, in which it suppressed the interferon gene signature in blood and had some effects in muscle tissue[114,115].

An alternative method for blocking IFNα using IFNα kinoid (IFN-K) has also been tested in phase II clinical trials in SLE. In this approach, inactivated IFNα is conjugated to a carrier protein and combined with an adjuvant to induce the production of endogenous anti-IFNα antibodies. Notably, this vaccine-like preparation induces polyclonal antibodies that might be more effective at neutralizing all IFNα subtypes than monoclonal antibodies. Immunization with IFN-K significantly reduced the interferon gene score and met secondary end points for clinical efficacy in patients with SLE[116–118]. However, inducing long-term immunity against interferons could increase the risk of infection[119] and the safety profile of IFN-K merits further study.

Overall, antibodies that target IFNα seem to have had mixed efficacy for rheumatic diseases. Because these antibodies specifically target IFNα, it is possible that other type I interferons (such as IFNβ or IFNκ) are still able to bind to the type I interferon receptor without interruption. Moreover, these antibodies have no effect on type III interferons. Of note, a soluble glycoprotein encoded by Yaba-like disease virus can effectively neutralize all human type I and type III interferons[120], demonstrating the possibility of developing a

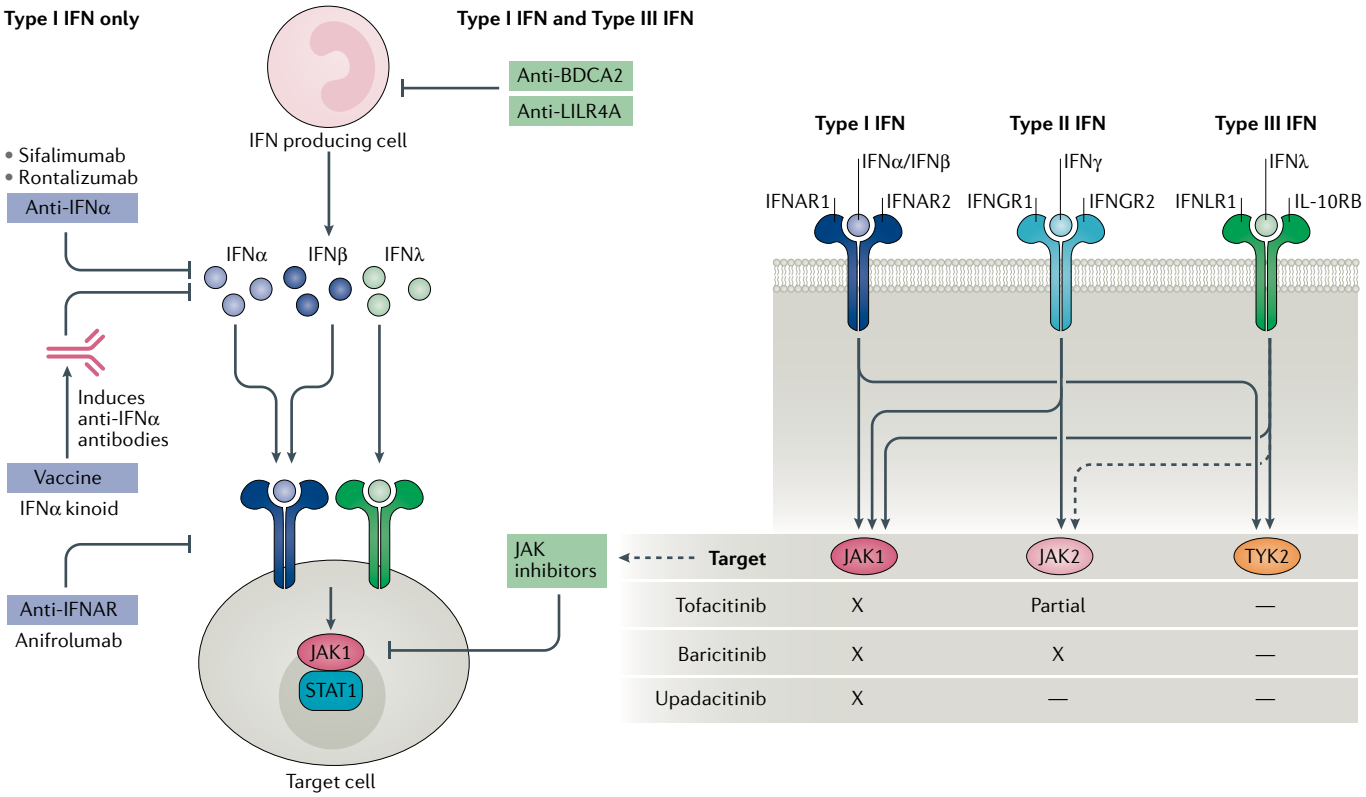

Fig. 3 | **Interferon blockade for autoimmune rheumatic diseases.** Biologic agents that target IFNα or IFNα receptor (IFNAR) can block the effects of type I interferons but have no effect on type III interferons. Drugs targeting interferon production by plasmacytoid dendritic cells (such as anti-BDCA2 or anti-LILR4A antibodies), or downstream Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling in target cells (such as JAK inhibitors), can block both type I and type III interferons. BDCA2, blood dendritic cell antigen 2; IFN, interferon; IFNGR1, IFNγ receptor 1; IFNGR2, IFNγ receptor 2; IFNLR1, IFNλ receptor 1; IL-10RB, IL-10 receptor subunit-β; LILR4A, leukocyte immunoglobulin-like receptor subfamily A member 4; TYK2, non-receptor tyrosine-protein kinase TYK2.

pan-interferon antagonist without also targeting signalling components shared by other cytokines (such as occurs with JAK inhibitors). Further research is needed to evaluate the safety and efficacy of this approach.

Targeting interferon receptors. Anifrolumab, a monoclonal antibody that targets IFNAR, is also being investigated in SLE. Data from phase III trials are encouraging, although results are somewhat conflicting. Anifrolumab failed to meet its primary end point of an SRI response in the TULIP-1 trial[121]. However, anifrolumab significantly reduced disease activity, as measured by a primary BILAG-Based Composite Lupus Assessment response, in the subsequent TULIP-2 trial[122]. Anifrolumab also reduced glucocorticoid use and improved skin disease in TULIP-2. Further analysis from a phase IIb trial showed that anifrolumab could reduce markers of cardiometabolic dysfunction in patients with SLE, suggesting that it might have additional benefit in SLE vasculopathy[123]. Anifrolumab is also being evaluated in a phase II trial for patients with active proliferative lupus nephritis[124].

In addition to SLE, anifrolumab is also being investigated in other autoimmune rheumatic diseases. Anifrolumab is currently being tested in a phase IIa proof-of-concept trial for patients with moderate-to-severe RA who have an increased interferon gene signature and who have not responded to other biologic DMARDs[125]. Anifrolumab was also tested in a phase I trial for SSc, in which it suppressed the interferon gene signature in whole blood and skin, which corresponded with a decrease in markers associated with T cell activation and collagen accumulation[126,127]. However, although targeting IFNAR should block signalling by all type I interferons, it will have no effect on IFNλ signalling. At present, no drugs are available that specifically target IFNλs or their receptor.

Targeting the JAK–STAT signalling pathway. JAK inhibitors are a promising new treatment for SLE that target and block the downstream signalling cascades of multiple cytokines involved in pathogenesis, including both type I and type III interferons. Moreover, these drugs can be given orally as opposed to intravenously like other biologic agents[128,129]. The JAK1 and JAK2 inhibitor baricitinib met its primary end point for clinical efficacy in a phase II trial in SLE, in which a higher proportion of patients receiving baricitinib achieved resolution of rash or arthritis compared with those who received placebo[130]. Phase III trials for baricitinib in SLE are ongoing[131–133]. Tofacitinib, a non-selective JAK1 and JAK3 inhibitor, has also shown potential in pre-clinical lupus models[121], and in a phase Ib/IIa trial

for mild-to-moderate SLE it demonstrated a good safety profile and improved cardiometabolic parameters[134,135].

JAK inhibitors have been extensively studied in RA, and several drugs (tofacitinib, baricitinib and upadacitinib) have been approved for clinical use by the FDA (reviewed elsewhere[128,129]). The efficacy of JAK inhibitors is probably related to their simultaneous targeting of multiple effector cytokines; however, there are currently no data on how JAK inhibitors modulate the interferon response in RA. JAK inhibitors are being investigated for pSS, SSc and myositis. Tofacitinib is currently in a phase I/II trial for pSS[136], and a phase I/II trial for early diffuse SSc[137] was recently completed, in which the drug was well tolerated and showed trends towards improvement of clinical outcome measures. Tofacitinib was also tested in a proof-of-concept study for refractory dermatomyositis in which it significantly reduced disease activity, as well as serum CXCL9 and CXCL10 concentrations and the interferon gene signature in skin[138]. Baricitinib is similarly being evaluated in a phase II trial for patients with idiopathic inflammatory myositis[139].

Table 3 | **Anti-interferon therapies for autoimmune rheumatic diseases**

Drug	Disease	Clinical development	Status	Refs
IFNα inhibitors				
Sifalimumab	SLE	Phase II	Completed	111
	DM and PM	Phase I	Completed	114,115
Rontalizumab	SLE	Phase II	Completed	112
IFNα kinoid	SLE	Phase II	Terminated	118
IFNAR inhibitors				
Anifrolumab	SLE	Phase III	Completed	121–123
		Phase III	Ongoing	151
	LN	Phase II	Ongoing	124
	RA	Phase II	Ongoing	125
	SSc	Phase I	Completed	126,127
JAK inhibitors				
Tofacitinib	SLE	Phase I	Completed	135
	RA	Approved	NA	152–155
	pSS	Phase I/II	Ongoing	136
	SSc	Phase I/II	Completed	137
	DM	Phase I	Completed	138
Baricitinib	SLE	Phase III	Ongoing	130–133
	RA	Approved	NA	156–158
	IIM	Phase II	Ongoing	139
Upadacitinib	SLE	Phase II	Ongoing	159
	RA	Approved	NA	160–163
Interferon production inhibitors				
BIIB059 (anti-BDCA2)	SLE and CLE	Phase II	Completed	145,146
VIB7734 (anti-LILR4A)	SLE, CLE, pSS, SSc, DM and PM	Phase I	Completed	147

BDCA2, blood dendritic cell antigen 2; CLE, cutaneous lupus erythematosus; DM, dermatomyositis; IFNAR, IFNα receptor; IIM, idiopathic inflammatory myositis; JAK, Janus kinase; LILR4A, leukocyte immunoglobulin-like receptor subfamily A member 4; LN, lupus nephritis; NA, not applicable; PM, polymyositis; pSS, primary Sjögren syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Targeting interferon production. pDCs are a primary source of both type I and type III interferons in SLE. Depletion of pDCs in mouse models of lupus attenuates autoimmunity[140–142], suggesting that targeting these cells might be an effective treatment option. Blood dendritic cell antigen 2 (BDCA2) is expressed specifically on pDCs and is a potent inhibitor of type I and type III interferon induction when ligated[39,143]. Notably, BDCA2 expression on pDCs from patients with SLE is decreased, and interferon production can be inhibited ex vivo by an anti-BDCA2 monoclonal antibody[144]. This approach was tested in a phase I trial for SLE. Treatment with BIIB059, an anti-BDCA2 monoclonal antibody, reduced ISG expression in peripheral blood and interferon-induced proteins in active skin lesions[145]. These findings were associated with improvements in cutaneous disease, as measured by the CLE Disease Area and Severity Index score, and were also associated with reduced $CD45^+$ immune cell infiltration into skin lesions. BIIB059 is still in development and has completed a phase II trial for SLE and CLE for which preliminary results have been announced[146].

Other biologic agents that target pDCs are also in development. VIB7734, an anti-leukocyte immunoglobulin-like receptor subfamily A member 4 (LILRA4) monoclonal antibody that targets pDCs for antibody-dependent cellular cytolysis, has completed a phase Ib trial in a variety of autoimmune diseases, including SLE, CLE, pSS, SSc, dermatomyositis and polymyositis[147]. Preliminary analysis of data in patients with CLE showed that VIB7734 significantly reduced pDCs in blood and skin, which corresponded with a decrease in interferon gene signature and improvement in CLE Disease Area and Severity Index score. Although these studies require further validation and did not discriminate between type I and type III interferons, the efficacy of anti-pDC therapies suggests that targeting upstream pathways could be advantageous over blocking type I interferons alone.

In addition to biologic agents that target BDCA2 or LILRA4, a variety of drugs can inhibit interferon production by other means. Antimalarial drugs such as hydroxychloroquine are commonly used to treat SLE and can inhibit type I and type III interferon production by pDCs in response to TLR7 or TLR9 stimulation[77,148]. Other TLR inhibitors are currently in various stages of development[149].

Conclusions

Type I interferons are central to the immunopathology of rheumatic diseases and are an important target for therapeutic intervention. By contrast, type III interferons are a new addition to the interferon family that have specialized functions, particularly at barrier surfaces. Early reports suggested that unlike type I interferons, type III interferons seemed to limit inflammation and host damage; as such, type III interferons have not been a major focus of research in rheumatology. However, data indicate that type III interferons are not strictly pro-inflammatory or anti-inflammatory; rather, they seem to have context-dependent functions in regulating immune responses. In autoimmune diseases such as SLE,

in which concentrations of IFNλs are abnormally elevated and their signalling is chronically activated, IFNλs might promote immune dysregulation and tissue inflammation. In other diseases in which the effects of IFNλs are more tightly regulated, endogenous or exogenously provided IFNλ might have an immunoregulatory function that suppresses inflammation. Although this difference is better understood in the context of infectious disease, improved understanding of the context-dependent functions of IFNλs will be important to optimize treatment and management for patients with autoimmune rheumatic diseases.

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Rheumatic diseases in Africa

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Abstract | Historically, rheumatic diseases have not received much attention in Africa, particularly in sub-Saharan Africa, possibly owing to a focus on the overwhelming incidence of infectious diseases and the decreased life span of the general population in this region. Global attention and support, together with better health policies and planning, have improved outcomes for many infectious diseases; thus, increasing attention is being turned to chronic non-communicable diseases. Rheumatic diseases were previously considered to be rare among Africans but there is now a growing interest in these conditions, particularly as the number of rheumatologists on the continent increases. This interest has resulted in a growing number of publications from Africa on the more commonly encountered rheumatic diseases, as well as case reports of rare diseases. Despite the limited amount of available data, some aspects of the epidemiology, genetics and clinical and laboratory features of rheumatic diseases in African populations are known, as is some detail on the use of therapeutics. Similarities and differences in these conditions can be seen across the multi-ethnic and genetically diverse African continent, and it is hoped that increased awareness of rheumatic diseases in Africa will lead to earlier diagnosis and better outcomes for patients.

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The growing burden of rheumatic diseases has been well documented all over the world[1]; however, there is relatively little information from Africa, particularly sub-Saharan Africa, and the limited health resources of many African countries are prioritized to address the challenges of infectious and communicable diseases. Non-communicable diseases, including rheumatic diseases, also contribute to poor health outcomes in Africa and are starting to receive more attention. Within this sphere, rheumatology has emerged as a slowly growing discipline in Africa. Over the past two decades, there have been an increasing number of reports of rheumatic diseases from all over Africa. The presence of a wide spectrum of rheumatic diseases and the challenges associated with their diagnosis and management has been previously reviewed elsewhere[2–4]. The extent of the burden of rheumatic diseases in Africa is unknown as there are limited epidemiological data; however, many patients with rheumatic diseases present to health-care facilities all over the continent. The burden of rheumatic diseases in Africa is probably greater than in other parts of the world in terms of morbidity and mortality, as patients often present at a later stage of disease[3,5].

The purpose of this Review is to provide an overview of the common types of chronic inflammatory arthritis (gout, rheumatoid arthritis (RA) and spondyloarthritis (SpA)) and connective tissue diseases (systemic lupus erythematosus (SLE) and systemic sclerosis (SSc)) reported among adults in Africa. To do so, we reviewed all the available publications from Africa in each of these diseases and selected studies in which there were sufficient data to permit comparison among the studies and with studies from other parts of the world. However, only limited data are available for some of these diseases or for some aspects of the diseases in different regions of Africa. As a result, we are unable to provide comparative data for all aspects of these diseases. In this Review, we also highlight the progress and challenges related to the study, diagnosis and management of rheumatic diseases in such a geographically vast and ethnically diverse continent as Africa, and discuss opportunities and advances made in the training of rheumatologists in Africa.

Gout

Gout has received increasing global attention as it has emerged as the most common cause of inflammatory arthritis in many parts of the world[6]. In Africa, however, many patients with gout currently present to primary care practitioners, who might not document them or might even miss the diagnosis. Thus, it is important to raise awareness of the occurrence of gout in Black Africans so that patients can be correctly diagnosed and receive appropriate treatment at an earlier stage.

Prevalence and risk factors. Before 1980, gout was uncommonly reported in Black Africans, and early reports comprised only small case series[7,8]. However, since then, an increasing number of publications on

Key points

- In the past, there has been an emphasis on communicable diseases in Africa, but attention has now shifted towards non-communicable diseases such as rheumatic diseases.
- Common rheumatic diseases are seen in Africa and are both comparable and different from presentations seen outside of Africa.
- Diverse genetic and environmental factors affect the presentation of common rheumatic diseases among different African nations.
- A shortage of appropriately trained staff, laboratory testing capacity and effective medications exists across the whole continent.
- Advocacy and research are needed to increase awareness of the risk factors, presentations and management of rheumatic diseases in Africa.
- Specialized treatment guidelines are needed for resource-poor countries in Africa.

gout have emanated from all over Africa. Many factors are likely to be responsible for the increased number of reports of larger series of patients with gout, including urbanization, the adoption of a Western lifestyle, increased alcohol consumption and the rising incidence of obesity, hypertension, diabetes and renal disease in African populations[9–11].

Limited epidemiological data are available on the prevalence of hyperuricaemia and gout in Africa. Clinical and radiographic surveys conducted in the 1970s of 1,185 Black individuals in a rural setting and of 424 Black individuals in an urban setting in South Africa failed to detect any patients with gout[12,13]. The authors also found that the mean serum uric acid levels were significantly higher in the urban community than in the rural community ($P < 0.001$)[12,13]. Subsequent community-based studies of rural and semi-urban populations have shown that gout is uncommon. The prevalence of gout was 0.1% in a survey of 2,484 people in a semi-urban setting in Nigeria, 0.06% in a survey of 1,500 people in a rural setting in the Democratic Republic of Congo (DRC) and 0.29% in a survey of 5,120 people in Egypt[14–16].

A genetic study of 46 South African patients with gout showed a statistically significant association with HLA-B14 alleles in patients with primary gout compared with healthy individuals[9]. To date, there have not been any other studies investigating the relationship between genetics and gout in Africans, which is not surprising given that Africans have a wide genetic diversity. The findings from some of the larger studies on gout from different parts of Africa[9–11,17–20] are shown in TABLE 1, including the prevalence of hypertension, obesity, diabetes, alcohol intake, kidney disease and diuretics (where this information was available). Alcohol intake ranged from 67% to 83% in most studies and is probably an important risk factor, except in Mali and Nigeria, where there was a lower prevalence of alcohol use of 5% and 17.8%, respectively[9–11,17–20]. This low rate of alcohol use might be attributable to abstinence on religious grounds. The other major comorbidities and risk factors identified in the studies were obesity, hypertension and the use of diuretics. One study also included a case–control analysis and found that obesity, 'white collar' occupation, hypertension and alcohol intake were risk factors for men, whereas alcohol intake was the only statistically significant risk factor for women[10].

Demographics, presentation and management. As most of the studies in TABLE 1 report data from hospital-based patients, the disease described is often a more severe form. The mean age at presentation ranged from 47.5 to 55 years in most studies, with a slightly younger age of 44 years reported in Togo[11], and there was a delay of between 3.4 and 7.5 years before patients presented to hospital. A wide variation exists in the sex distribution of patients among the studies, with most showing a higher prevalence of gout in men; however, an almost equal number of men and women were reported in Cameroon, and a greater number of women than men had gout in Mali[17,19]. Although most patients had a monoarticular or oligoarticular presentation, 27–44% of patients had polyarticular involvement. The joints most commonly involved were the large joints such as the knees and ankles and, less often, the first metatarsophalangeal joints. A lower prevalence of tophi occurred in Mali and Nigeria (4.0% and 6.2%, respectively), whereas tophi occurred in between 21.9% and 51% of individuals in most of the other studies. In many patients, the tophi are large and can be numerous. Little information is currently available on the socioeconomic effects of gout, quality of life, course of the disease and adherence and response to therapy. Treatment is mostly with NSAIDs, colchicine and glucocorticoids for acute attacks, and although the urate-lowering therapies allopurinol and probenecid are available, febuxostat is only available in some African countries. Drugs such as pegloticase, rilonacept and canakinumab are unavailable. A clear need exists to treat gout at an early stage — an approach that would be of good economic benefit to the governments concerned — and there is also a need for longitudinal studies on the outcomes of gout.

Rheumatoid arthritis

Although RA was first recognized as a distinct entity in 1800 (REF.[21]), it was only in 1956 that the first two cases of RA were reported in Black Africans in Malawi[22]. Subsequently, small case series from Zimbabwe, Uganda, Kenya, Nigeria and Lesotho were published[5,23]. However, since then, larger series of patients with RA have been reported from all regions of Africa. The reasons for these observations are probably multifactorial and include the increasing urbanization of the population, improvements in access to health services, a greater awareness of the disease alongside the development of rheumatology services in major academic centres and the possibility that autoimmune diseases such as RA are truly emerging diseases on the African continent. The increase in number of patients with RA has provided an opportunity to study the clinical expression, serological manifestations, disease course, response to treatment and outcomes compared with patients from other ethnic groups around the world.

Prevalence and risk factors. Limited epidemiological data are available on the prevalence of RA in Africa. A systematic review on the burden of RA in Africa published in 2012 noted that there were limited epidemiological data on the prevalence of RA in Africa[24]. Similarly, a meta-analysis on the prevalence of arthritis

Table 1 | **Clinical features of gout in different sub-Saharan African countries**

Region	Southern Africa		East Africa	West Africa			
Country	South Africa	South Africa	Kenya	Togo	Nigeria	Mali	Cameroon
Study	Cassim et al. (1994)[9]	Tikly et al. (1998)[10]	Oyoo et al. (2004)[18]	Mijiyawa et al. (2000)[11]	Adelowo et al. (2014)[20]	Kodio et al. (2015)[17]	Doualla-Bija et al.(2018)[19]
Demographics							
Number of participants	107	90	21	160	146	100	174
Female-to-male ratio	1.0:6.6	1.0:3.3	1.0:9.5	1.0:159.0	1.0:2.8	1.2:1.0	1.0:1.1
Family history (%)	NR	8.8	NR	10.6	NR	NR	NR
Mean (s.d.) age (years)	Total 50.5 (11.5); 49.3 F; 57.0 M	54.3 (30–86) M; 55.3 (43–64) F	47.5	44.0	Total 53.4 (11.0); 52.8 (10.3) M; 54.9 (13.0) F	57.3 (10.0)	55.0 (14.3)[a]
Mean (s.d.) duration of symptoms (years)	3.4 (4.4)	5.4 (0.02–0.08) M; 2.7 (0.05–0.04) F	NR	6.0	3.6	NR	7.5 (10.0)[a]
Pattern of joint involvement (%)							
Monoarthritis	37.4	4.4	47.6	NR	50.0	36.0	NR
Oligoarthritis	28.0	51.2	19.1	NR	35.6	30.0	NR
Polyarthritis	34.6	44.4	33.3	NR	14.4	27.0	NR
Joints involved (%)							
Knee	85.0	57.8	14.1	NR	55.5	92.0	NR
1st MTP joint	74.8	60.0	34.9	NR	14.4	22.0	NR
Ankles or feet	61.7	66.7	18.6	NR	34.4	45.0	NR
Wrists	NR	45.6	NR	NR	9.6	20.0	NR
Other features							
Presence of tophi (%)	36.4	51.1	NR	21.9	6.2	4.0	35.1
Comorbidities (%)							
Hypertension	40.2	60.6	61.5	26.3	49.3	76.0	35.6
Obesity	NR	74.0	90.5	40.0	12.3	83.0	84.5
Diabetes	NR	3.3	7.7	NR	4.1	13.0	13.2
Alcohol	79.0	75.3	100.0	83.1	17.8	5.0	67.2
Kidney disease	16.8	31.1	NR	NR	0.7	37.0	NR
Diuretic therapy	44.9	36.8	NR	12.5	NR	64.0	NR

F, female; M, male; MTP, metatarsophalangeal; NR, not reported; s.d., standard deviation. [a]Median (interquartile range).

in Africa in 2015 found that there was a paucity of prevalence data on arthritis[25]. TABLE 2 shows a summary of the published population-based epidemiological studies on RA and describes the setting in which each study was conducted[14,26–35]. Most of the studies shown in TABLE 2 had small numbers of participants, ranging from 543 to 1,070. In 1975, a South African study reported a prevalence of RA of 0.90% in an urban Black population, which was similar to the ~1% prevalence of RA reported in most parts of the world[27]. However, the picture seems quite different in rural populations. In the same year, another South African study reported a prevalence of RA of only 0.12% in a rural Black population[28]. A 1993 survey of 1,994 people in rural Nigeria failed to detect any patients with RA[32] and, similarly, a 2017 study of a semi-urban Nigerian population identified only three individuals with RA (0.12%) among the 2,454 people studied[14]. Other studies with larger numbers of participants reported prevalence of 0.29% in Egypt and 0.60% in the DRC[33,34]. These findings are similar to observations in other parts of the world, where the prevalence of RA ranges from 0.2% to 0.5% in most rural communities in low-income and middle-income countries[16]. An Algerian study of 52,504 urban residents also reported a low prevalence of 0.13%[35].

Early genetic studies showed that an increased susceptibility to RA was associated with different alleles of *HLA-DRB1* (REF.[36]). In 1987, Gregersen et al. found that these alleles carried the 'shared epitope' (a common sequence of five amino acids at positions 70–74), which was associated with increased susceptibility to RA[37]. Studies from South Africa and Zimbabwe have reported a genetic association between *HLA-DR4* and RA in Black individuals[23]. Genetic studies from Nigeria showed that *HLA-DR4* was present in <1% of the population, suggesting that different genetic factors are associated with RA in different populations[38]. In the DRC, the prevalence of *HLA-DRB1*04:01* alleles is low among both patients with RA and healthy individuals, but there is a higher prevalence of other *HLA-DRB1* alleles, suggesting a different genetic risk profile compared with patients in Southern Africa and those of European ancestry[39].

A study from Cameroon confirmed the association between susceptibility to RA and *HLA-DRB1*-shared epitope alleles; however, the allele frequency was only 30%, which is much lower than the 50–70% reported in individuals with European ancestry[40]. Genome-wide association studies have revealed over 100 susceptibility loci that have important variations among different ethnic groups[36]. A high-resolution HLA-typing study in 266 Black patients with RA from South Africa revealed an increased risk of RA in patients with a histidine at position 13 or a valine at position 11 of the third hypervariable region of the beta chain of HLA-DRB1 (REF.[41]). Conversely, alleles that carried a serine at position 11 conferred protection in this population. These findings are in agreement with the earlier observations in North America and Europe[42]. Outside the HLA region, a polymorphism in *PTPN22* is associated with the highest risk of RA in patients of European ancestry[43]. However, this gene was non-polymorphic in Black South Africans and therefore not associated with RA in this population[44]. Previous studies have acknowledged that despite Africa having the highest genetic diversity in the world, very few genetic studies in African populations have been published[45,46]. As highlighted by these findings, a great need exists for large-scale genetic studies across Africa. Such studies will help researchers to identify the similarities and differences within African populations compared with other populations, and to understand the role of genetic factors in disease severity and response to drugs such as methotrexate.

Environmental risk factors for RA include smoking, which is linked to the development of RA and is associated with more severe disease[47]. A low prevalence of smoking has been reported in African countries including Sudan (1.2%) and the DRC (1.6%)[39,48]. However, a South African study noted a likely under-reporting of smoking, as many patients had high nicotine levels, despite reportedly being non-smokers[49]. In addition, many of the patients in this study were using smokeless tobacco, which can be sniffed, sucked, chewed or just applied to the teeth or gums[50]. The use of smokeless tobacco varies widely in Africa, ranging from 24.7% in men and 19.6% in women in Madagascar, to 3.8% in men and 0.5% in women in Nigeria, and 0.03% in men and 0.31% in women in Burundi[51]. Although smokeless tobacco is also considered a risk factor for RA, a Swedish study of 1,998 patients with RA and 2,252 healthy individuals did not find any increase in moist snuff (smokeless tobacco) users among those with RA[52]. Further studies are required to determine whether smokeless tobacco is associated with an increased risk and/or severity of RA. Another environmental risk factor for RA is periodontal infection, which shows a significant association with RA in many systemic reviews and meta-analysis studies[53,54]. Statistically significant associations between periodontal infection and RA have also been reported in studies from Senegal and Sudan[55,56].

Demographics, presentation and management. The reported manifestations of RA varied in early studies in Africa, but seemed to be characterized by a young age at onset, a low prevalence of subcutaneous nodules and extra-articular manifestations, and mild disease with less severe radiographic changes[5,23]. Many studies involving larger numbers of patients have been published from all over Africa in the past two decades[39,40,48,57–63], the results of which are summarized in TABLE 3. Most of the studies in TABLE 3 show a higher prevalence of RA in women than in men, with a ratio of nearly 6:1, which is greater than the ratio of 3:1 in patients of European descent[64]. Long delays often occurred before referral to a specialist, ranging from 3.0 years to 12.9 years. As a result of a delay in referral, untreated or inadequately treated active disease results in high disease activity, greater functional impairment and more severe joint damage. Notably, the shorter mean duration of 11 months (standard deviation (s.d.) 7.1 months) in the 2012 study in South Africa resulted from the inclusion of only patients with early RA (disease duration of <2 years)[58].

Subcutaneous nodules are among the most common extra-articular manifestations and occur in up to 30% of patients with RA across Africa (TABLE 3); however, low prevalence of subcutaneous nodules (3.0–14.4%) was reported in Senegal, the DRC, Cameroon, Egypt

Table 2 | **Epidemiological studies of rheumatoid arthritis in Africa**

Study	Country	Study population	Number of participants	Prevalence (%)	Ref.
Muller et al. (1972)	Liberia and Nigeria	Rural	1,027	0.97	26
Solomon et al. (1975)	South Africa	Urban	551	0.90	27
Beighton et al. (1975)	South Africa	Rural	801	0.12	28
Meyers et al. (1977)	South Africa	Rural	577	0.68	29
Moolenburgh et al. (1986)	Lesotho	Rural	1,070	0.28	31
Brighton et al. (1988)	South Africa	Rural	543	0.00	30
Silman et al. (1993)	Nigeria	Rural	1,994	0.00	32
Abdel-Nasser et al. (1997)	Egypt	Rural	5,120	0.29	33
Malemba et al. (2012)	Democratic Republic of Congo	Community	5,000	0.60	34
Slimani et al. (2014)	Algeria	Urban	52,504	0.13	35
Courage et al. (2017)	Nigeria	Semi-urban	2,454	0.12	14

Table 3 | **Clinical features of rheumatoid arthritis in different African countries**

Region	Southern Africa		West Africa		Central Africa		North Africa		East Africa	
Country	**Zimbabwe**	**South Africa**	**Senegal**	**Nigeria**	**Democratic Republic of Congo**	**Cameroon**	**Algeria**	**Egypt**	**Sudan**	**Kenya**
Study	**Chikanza et al. (1994)[57]**	**Hodkinson et al. (2012)[58]**	**Ndongo et al. (2009)[59]**	**Adelowo et al. (2010)[60]**	**Malemba et al. (2013)[39]**	**Singwe-Ngandeu et al. (2010)[40]**	**Slimani et al. (2014)[61]**	**Sakr et al. (2018)[62]**	**Elshafie et al. (2016)[48]**	**Owino et al. (2009)[63]**
Demographics										
Number of participants	84	171	100	200	128	56	249	3,219	281	60
Female-to-male ratio	4.0:1.0	4.5:1.0	7.3:1.0	2.4:1.0	8.0:1.0	NR	5.9:1.0	6.2:1.0	8.4:1.0	6.5:1.0
Mean (s.d.) age at presentation (years)	39.2 (10.7)[a]	47.1 (12.4)	40.3 (15.5)	46.9[b]	51.2 (14.9)	53.5 (39.0–61.5)[c]	50.1 (14.5)	40.5 (12.6)	48.3 (13.0)	41.4 (16.8)
Mean (s.d.) duration of symptoms	5.8 (4.1) years	11.7 (7.1) months	3.0 (3.6) years	63.4 months[b]	48.0 (2.0–108.0) months[c]	3.0 (2.0–6.0) years[c]	8.4 (7.8) years	12.9 (7.9) years	48.0 months[d]	NR
Smoking (%)	NR	23.4	NR	NR	1.6	2.0	NR	9.4	1.2	NR
Clinical, laboratory and radiographic findings										
Nodules (%)	25.0	20.0	3.0	29.5	13.3	7.0	22.7	14.2	NR	13.3
Positive rheumatoid factor (%)	77.4	83.6	78.0	38.5	34.7	NR	78.5	52.0	52.4	78.9
Positive ACPA (%)	NR	80.1	89.7	NR	47.2	NR	69.3	NR	NR	NR
Mean (s.d.) ESR (mm/h)	45.6 (29.0)	46.0 (30.8)	44.5 (4.0–120.0)[c]	NR	NR	NR	40.6 (26.3)	NR	55.0[b]	NR
Mean (s.d.) CRP (mg/l)	37.8 (21.0)	30.2 (38.7)	12.0 (0.0–96.0)[c]	NR	NR	12.0 (6.0–35.0)[c]	11.1 (15.6)	NR	NR	NR
Mean (s.d.) DAS28	NR	39.4 (16.2)[e]	6.5 (1.3)	NR	5.1[b]	4.72[b]	4.3 (1.4)	NR	NR	4.4 (1.7)
Erosions (%)	NR	50.9	56.0[f]	29.2	55.1[g]	44.0	68.1	20.6	56.7	NR
DMARD use (%)										
Methotrexate	NR	91.0	NR	88.5	NR	NR	72.2	82.8	52.1	43.3
Sulfasalazine	NR	6.0	NR	18.0	NR	NR	8.6	NR	3.7	5.0
Hydroxychloroquine	NR	30.6[h]	NR	20.0	NR	NR	15.3	51.0	2.3	NR
Leflunomide	NR	NR	NR	8.0	NR	NR	8.6	18.8	0.8	NR
Biologic DMARDs	NR	0.0	NR	2.0	NR	NR	4.0	0.0	NR	0.0

ACPA, anti-citrullinated protein antibody; CRP, C-reactive protein; DAS28, 28-joint disease activity score; ESR, erythrocyte sedimentation rate; NR, not reported; s.d., standard deviation. [a]Age at onset. [b]s.d. not reported. [c]Median (interquartile range). [d]Median without interquartile range. [e]Simplified disease activity index. [f]Structural changes. [g]Erosions and/or joint space narrowing. [h]Chloroquine.

and Kenya[39,40,59,62,63]. The prevalence of rheumatoid factor is low in the DRC (34.7%) and Nigeria (38.7%)[40,60], but is nearly 80% in South Africa, Senegal, Algeria and Kenya, similar to reports in most European and North American populations[58,59,61,63]. Early radiographic studies from Africa noted that erosions were uncommon; however, most of the reports shown in TABLE 3 found erosions in nearly 50% of patients. Compared with the early observations of low disease activity, all the studies in TABLE 3 show the presence of moderate or high disease activity based on the 28-joint disease activity score.

The most commonly used DMARD to treat RA is methotrexate, which is often used in combination with hydroxychloroquine or chloroquine. Biologic DMARDs (bDMARDs) are not available in most public-sector hospitals and are used only by patients with private health insurance or those who can afford to pay for them (BOX 1), as exemplified by data from the South African Biologics Registry, which mostly comprises patients with private health insurance[65].

Analysis of the reports of RA in Africa show that patients with RA often present at a later stage in the course of their disease. Therefore, many patients already have erosive arthritis with considerable functional limitation at presentation. Heterogeneity exists with respect to age of onset, extent of female predominance, prevalence of subcutaneous nodules, seropositivity and genetic associations in different parts of Africa. For RA, there is an urgent need to raise awareness and promote education and training of health professionals so that patients are diagnosed and treated at an early stage and so that better outcomes can be achieved for patients.

Box 1 | **Biologic DMARD use in Africa**

Unlike most other continents, biologic DMARD (bDMARD) penetration in African countries, particularly sub-Saharan Africa, is low. This low penetrance is caused by the non-availability of bDMARDs, as well as the lack of affordability. Patients in many African countries pay out of pocket for their medications and, as a result of the high cost of bDMARDs, such patients are unable to benefit from these agents. South Africa, Kenya and North African countries have access to many of the bDMARDs that are available. These are also the countries that have robust health insurance schemes. By contrast, Nigeria, for example, has a less than desirable health insurance scheme, and most patients therefore have to pay for their medicines themselves. Most of the other 54 African countries, especially those in Sub-Saharan Africa, do not have access to a single bDMARD.

At an International League Against Rheumatism-sponsored symposium on "Patient access to biologics across the globe" during the 2014 ACR Congress, it was noted that Africa had the lowest regional uptake of bDMARDs. Registries of bDMARDs were reportedly available in only three countries (Algeria, Morocco and South Africa) and, at the time, only a limited number of patients were being treated with bDMARDs. The number of different biological agents available was also widely variable, ranging from just two in Nigeria and four in Kenya, to the majority of agents being available in North African countries and South Africa. Although many African countries have started using biosimilars and biomimics because of their relatively lower costs, no published data are available on their efficacy and safety in African populations.

Tuberculosis ocurrence is a barrier to the use of bDMARDs, especially in countries where tuberculosis is endemic. Reports from registries in France and the UK have shown twofold to sixfold increases in rates of tuberculosis reactivation in patients receiving TNF inhibitors, with lower rates among those receiving soluble TNF receptors[129,130]. However, a 2020 study of 4,830 South Africans from a bDMARDs registry found a tuberculosis incidence rate that is ten times higher than that in European countries[65]. The prevalence of tuberculosis was 2% or 1,240 per 100,000 patient-years of treatment. Reactivation occurred in approximately 50% of patients and was associated with Black race, male sex and a younger age[65]. Therefore, in countries with endemic infection, it is mandatory to screen for tuberculosis before administering any bDMARD, particularly TNF inhibitors.

Spondyloarthritis

Although SpA is commonly reported in North African countries and among white individuals, people of Indian descent and mixed ethnicity populations in South Africa, it is rarely reported among Black Africans. This rarity might be due to the low prevalence of *HLA-B27*, an important genetic risk factor for certain types of SpA, in Black Africans (<1%) compared with the prevalence of 3–5% in North Africans and 8% in patients with European ancestry[66]. The association between HLA-B27 and SpA, particularly ankylosing spondylitis (AS), might be more complex in Black Africans. Although higher frequencies of HLA-B27 have been reported in certain ethnic groups in Gambia (up to 7.8%), and in Mali (9.7%), AS is rarely seen in these populations[67,68]. A prospective survey among 900 adult Fula men in Gambia (of whom 6 of the 100 tested were *HLA-B27* positive) did not identify any patients with AS[69]. Greater awareness of SpA in Africa is needed, as is further research into genetic heterogeneity among different ethnic groups.

Prevalence and risk factors. Few community studies on SpA have been performed in African countries, with the most notable being the Community-Oriented Program for Control of Rheumatic Diseases (COPCORD) studies, in which Egypt had an overall prevalence of SpA of 0.15%[70]. The prevalence of AS in Egypt was reported in another COPCORD study to be 0.09%, whereas the DRC had a prevalence of SpA of 3.8%[15,16]. No cases of SpA were reported in other studies in Nigeria and Gambia[14,69]. Hospital-based studies in rheumatology clinics in Central African countries also noted that AS was uncommon[71,72]. Studies of patients seen in rheumatology clinics identified only 3 with AS among 2,370 patients in Kinshasa, DRC, 4 patients with AS among 10,000 patients in Brazzaville, Congo and 8 patients with AS among 9,065 patients in Togo[71–73]. A prospective survey of 984 rheumatology outpatients in the DRC found 105 (10.7%) with SpA; the sub-types were non-radiographic axial SpA (5.0%), reactive arthritis (4.3%), AS (1%) and psoriatic arthritis (0.1%)[74].

The prevalence of HLA-B27 is 90–95% in patients of European ancestry with AS, compared with only 29–64% in individuals from Algeria, Egypt, Morocco and Tunisia with the disease[75–78]. One study from Togo has reported an association between HLA-B14:03 and AS in Black Africans, but this association has not yet been verified in other studies[73]. Interestingly, an upsurge of SpA accompanied outbreaks of HIV infection in East Africa and Southern Africa[79], suggesting HIV infection could be a risk factor for some types of SpA, in particular reactive arthritis and undifferentiated SpA.

Demographics, presentation and management. An audit of 518 patients with SpA in three North African countries (Tunisia, Algeria and Morocco) did not reveal much difference from data from Europe on SpA[80]. The mean age at onset in this study was 26.6 years (s.d. 10.7 years). The male-to-female ratio was 3:1, and inflammatory back pain was reported in 90% of the cohort. Symptoms of sacroiliitis were reported by 97% of patients, whereas peripheral oligoarthritis was reported by 42% and dactylitis by 10%. However, extra-articular features were infrequently reported; uveitis occurred in 13%, psoriasis in 6% and inflammatory bowel disease in 3%[80]. In a separate Algerian study, a mean delay of 4.3 years occurred before a diagnosis could be made, and there was a high frequency of hip involvement, which differed from what is usually seen among Europeans[81]. Although commonly reported in North Africans, familial occurrence has rarely been documented in South Africa[82]. In addition, non-radiographic axial SpA has been documented in North African countries, the DRC and in South Africa[74,82].

The Assessment of Spondyloarthritis International Society criteria for diagnosing early axial disease have been suggested to not be particularly useful in Black individuals because of the low frequency of HLA-B27 in this population. The limited availability and high cost of MRI in Africa also makes implementing the criteria difficult. Conventional synthetic DMARDs were available to patients in all the aforementioned studies, whereas only 14% of patients with SpA in Egypt received bDMARDs[76]. The 2016 Assessment of Spondyloarthritis International Society–EULAR management recommendations for axial SpA note that, in principle, conventional synthetic DMARDs should not be used for purely axial disease[83]. Although sulfasalazine, methotrexate and leflunomide are not effective for axial symptoms, they can be used in exceptional situations where no other treatment option is available owing to toxicity, contraindications or costs. Therefore, in the absence of access

to bDMARDs, patients with purely axial symptoms in African countries cannot currently be treated adequately.

Systemic sclerosis

SSc (also known as scleroderma) is a rare autoimmune rheumatic disease in which cutaneous and visceral organ fibrosis and vasculopathy are the predominant features. Historically, one of the ground-breaking observations in SSc research was made in Africa. Robert Goetz, a vascular surgeon working in Cape Town, South Africa in 1945, was the first to show in autopsy studies that the disease is not confined to the skin and that fibrotic and vascular changes occur in several visceral organs[84]. He coined the term 'progressive systemic sclerosis' in preference to 'scleroderma'. Subsequently, the term 'systemic sclerosis' was adopted as the most appropriate description of the disorder as the disease is not 'progressive' in all individuals.

Prevalence and risk factors. Except for some small studies in South Africa, which have shown that underground gold miners have around a 25-fold higher annual incidence of SSc than the general population (7.73–8.1 per 10,000 versus 0.33 per 10,000)[85,86], there have been no formal epidemiological studies in Africa. To date, none of the COPCORD studies in rural Africa has reported any cases of SSc. Although familial clustering of SSc is uncommon, the interplay of genetic factors and environmental triggers is known to be important in the aetiopathogenesis of SSc[87]. The seminal work of L.D. Erasmus in 1957 showed that white gold miners exposed to silica were at an increased risk of developing SSc[88]; observations that were subsequently confirmed in Black gold miners in South Africa[85]. Only a handful of studies have been performed on genetic risk factors for SSc in Africans, and in the only study to date on HLA associations with SSc, *HLA-DRB1*15:01* was associated with SSc overall in South Africans, *HLA-DQB1*03:01* was associated with diffuse cutaneous SSc (dcSSc) and *HLA-DRB*11:01* was associated with anti-U3RNP antibodies[89].

Demographics, presentation and management. A distinctive feature of SSc in Africa is that dcSSc is the predominant subset in sub-Saharan Africa[90] and in North Africans[91,92], whereas limited cutaneous SSc (lcSSc) is more prevalent in white populations. These ethnic differences are mirrored in the USA, where dcSSc is more common than lcSSc in African Americans[93]. A 2020 systematic review of 1,866 patients with SSc in sub-Saharan Africa based on 90 publications (mainly from South Africa, Nigeria and Senegal) showed that two-thirds of patients have dcSSc and that most patients are female (84%)[90]. The most striking clinical feature in these patients was the mixed hyper-pigmentary and hypo-pigmentary skin changes, commonly referred to as 'salt and pepper' depigmentation[94]. Raynaud's phenomenon occurred in 79% of individuals, oesophageal reflux in 70% and interstitial lung disease in ~50%. Digital ulcers, pulmonary hypertension and cardiac involvement were less common, and scleroderma renal crisis was rare.

Antinuclear antibodies (ANAs) were present overall in 65% of individuals, with anti-U3RNP, anti-U1RNP and anti-topoisomerase-1 (Scl-70) antibodies the most common[90]. The high prevalence of anti-U3RNP antibodies ties in with the predominance of dcSSc and associated 'salt and pepper' depigmentation[95]. By contrast, anti-centromere antibodies are rare[96], consistent with lower prevalence of lcSSc in African populations. A report from Tunisia also showed that Raynaud's phenomenon was more common among those with dcSSc (91%) than in those with lcSSc (43%)[92]. Anti-centromere antibodies were also uncommon in this population, and renal and cardiac involvement were rarely reported, similar to elsewhere in Africa; however, interstitial lung disease and gastrointestinal manifestations were frequent[92].

Treatment of SSc in sub-Saharan Africa has mainly focused on symptom relief, such as NSAIDs for pain relief and proton pump inhibitors for gastro-oesophageal reflux. Glucocorticoids have been prescribed less frequently than NSAIDs to control inflammation, sometimes at high doses of 40–60 mg daily. Other immunosuppressive agents such as methotrexate, cyclophosphamide and azathioprine are rarely prescribed[90]. No reports have been published on autologous stem cell transplantation in Africa or on the use of agents such as prostanoids or endothelin receptor antagonists for the treatment of pulmonary hypertension. Overall, although SSc is rare in Africa, a need exists for more effective and less costly medications for the treatment of severe skin disease, as well as for the serious vascular and lung complications that are often major causes of morbidity and mortality.

Systemic lupus erythematosus

Historically, SLE has rarely been reported among Africans, especially Black Africans. Most of the early reports comprised single cases or small case series. In an article in 1995, Deborah Symmons proposed a gradient theory that suggested that SLE is rare in sub-Saharan Africa and that the prevalence rises moving upwards from North Africa into Europe[97], a theory that was corroborated by other analyses[98,99]. Although early studies found few cases of SLE, subsequent studies have reported larger numbers. This underreporting might have been caused by under-diagnosis, as many individuals with SLE are wrongly diagnosed with infections such as malaria and tuberculosis in areas where these diseases are endemic[100].

Prevalence and risk factors. Several rural community COPCORD studies have not documented any patients with SLE[14,15,70,101]. Similarly, a 1998 study found a cumulative total of only 413 patients with SLE over the period of 1971–1984 in African countries[99]. However, since 1984, an increasing number of reports of SLE have emerged from all over Africa[100,102–108]. A 2020 systematic review and meta-analysis of a pooled population of 28,375 individuals in hospitals revealed a prevalence of SLE of 1.7% (95% CI 0.8–2.9%) across general medicine and rheumatology units[109]. The authors concluded that there was an increasing prevalence of SLE across sub-Saharan populations[109]. A study from South Africa

had also reported that SLE might be more prevalent in Black Africans than had previously been thought[110]. This report also identified a high prevalence of comorbid tuberculosis among patients with SLE[110].

Although many studies on the genetics of SLE have been performed in other parts of the world, there have been very few studies among Black African populations, even though these populations have wide genetic diversity and complex disease mapping[45,46]. In addition, the clinical heterogeneity of SLE has led to efforts to identify genetic variants that might account for the different clinical phenotypes of SLE. In a study in South Africa, the prevalence of *HLA-DRB1*02* was higher in Black individuals with SLE than in ethnically matched healthy individuals, and *HLA-DQB1*02:01* was associated with the presence of anti-Ro antibodies, rather than with SLE itself[111]. An additive genetic model, rather than environmental factors, has been proposed to be responsible for the differences between Black African, African American and Afro-Caribbean patients with SLE and those of European ancestry[112,113]. The association between apolipoprotein L1 and non-diabetic renal diseases, such as lupus nephritis, in African Americans might also be applicable to Black Africans[114]. Looking at epigenetics, a South African study in Black individuals with SLE or SSc revealed more hypomethylated genes in both diseases than in healthy individuals[115].

Demographics, presentation and management. The demographic data, clinical manifestations and autoantibody profiles of patients with SLE in Africa, as reported in some of the larger studies published between 2009 and 2020, are shown in TABLE 4[100,102–108]. In a South African study of a multi-ethnic cohort of 408 patients comprising mostly Indians (58.1%) and Black Africans (33.6%), no significant differences were reported in the age at onset or gender between the two groups[103]. The mean age at presentation ranges from 29.2 years in a large Egyptian study[105], to around 33–36 years in most of the other studies[105] (TABLE 4). The female-to-male ratio is overwhelmingly high in many African populations, with ratios of between 18:1 and 32:1 reported in South Africa, Nigeria, Sudan and Kenya[100,104,107,108]. In addition, there is a striking delay before presentation to a rheumatologist (15.6 months in Tunisia and 30 months in Nigeria). In Egypt there was a similar delay, with the mean age

Table 4 | **Clinical features of systemic lupus erythematosus in different African countries**

Region	**Southern Africa**		**East Africa**		**West Africa**		**North Africa**	
Country	**South Africa**	**South Africa**	**Kenya**	**Sudan**	**Nigeria**	**Nigeria**	**Tunisia**	**Egypt**
Study	**Wadee et al. (2007)[108]**	**Budhoo et al. (2018)[103]**	**Genga et al. (2015)[104]**	**Elbagir et al. (2020)[107]**	**Adelowo et al. (2009)[100]**	**Adelowo et al. (2012)[a102]**	**Khanfir et al. (2013)[106]**	**El Hadidi et al. (2018)[105]**
Demographics								
Number of participants	226	408	100	115	66	95	749	1,109
Female-to-male ratio	18.0:1.0	10.3:1.0	32.3:1.0	22:1.0	21.0:1.0	22.7:1.0	9.3:1.0	8.7:1.0
Mean (s.d.) age at presentation (years)	34.0 (12.5)	32.9 (13.7)	36.6 (10.7)	34.9[b]	33.0[b]	33.4[b]	30.7[b]	29.2 (11.5)
Clinical features (%)								
Arthritis	70.4	80.6	90.0	85.5	87.0	NR	87.1	76.7
Mucocutaneous manifestations	NR	NR	78.0	NR	NR	NR	81.7	78.1
Malar rash	58.4	49.0	54.0	52.2	21.2	NR	68.7	48.5
Discoid rash	41.5	27.7	22.0	6.1	43.9	NR	11.9	5.4
Photosensitivity	38.9	67.2	44.0	53.0	9.0	NR	67.6	45.6
Oral ulcers	38.5	50.0	36.0	63.5	33.0	NR	23.3	34.5
Haematological manifestations	52.2	74.8	67.0	17.4	47.0[c]	NR	81.0	55.0
Renal manifestations	43.8	39.2	24.0	22.8	50.0	NR	49.5	33.1
Neuropsychiatric manifestations	15.9	15.2	19.0	15.6	50.0	NR	37.0	6.4
Serositis	18.1	19.4	28.0	24.3	25.8	NR	49.7	32.2
Autoantibodies (%)								
ANA	99.1	96.8	82.0	96.9	98.5	95.7	98.0	96.9
Anti-dsDNA	55.3	45.3	52.0	35.5	53.8	54.4	77.3	79.3
Anti-Sm	40.7	33.8	NR	32.3	63.6	75.7	44.8	22.5
Anti-RNP	NR	59.1	NR	24.7	66.7	81.8	45.5	NR
Anti-Ro	NR	NR	NR	45.2[d]	46.7	69.7	56.4	20.4
Anti-La	NR	NR	NR	23.7	9.0	15.2	32.5	11.6

ANA, antinuclear antibody; dsDNA, double stranded DNA; NR, not reported; RNP, ribonucleoprotein; s.d., standard deviation. [a]Study specifically looking at autoantibodies. [b]s.d. not reported. [c]Anaemia only. [d]Anti-Ro60.

at onset being 25.9 years (s.d. 10.8 years) and the age at the first visit being 29.2 years (s.d. 11.5 years)[105]. The delay has been attributed to mistaken diagnoses by general practitioners of malarial fever, bacterial infections, tuberculosis and even HIV. Valuable time is therefore lost before these patients are seen by rheumatologists.

The most common manifestations of SLE in the studies included in TABLE 4 are arthritis (70–90%) and mucocutaneous manifestations (78–82%). Among the cutaneous manifestations, discoid lupus was less common in North Africa (5.4–11.9%) than in sub-Saharan Africa, specifically Nigeria, South Africa and Kenya (22–44%)[100,104,108]. In the USA, discoid lupus is also more common in African Americans than in white Americans, at a ratio of 5.4:1.0 (REFS[116,117]). A surprisingly low prevalence of photosensitivity was reported in Nigeria (9%) compared with the prevalence in most of the other studies, which ranged from 39% to 68%. Oftentimes, patients with a malar rash or indeed, other skin rashes, apply various astringents to the skin, thus presenting with atypical rashes. The prevalence of renal disease is also high, ranging from 33 to 50% in most of the studies (TABLE 4).

Autoantibody testing is not readily available in many hospitals, particularly in sub-Saharan Africa. ANAs were found in 97–99% of individuals in all studies in TABLE 4, except in Kenya, where a lower prevalence of 82% was reported. A study in a cohort of patients with SLE in Nigeria showed a speckled ANA staining pattern to be predominant (77.5%), compared with homogeneous (16.2%), speckled with homogeneous (3.8%) and ribosomal (2.5%) patterns[102]. Anti-double stranded DNA antibodies were present in 45–55% of patients with SLE in Kenya, Nigeria and South Africa, with a lower prevalence of 35% in Sudan and a higher prevalence of 77–79% in Tunisia and Egypt (TABLE 4). The prevalence of anti-Sm antibodies was similarly variable, ranging from 22.5% in Egypt to 63.6% in Nigeria. A comparative study of cohorts of patients with SLE from Sudan and Sweden found that anti-histone antibodies and anti-double stranded DNA antibodies were more common in Sweden than in Sudan, whereas anti-Sm antibodies were more common in Sudan than in Sweden[107], thus confirming the importance of anti-Sm antibodies in Black populations. Reports from Tunisia have also shown a relatively high occurrence of anti-Sm antibodies, among others[118]. The ACR–EULAR SLE classification criteria (which are often used for diagnosis) require an ANA titre of 1:80, yet serologies are unavailable in many centres or are too expensive for patients to afford. Therefore, the argument could be made for developing a set of clinical criteria to be used when diagnosing SLE in sub-Saharan Africa that does not require serology, to avoid missing potential patients.

Antimalarial agents such as chloroquine and, to a lesser extent, hydroxychloroquine, are readily available; chloroquine is often prescribed for endemic malarial fever in many African countries. Glucocorticoids are also readily available, as are immunosuppressive agents, such as methotrexate, azathioprine, mycophenolate mofetil and cyclophosphamide, although the cost of such medications can be prohibitive, especially for the majority of patients who pay out of pocket. bDMARDs are mostly unavailable (BOX 1).

Challenges and unmet needs

Rheumatic and musculoskeletal diseases have been estimated by the World Health Organization (WHO) to be the second largest cause of disability worldwide, as measured by years lived with disability[119]. The burden of these diseases is projected to be higher in low-income and middle-income countries owing to limited access to clinical services and treatments[120]. However, over the past two decades, great strides have been made in the diagnosis and management of rheumatic diseases in Africa, and the number of rheumatologists has increased, albeit from a low base.

Many challenges to the delivery of optimal health-care in Africa exist. Foremost among them is the fact that more than half of the countries in Africa are among the poorest nations in the world[121]. Many interests compete for the limited health-care resources available, including poverty-associated nutritional diseases, vaccination programmes and the triple burden of HIV, tuberculosis and malaria. A lack of education, low socioeconomic status, limited access to health care and some religious and cultural beliefs also contribute to a reluctance or delay in seeking medical care. Many patients might attribute their illness to cultural influences and seek help from traditional healers or alternative care practitioners. When patients do seek conventional medical attention, they are often seen at community health centres and receive symptomatic treatment only. As such, their condition might worsen and they can lose faith in traditional medical care. In addition, there is often a long delay before a patient is seen by a rheumatologist, during which time they can experience considerable disability and tissue damage. Thus, it is not surprising that many patients, especially those living in rural communities, are seen at an advanced stage of their illness and account for some of the extreme phenotypes that are reported. The possible contribution of genetic factors to this more severe disease still needs to be studied. The African League Against Rheumatism (AFLAR) has provided recommendations on the management of rheumatic diseases during the COVID-19 pandemic[122]. However, a report from a survey of African rheumatologists concerning the effect of the COVID-19 pandemic on rheumatology practice concluded that rheumatologists are generally avoiding performing physical examinations on patients and are relying mostly on telephone calls, messaging apps or video calls, and that there is a lack of national rheumatology guidelines or registries, as 57% of the respondents reported the presence of specific COVID-19 recommendations, and only 13% confirmed the availability of a national rheumatology COVID-19 registry in their country[123].

Human resources. Africa, particularly sub-Saharan Africa, has among the lowest ratios of health-care personnel to the size of the populations in the world[124], and there are shortages in all categories of health-care personnel, especially doctors. North African countries and South Africa have a better infrastructure for patients seen in public sector facilities than other African

countries, and there is a relatively greater number of doctors and rheumatologists who have access to laboratory tests, imaging facilities and essential medicines. Many countries in Africa do not have any rheumatologists, and others have only a few. Accurate information about the number of rheumatologists in each African country is not available, but on the basis of information provided by rheumatologists in their respective countries, it is estimated that there is about 1 rheumatologist per 110,000 people in Algeria, 1 per 590,000 in South Africa, 1 per 4,120,000 in Nigeria and 1 per 5,300,000 in Kenya (O.A., G.M.M., M.T., O.O. and S.S. unpublished data). As a result, many patients with rheumatic diseases in sub-Saharan Africa are managed by orthopaedic surgeons, internists, family practitioners and at community health-care centres. These practitioners often have limited training and support, which can result in conservative management approaches and in a failure to achieve treatment goals.

Education and training. Rheumatology training programmes have existed for a long time in South Africa, Nigeria and North African countries. Over the past two decades, however, additional training centres have been established in East African countries such as Kenya and in West African countries such as Burkina Faso, Ghana, Senegal and Cameroon. In many countries, rheumatology fellows in training take part in the 2-year EULAR online postgraduate courses, the successful completion of which is a pre-requisite for certification as rheumatologists in the English-speaking West African countries Nigeria, Ghana, Sierra Leone and The Gambia. Planning is currently underway for a EULAR–AFLAR-led webinar series for rheumatology trainees from all over Africa, and there have been major strides in the training of paediatric rheumatologists in Africa, with the formation of a paediatric rheumatology society under AFLAR, known as PAFLAR, that will no doubt help to improve outcomes for children with rheumatic diseases. The major advances in knowledge and management strategies for rheumatic diseases have resulted in improved outcomes for patients in Africa[103,125,126].

It is unlikely that there will be enough rheumatologists, or even internists and doctors, to identify and care for patients with rheumatic diseases in Africa; therefore, it could be expedient to train nurses and community health workers to identify patients with common rheumatic diseases. In a 2020 review of the global literature on nurse-led management of RA, the authors proposed strategies to implement programmes in the Middle East and Africa[101,127]. This proposed model would involve nurses performing an extended role, assuming their own patient caseloads and treating, educating, monitoring and referring as appropriate. Such initiatives should be implemented in parallel with initiatives to improve knowledge about rheumatic diseases among medical students and during the training of internists. The development of digital means of communication, education and training that has occurred during the global COVID-19 pandemic has led to an explosion of opportunities for remote learning that should feature prominently as we strive to address future challenges.

Advocacy. Urgent needs exist to raise awareness of the burden and effects of rheumatic diseases in Africa, promote the education of community and health-care workers, undertake research to inform clinical practice and improve outcomes for patients. In addition, representation to health-care administrators is required to lobby support for patients with rheumatic diseases. Globally, increased attention is being paid to the growing burden of non-communicable diseases, even in low-income and middle-income countries within Africa. Cardiovascular diseases, diabetes, cancers and chronic lung diseases, among others, were identified as priorities in this category[128]. Unfortunately, rheumatic diseases, including musculoskeletal diseases, are not included in this list. However, global initiatives such as the Bone and Joint Decade initiative in 2000–2010 drew attention to the burden and effects of musculoskeletal diseases, and its successor, the Global Alliance for Musculoskeletal Health, is continuing efforts to get musculoskeletal diseases included in the priority list by the WHO and the United Nations. Such initiatives are important because the health policies and funding of many low-income and middle-income countries are influenced by the health priorities adopted by the WHO and the United Nations.

Research. The shortage of rheumatologists has resulted in limited amounts of high-quality research being undertaken, particularly in sub-Saharan Africa. With the exception of South Africa and North African countries, little funding is available for research. To get around this problem, rheumatologists from some countries in Africa have partnered with colleagues in other parts of the world to receive mentorship and support for their work. However, capacity urgently needs to be developed to enable applications for globally funded competitive research grants to be submitted. The increase in the number of rheumatologists in Africa, especially sub-Saharan Africa, over the past 20 years has resulted in an increased number of research publications. However, most of the work has been undertaken by a small group of rheumatologists from some of the larger academic centres and might not be representative of Africa as a whole. Ongoing challenges to data collection include limited access to laboratory tests, such as serological tests, and difficulties in obtaining a regular supply of conventional synthetic DMARDs, such as methotrexate, not to mention their affordability. However, we now have an opportunity to collect our own information about the standard of care in different African countries, identify risk factors for rheumatic diseases, assess responses to therapy and study patient-reported outcome measures and quality of life. Once this information is available, it will be possible to develop evidence-based guidelines for diagnosis and management in settings with limited resources and a high burden of infectious diseases. A major need also exists for more research into genes that are important in the clinical phenotypes and response to therapies that occur among African individuals, particularly among those in sub-Saharan Africa. The West African heritage of many African Americans adds to the necessity of this research need.

Conclusions

Rheumatology in Africa has advanced substantially from an era of poor knowledge to one of increasing information. Common rheumatic diseases such as gout, RA, osteoarthritis and SLE are increasingly being reported, as are conditions previously considered to be rare such as SSc, psoriatic arthritis and osteoporosis. The clinical presentations and laboratory features of rheumatic diseases are often similar to those seen in other regions of the world, but there are certain differences too. More and better genetic studies might go some way to explaining these differences. Patients are managed according to generally accepted guidelines, although newer therapies such as bDMARDs are invariably unaffordable and unavailable. Future progress depends on education, early diagnosis, the training of more health professionals to diagnose and manage patients and reductions in the costs of newer therapies; greater expenditure on investigations into non-communicable diseases in general will be required to facilitate future advances.

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